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Physiological studies in cellulose fermentation

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Physiological Studies in Cellulose Fermentation

J. Raymond Sanborn

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"PHYSIOLOGICAL STUDIES IN CELLULOSE FERMENTATION"

J. RAYMOND SANBORN.

Thesis submitted for the Degree of

Doctor of Philosophy.

Massachusetts Agricultural College,

Amherst, Mass.

June, 1926.

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GENERAL INTRODUCTION.

Cellulose has been called the "structural basis of the vegetable world" (1), and the preponderating constituent of all vegetable tissues (2). In view of man's dependence, directly or indirectly, upon plant materials for the fulfilment of his wants and contributions to his comforts, the importance of cellulose as the structural basis of the vegetable world is unquestioned. In industry, agriculture and physiology, the conspicuous and significant rôles played by cellulose materials are beginning to be realized.

The World War gave tremendous impetus to the study and utilization of these materials, and the manufacture of useful and necessary products from them. One has only to note, aside from the printed page, the advent of paper utensils, blankets, towels, dishes, and articles of apparel, to appreciate the progress that is being made not merely from the standpoint of economy, but also of comfort and sanitation. In the field of textiles which, of course, is largely concerned with vegetable fibers, the advance in the production of artificial fibers is noteworthy. The contribution of cellulose through its nitrates to the manufacture of explosives should be mentioned, but probably of greater significance are the products celluloid and collodion also derived from

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- (1) Cross, C. F. and Bevan, E. J. "Cellulose" (1916), London, 1.
(2) Dict. of Applied Chem., Thorpe, (1912) 1; 705.

nitro-cellulose. Both of these have a variety of uses.

From the distillation of cellulose (1) and other processes of cellulose decomposition, products are being obtained which may prove to be of tremendous importance; such, for example, as may be used for fuel.

An enumeration here of all of the individual products directly or indirectly derived from cellulose would be a time-consuming and laborious task. Such a compilation is hardly necessary at this time. The division of cellulose chemistry has already drawn together a fairly complete and graphic report of the chemical products of cellulose (2).

Numerous manuals and textbooks of industrial chemistry will treat the subject of cellulose industries in greater detail (3).

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- (1) Heuser, E. "Textbook of Cellulose Chemistry", trans. by West and Esselen, (1924) 164.
- (2) "Chemical Products of Cellulose", Div. of Cellulose Chemistry, Jour. Ind. Eng. Chem. (1925) 17; 33.
- (3) Rogers, A. "Industrial Chemistry", (1921) 982, 1033, 1048.
Slosson, E. E. "Creative Chemistry", (1920) 110.
Cross, C. F. and Bevan, E. J. "Cellulose", (1916), London.
"Researches on Cellulose",
(1895-1900), (1900-1905).
Cross, C. F. and Doree, C. "Researches on Cellulose",
(1910-1921).
Worden, E. C. "Nitrocellulose Industry". (1911).
Duncan, R. K. "The Chemistry of Commerce". (1907), 219.
Bogue, R. H. "Colloidal Behavior", Ch. 27 "Cellulose and its Derivatives", G. J. Esselen, (1924).
Congress of App. Chem. (1912), 13; 101.
Armstrong, E. F. "Chemistry in the Twentieth Century". (1924), 159.
Bersch, I. "Cellulose, Cellulose Products, etc." (1904).
Trans. by Brannet.

Cellulose is also of great importance to agriculture. In a general way one may enumerate possible benefits to the soil resulting from the transformation and decomposition of recent vegetation and older plant residues. The secrets of this phenomenon apparently rest with nature, and investigators of cellulose fermentation will do well to study and observe the process as it is carried on there. A great deal of uncertainty surrounds present day conceptions not only of the rôle of cellulose in nature, but also of the physical and chemical character of cellulose itself. A statement made by Lochhead (1) with reference to soil microbiology may be applied to cellulose investigations in general. He states that much preliminary fundamental research in pure science is indispensable for a sufficient knowledge of the subject to permit direct application to the problems of agriculture. It would seem desirable, therefore, in dealing with the problems of cellulose, whether primarily chemical, industrial, physical, or biological, to bring into play the concerted efforts of the chemist, physicist, microbiologist, and plant physiologist.

One of the foremost cellulose problems confronting the microbiologist is the utilization of the huge quantities of straw which accumulate in the wheat-growing regions. The wholesale burning of the straw would seem to be a wasteful annual loss (2).

(1) Lochhead, A. G. "Some Trends in Soil Microbiology".
Scientific Agr. (1924) 4; 308.

(2) "The Utilization of Straw". Can. Chem. Metallurgy, (1924)
8; 231.

Steps are being taken to utilize this material for industrial purposes as in the manufacture of strawboard. At the Rothamsted Experimental Station, straw has been converted into an artificial farmyard manure through microbiological agencies (1). This procedure, if applicable to field conditions, may prove to be an effective solution of the problem and at the same time be a signal benefit to agriculture.

The subject of cellulose as a food of greater or lesser nutritive value is ever conducive to debate. It is probably true that the presence and subsequent decomposition of the cellulose of plant materials in the animal body are factors of importance in the intestinal functionings and in the digestive and possibly the metabolic processes of the body.

It may be that the dissolution or destruction of the cell walls of plant tissue is, in itself, a valuable aid to the animal in rendering accessible certain nutriments. There are those who consider cellulose to be a substance of high nutritive value, and various forms have been investigated and recommended as suitable for animal feeding. In this connection the pressure of the war in certain countries made it advisable to utilize cellulose in the form of wood and paper pulp, sawdust and straw, not only in cattle food, but also in war bread. Since the war, however, there is evidence that, while the human stomach may be incapable

(1) Hutchinson, H. B. and Richards, E. H. "Artificial Farmyard Manure". Jour. Min. Agr. (1921) 28; 398.

of deriving much nourishment from cellulose, cattle are able to utilize certain forms of cellulose in a somewhat predigested condition, such, for example, as hydrolyzed sawdust (1). This short resumé of a few of the outstanding features in connection with cellulose, is presented primarily to indicate the importance and scope of the subject. In view of the more detailed discussion to follow, the comments made were of necessity very brief.

This thesis is to be presented in three parts. Part one is devoted to a critical review of the subject of cellulose from the physical, chemical, and biological aspects. The literature available on the various phases of cellulose study has been covered with sufficient completeness to enable the author to view the situations existing in what seems to him to be their true light, and with an appreciation of the problems involved. Obviously it is quite impossible to exhaust the literature on any one subject. Before undertaking an investigation of cellulose fermentation, it would seem desirable to know something of the physical and chemical as well as the biological aspects of cellulose. Therefore the critical review starts with the early work upon the plant cell membrane and the identification of cellulose as its foremost constituent. With the recognition of true cellulose as a definite cell wall constituent, certain characteristics and criteria have been discovered and adopted by which one may recognize true cellulose from other constituents of the cell wall of plants. The combinations, in nature, of true

(1)

Sherrard, E. C. and Blanco, G. W. "The Preparation and Analysis of a Cattle Food Consisting of Hydrolyzed Sawdust". Jour. Ind. Eng. Chem., (1921), 13; 61.

cellulose and these non-cellulose substances are often so intimate that, in certain cases, it would seem as if new substances had been formed. These intimate combinations are included under the term "compound celluloses". It has proven to be exceedingly difficult to isolate true cellulose from these compound celluloses. In the interests of pure or industrial chemistry numerous attempts have been made to purify the true cellulose constituent of the cell membrane with varying degrees of success. Various grades of wood pulp and filter paper are results of these chemical procedures.

The dissolution and decomposition of cellulose in nature and in the laboratory by microbiological agents is treated in that portion of the critical review which deals with the biological aspects of cellulose. Arising from and suggested by this critical survey of the literature, a specific problem presents itself for investigation.

Part two of the thesis introduces the problem, states the purpose, and outlines the plan of investigation. Certain preliminary considerations necessary for effective research along the lines indicated, are also discussed.

Part three summarizes the existing situation and focuses the investigation upon specific problems formulated in accord with the purpose and objective.

P A R T I .

CRITICAL REVIEW.

1. Physico - Chemical Aspects of Cellulose.

(a) The Cell Membrane.

Early conceptions of cellulose are intimately associated with the pioneer work on the cell membrane of plants. This membrane, observed by Schleiden (1) in 1838 was studied more in detail by Payen (2) in 1842. The latter concluded that cellulose constituted the network or webbing (trame) of plant cells, and revealed a homogeneous chemical composition throughout the plant kingdom. Payen purified plant tissues from foreign deposits by chemical treatments and presented tables showing that the resulting materials possessed, when analyzed, very similar compositions.

There is a great deal of confusion and contradiction manifest among the early workers regarding the physical and chemical nature of the cellulose membrane. The iodine - sulphuric acid color reaction is classic as a test xxxx of the presence of cellulose in the cell membrane. Payen (3) describes this reaction which he obtained in plant sections under the microscope. Von Mohl, however, considered this reaction to be quite unreliable as

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- (1) Schleiden, M. J. "Beitr. z. Phytogenesis", Müller's Arch., (1838).
(2) Payen, M. "Mémoires sur les Développements des Végétaux", (1844), 211.
(3) Loc. cit., 215, 216.

an indication of the presence or absence of cellulose (1). One other color reaction of importance among the early attempts to confirm the presence of cellulose in the cell membrane is the blue-violet color obtained with chlor-zinc-iodide, a reaction successfully used by Strasburger (2). These two reactions were generally considered characteristic of cellulose, as the following quotation from Schütt suggests (3):-

"Die Substanz der Membran besteht aus Cellulose, wie die Violetblaufärbung mit Chlorzinkjod und die Blaufärbung mit Jod und Schwefelsäure anzeigt".

"The membrane consists of cellulose, as the bluish-violet color with chlor-zinc-iodide and the blue color with iodine and sulphuric acid indicate".

A number of investigators including Klebs (4) and Strasburger (5) have attempted to explain the formation of new cell walls by observing naked protoplasts and plasmolyzed cells. Strasburger caused plasmolysis in Vaucheria repens and demonstrated the formation of the new cell wall by means of Congo red, which colored the edge of the protoplasm. That this is, in reality, a secretion of cellulose may be confirmed by the chlor-zinc-iodide reaction, according to the author. Strasburger concluded that the cell membrane materials are products of the protoplasm which are secreted at the surface of the protoplast, or, remaining within, undergo varied changes of state.

According to Timberlake (6) it is probable that the substance

-
- (1) Mohl, H. von. "The Vegetable Cell". (1851) 27.
 - (2) Strasburger, E. "Die pflanzlichen Zellhäute". Jahrb. f. wiss. Bot., (1898) 31; 595.
 - (3) Schütt, F. "Centrifugales Dickenwachsthum der Membran und Extra-membranöses Plasma". Ibid., (1899) 33; 599.
 - (4) Klebs, G. "Beiträge zur Physiologie der Pflanzenzelle". Untersuch. aus d. botan. Institut zu Tübingen, (1888) 2; 489.
 - (5) Loc. cit.
 - (6) Timberlake, H. G. "The Development and Function of the Cell Plate in Higher Plants". Bot. Gaz., (1900) 30; 73, 154.

for the formation of cell wall is held in a reserve form in the protoplasm before it is actually needed for this process. Actual growth of cell walls may be measured microscopically. Krabbe (1) has made measurements of walls in various bast cells which have grown in width on the surface. The two theories advanced in explanation of the increase in thickness of cell walls, the theories of intussusception and accretion, are attributed to Nageli (2) and von Mohl (3) respectively. The former process involves an interpolation of invisible crystalline particles or "micellae" between those already existing. According to the latter view, increase in thickness takes the form of a deposition of particles in layers. It is probable that both processes may take place simultaneously, as Strasburger suggests (4).

As early as 1842, Payen expressed the opinion that in very young cells the cellulose exists in a more nearly pure state than in the later stages of maturity (5). He also recognized the prevalence of foreign or incrusting substances within the cell of mature plants. Payen states:-

"Ce fut encore le mélange ou l'interposition de corps étrangers qui, donnant à la trame résistante de certains organismes des propriétés nouvelles et une composition variable détermina l'adoption des noms de fungine, de lignine, de lichénine, de médulline, qu'il faut actuellement rayer des nomenclatures scientifiques".

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- (1) Krabbe, G. "Ein Beitrag zur Kenntnis der Structur und des Wachstums vegetabilischen Zellhäute". Jahrb. f. wiss. Bot., (1887) 18; 347.
(2) Nageli, C. "Die Stärkekörner". Zurich; (1858). Bot. Mittheilungen (1864) München, Bd. 2.
Von Mohl, H. Loc. cit. also.
(3) Bot. Zeit., (1853) 11; 753, 769. Ibid. (1858) 16; 373.
(4) Loc. cit., 596.
(5) Loc. cit.

"It was again the mixture or the interposition of foreign materials, giving to the resistant framework of certain organisms new properties and a variable composition which determined the adoption of the names of "fungine", "lignine", "lichenine", "medullene", which it is now necessary to eliminate from scientific nomenclatures".

This view finds expression today in the words of Haas and Hill, who state that in the course of time the cellulose originally formed is altered by the addition to it of various secondary products known as incrusting substances (1). These substances will be discussed more in detail later. König makes the statement that in its early development the cell membrane consists throughout of pure cellulose:-

"Die Zellmembran besteht in der ersten Entwicklungszeit der Pflanzen durchweg aus reiner Zellulose" (2)

According to other authorities cell walls never consist of pure cellulose (3), and, as a matter of fact, there is no proof of the existence of such a substance. Early conceptions were largely hypothetical, and a great deal of uncertainty attended the use of the word "cellulose". Even today a similar confusion is manifest. For example, a reserve food of the plant known as the hemicelluloses, probably constituting a major part of the cell walls of seeds, is sometimes considered practically synonymous with cellulose. If these reserve materials actually exhibited similar properties and reactions to true cellulose, it might be possible to form a group of celluloses. The hemicelluloses will be taken up later more in detail, when it will be shown that the members of the group are quite unlike

(1) Haas, R. and Hill, T.G. "An Introduction to the Chemistry of Plant Products". (1921) 1; 148.

(2) König, J. "Die Zellmembran und Ihre Bestandteile in Chemischer und Physiologischer Hinsicht". Die landw. Versuchs-Stationen. (1907) 65; 55.

(3) Strasburger's Textbook of Botany, (1921) 35.

true cellulose in many respects. It is possible that some of the confusion apparent among the early workers was due in part to the presence within the cell wall of this reserve material. This seems quite likely when one takes into consideration the fact that in spite of the dissimilar characteristics exhibited by true cellulose and the hemicelluloses, apparent even among the members of the latter group itself, many of the hemicelluloses take the same stain with chlor-zinc-iodide as true cellulose (1). The purest form of cellulose according to the present conception is found in Swedish filter paper and cotton. Most of the purified celluloses, however, have been subjected to more or less drastic chemical treatments which probably alter to some degree the original cellulose. Therefore, while it may be impossible to obtain an unaltered cellulose, the use of the term pure or true cellulose is admissible.

(b) Characteristics of True Cellulose.

According to Esselen (2), the property of cellulose on which its greatest usefulness depends is probably its general chemical inertness. In spite of this fact, he cites an observation of Cross and Bevan which would indicate an almost "paradoxical behavior" on the part of cellulose. It was found that two samples of cellulose, identical except for the fact that one had

(1) Strasburger's Textbook of Botany. (1921), 35.

(2) Esselen, G. J. Chap. 27, "Colloidal Behaviour". Bogue, (1924) 2; 632.

been boiled in distilled water for two hours and then dried, exhibited markedly different chemical characteristics. Esselen states:-

"Indeed there seems to be evidence that any treatment to which cellulose is subjected, either physical or chemical, modifies its chemical activity".

Cross and Dorée suggest that cellulose is constitutionally changed by any and every treatment to which it is exposed (1).

The resistance manifested by cellulose toward solvents, however, is one of the most common indications of its stability. Schweitzer's reagent has been for a long time one of the best known solvents of cellulose. Several others have been added to the list, particularly through the efforts of Deming (2). A few of the most important ones follow:-

- (1) Zinc chloride in concentrated aqueous solution.
- (2) Zinc chloride and hydrochloric acid.
- (3) Ammoniacal cupric oxide.
- (4) Antimony tri-chloride.
- (5) Stannous chloride and zinc bromide in either concentrated aqueous or hydrochloric acid solutions.

The cellulose-glucose relationship has awakened a great deal of discussion. The view that cellulose is a polyglucose anhydride (3) is commonly accepted. Numerous attempts at

(1) Loc. cit., 4, 12.

(2) Deming, H. G. "Some New Solvents for Cellulose and Their Action on This Substance". Jour. Amer. Chem. Soc., (1911) 33; 1515.

(3) Irvine, J. C., and Soutar, C. W. "The Constitution of Polysaccharides". Jour. Chem. Soc. (1920) 117; 1490.

quantitative resolutions of cellulose into glucose have been made (1).

Because of the severity of many of the hydrolytic treatments it is necessary to exercise great care in the procedure to avoid profound alteration in the sugar liberated and to definitely identify the products. The series of recent investigations dealing with: "The Constitution of the Polysaccharides", carried on by Irvine and his collaborators, are deserving of special mention because of their judicious and painstaking approach. Irvine and Soutar point out that pure crystalline glucose has never been obtained from cellulose and that the evidence of specific rotation and reducing power "even when apparently consistent, cannot be held to characterise an uncrystallisable syrup as a definite sugar"(2). The procedure adopted by these authors involved treatment of normal cotton cellulose with acetic anhydride containing acetic and sulphuric acids. Polysaccharide acetates were formed which could be converted into a crystalline methyl glucoside by heating with methyl alcohol and hydrochloric acid. On hydrolysis pure crystalline glucose was readily obtained from the glucoside.

Irvine and Soutar state:-

-
- (1) Ost, H. and Wilkening, L. "Die Versäuerung des Zellstoffs". *Chemiker-Zeitung*, (1910) 34; 461.
Wilstätter, I.R. and Zechmeister, L. "Hydrolysis of Cellulose". *Ber.*, (1913) 46; 2401.
Lindsay, J.E. and Tollens, E. "Ueber Dextrose aus Sulfit-cellulose und aus Tannenholz". *Ann. d. Chemie* (1892) 267; 370.
Bersch, J. "Cellulose, Cellulose Products, etc." (1904) Trans. by Brann, 93.
(2) *Jour. Chem. Soc.*, (1920) 117; 1489, 1492.

"In the work now described we adhered to the principle that the yield of hexose should be ascertained from the weight of crystalline compounds, obtained in a condition of analytical purity, and in well-defined stereochemical forms. Adopting this standard we have been able to show that, as a minimum, the yield of glucose obtained from cellulose is 85 per cent. of the theoretical amount. The method used by us embodies the same principle as acetolysis in that it involved hydrolysis of cellulose and simultaneous condensation of the sugar liberated, so as to give a stable derivative which thereafter remained unaffected. In this way the glucose was protected from the destructive effect of the hydrolytic agents".

Further work had been carried on by Irvine and Hirst and Haworth and Hirst based upon the methylation of cellulose described by Denham and Woodhouse (1). The methylation is effected in this case by the agency of sodium hydroxide and methyl sulphate. According to Irvine and Hirst (2) the transformation of cellulose into pure crystalline methyl glucoside can be effected in yields which are at least 95 per cent. of the theoretical amount. These investigators were able to obtain an 86 per cent. yield in the conversion of tri-methyl methylglucoside to tri-methyl glucose. The disacchurose cellobiose is probably one of the intermediate products in the conversion of cellulose to glucose. The formation of this sugar was mentioned by Pringsheim in his study of cellulose fermentation (3) and has been definitely obtained through the efforts of Haworth and Hirst (4) in the interest of

(1) Denham, W. S. and Woodhouse, H. "The Methylation of Cellulose". Jour. Chem. Soc., (1913) 103; 1735, (1914) 105; 2357.

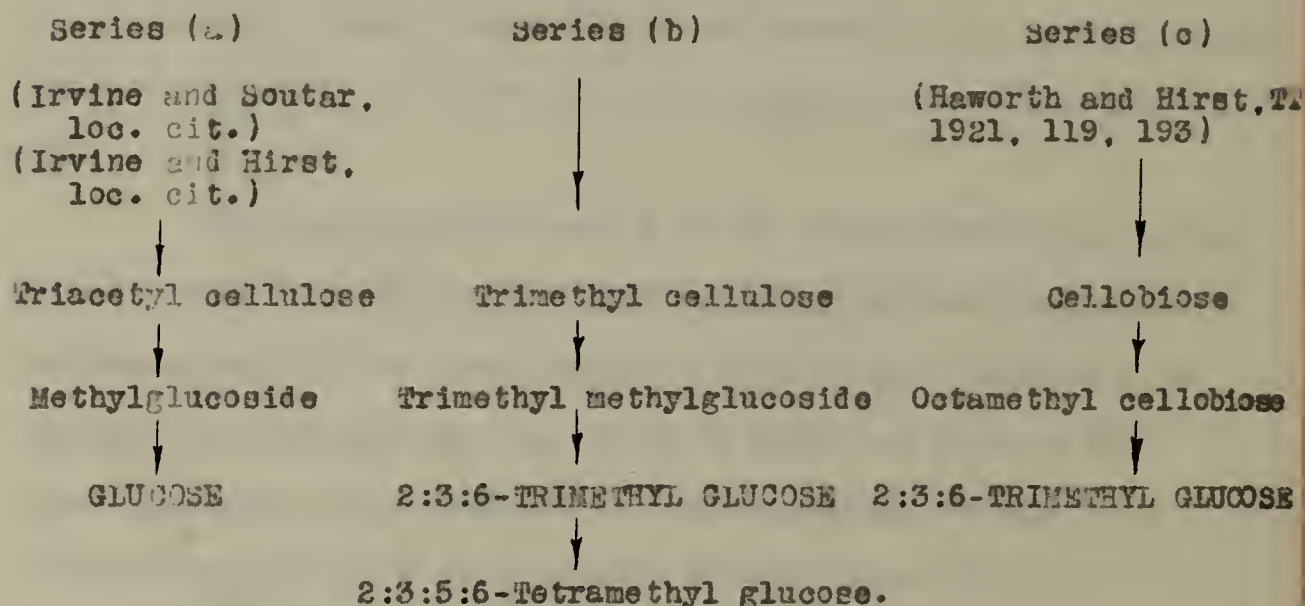
(2) Irvine, J. C. and Hirst, E. L. "The Constitution of Polysaccharides". Jour. Chem. Soc., (1923), 123; 518.

(3) Pringsheim, H. "Über den Fermentativen Abbau der Cellulose". Zeitschr. physiol. Chem., (1912) 78; 266.

(4) Haworth, W. N. and Hirst, E. L. "The Constitution of the Disaccharides". Jour. Chem. Soc., (1921) 119; 193.

the constitution of cellulose. In their opinion, cellobiose bears much the same relationship to cellulose that maltose does to starch and gives rise on hydrolytic cleavage to two molecular proportions of glucose. Irvine and Hirst offer a synopsis of the reactions of primary importance involved in these series of investigations (1).

CELLULOSE (2).



It is obvious that a knowledge of these reactions will aid materially in arriving at the molecular unit of cellulose. In this connection Irvine and Hirst make the following statement (3):-

"The constitution of the cellulose molecular unit proposed by us depends on three factors taken in conjunction. These have all been investigated here and are: (3) the proof that one fragment of the molecule consists of 1:5 - anhydroglucose (Denham); the proof that the same fragment is present

(1) Loc. cit.

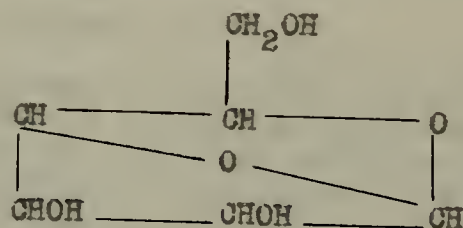
(2) Jour. Chem. Soc., (1923) 123, 523.

(3) Loc. cit. 528.

in cellobiose, which is thus an integral part of the cellulose molecule (Haworth and Hirst); and the proof now contributed that all the glucose residues in cellulose are identical. A constitutional formula arrived at in the absence of any one of these factors is logically invalid".

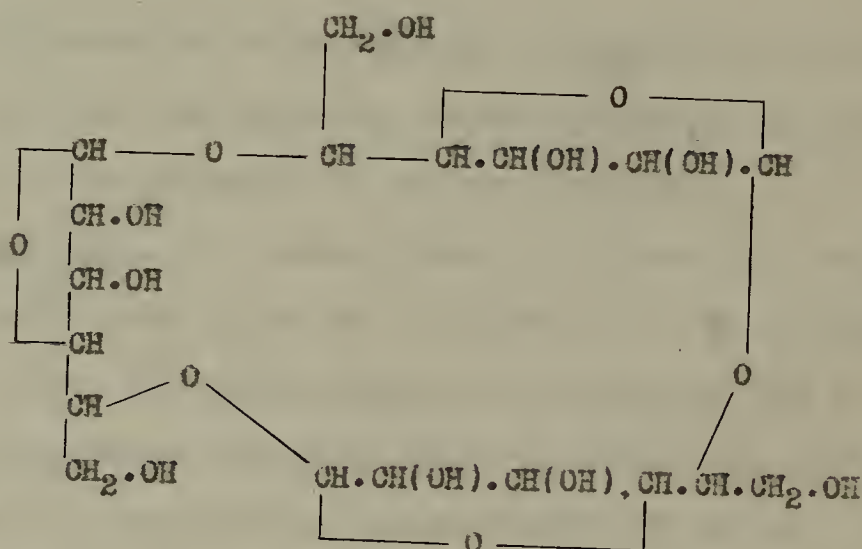
The procedure of Ost and Wilkening involving treatment with 72 per cent. sulphuric acid was repeated by Monier-Williams in the hydrolysis of cotton cellulose. This method invariably gave a syrup from which no crystalline glucose could be prepared. An independent method adopted by this author yielded a crystalline glucose amounting to 90.67 per cent. of the theoretical quantity (1).

The work reviewed above on the quantitative conversion of cellulose to glucose is a most important advance towards an understanding of the constitution of cellulose. According to Esselen (2), Hibbert was the first to point out that of the three hydroxyl groups present in the cellulose molecule, two are secondary and one is a primary alcohol group (3). Hibbert's formula is as follows:-



- (1) Monier-Williams, G. W. "The Hydrolysis of Cotton Cellulose". Jour. Chem. Soc., (1921) 119; 803.
 (2) Loc. cit.
 (3) Hibbert, H. "Studies on the Chemistry of Cellulose". Jour. Ind. Eng. Chem., (1921) 13; 256, 334.

According to Irvine (1) it is probable that cellulose is composed entirely of glucose units. In the light of recent investigations along the lines referred to above by Irvine and Hirst, it has been possible to build up several structural formulae. Irvine offers one quite in keeping with the experimental evidence which also satisfies the essential reactions of cellulose (1):-



Irvine and Hirst, in their discussion of possible formulae and the adherence of these to the evidence at hand, make the following statement (2):-

"Our work gives no indication of the degree of polymerisation undergone by the molecular unit, but the extreme insolubility of trimethyl cellulose compared with the ready solubility of methylated starch, inulin, and glycogen, points to the idea that cellulose is the most highly polymerised of the known polysaccharides".

These authors also discuss other formulae for cellulose

-
- (1) Irvine, J. C. "Some Constitutional Problems of Carbohydrate Chemistry". Jour. Chem. Soc., (1923) 123, 124; 908.
 (2) Irvine and Hirst, Loc. cit., 526.

which express a relationship between cellulose and glucose. While, in the words of Irvine, "the outlines of the cellulose molecule have been defined", further work is necessary along quantitative lines particularly with other forms of cellulose and the degradation products. Irvine and his workers are engaged in such problems of research at present. A detailed discussion of the constitution of cellulose may be found in a recent volume by Heuser (1).

The third characteristic of true cellulose to be considered will be the formation of the so-called "cellulose derivatives" when subjected to certain treatments.

Amyloid. If unsized paper is plunged into cold sulphuric acid (1.5-1.6 sp. gr.) diluted, and then transferred quickly to water, the acid compound is decomposed, and a gelatinous hydrate of cellulose known as amyloid is precipitated (2). This is a starch-like, gummy product and stains blue with iodine. The name is also applied to a mucilaginous constituent of the cell walls of certain seeds (3).

Hydrocellulose and Hydracellulose. The action of concentrated hydrochloric acid upon cellulose is rapid, and according to Cross and Bevan, disintegrate cellulose tissues. To quote from the authors (4):-

"The product obtained from cotton, after washing and drying, is a white powder which under the microscope is seen to

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- (1) Heuser, E. "Textbook of Cellulose Chemistry"., Trans. by West and Esselen (1924).
(2) Cross and Bevan, "Cellulose", 53, 54.
(3) Ibid., also Molisch, H. "Microchemie der Pflanze", (1921) 331.
(4) Loc. cit.

consist of annular fragments of the original fibers. It has been termed hydrocellulose (1) by Girard who first described this product, and hydracellulose (Witz), the latter term being perhaps preferable".

More or less of a distinction is made between these words by Otto (2) in his work on the dissolution of cellulose and cell walls by fungi. According to a more recent view (3), hydrocellulose does not represent a definite product but consists of unchanged cellulose, a little glucose, and all the intermediate products.

Oxycellulose. Several oxidants act characteristically upon cellulose (4). Nitric acid (1.1-1.3 sp. gr.) attacks cellulose at 80° to 100° with the formation of oxycellulose. Chromic acid in dilute solutions or in the presence of mineral acids acts more slowly, forming a similar oxycellulose (5). Other oxidants also yielding oxycellulose are Cl gas in the presence of water, hypochlorous acid, hypochlorites, and permanganates (6). The status of hydro- and oxycellulose is extremely uncertain. Cross and Bevan state that they have "no claim to be considered as individuals of definite identity", and that "the terms rather connote reactions, and the products represent phases of equilibrium of complex effects, in part determined by the reagent and conditions

(1) J. Textile Inst. (1923) 14; 69-77. Cellulose Chemie. (1922) 3; 89-96, 101-7.

(2) Otto, H. "Beiträge zur Allgemeinen Botanik", Haberlandt, Berlin, (1918) Erster Band, 202.

(3) Esselen, G. J. "Colloidal Behavior", Bogue, 634.

(4) Cross and Bevan, "Cellulose", 56-62.

Esselen, G. J. Loc. cit. 638.

Birtwell, C., Clibbens, D. A. and Ridge, B. P. "Chemical Analyses of Cotton". Oxycellulose I. J. Text. Inst. (1925) 16; 13-52T.

(5) Cross and Bevan, loc. cit.

(6) Ibid.

of action, in part the result of re-arrangements, through the weakening or liberation of internal strains" (1). On the other hand Otto considers the oxycelluloses to be a fairly definite group consisting of cotton and other derivatives of celluloses which are formed by oxidizing agents such as chloride of lime (Chlorkalk), bromine water, nitric acid, potassium chlorate (Kaliumchlorat), chromic acid, and permanganate (2).

Alpha- Beta- Gamma- Celluloses. The Mercerization test of Cross and Bevan (3) has been used as a standard of purity for cellulose (4). This test, in the words of Dore (5), depends upon the fact that the "normal cellulose unites with the sodium hydroxide of mercerizing strength to form alkali cellulose and is regenerated without loss of weight upon diluting and washing out the lye". In practise the fiber is treated with 17.5 per cent. NaOH (6), diluted and filtered. After copious washing the residue is dried and weighed as normal or alpha cellulose. According to Dore a diminished yield of normal cellulose "indicates that the treatment has been too severe and has attacked the most resistant member of the series".

Other celluloses are supposedly dissolved by the alkali treatment. When the filtrate is neutralized, however, a

(1) Cross and Doree, "Researches on Cellulose", (1922) 112,140.

(2) Otto, H. Loc. cit.

(3) Loc. cit., 23, 120.

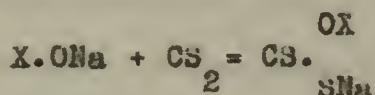
(4) Uyeda, Y. "Studies on Bast Fibers. II Cellulose in Bast Fibers". Jour. Ind. and Eng. Chem., No.2 (1921) 13; 141.

(5) Dore, W. H. "The Determination of Cellulose in woods". Jour. Ind. and Eng. Chem., No.3 (1920) 12; 264.

(6) Ibid. Also Mahood, S. A. and Cable, D. E. "Comparison of Wood Cellulose and Cotton Cellulose". Jour. Ind. and Eng. Chem., No.8, (1922) 14; 727.

precipitate appears which is called beta cellulose. A third portion of the cellulose is considered to remain permanently in solution as gamma cellulose. An improved method for the determination of alpha- beta- and gamma- celluloses has recently been suggested by Bray and Andrews (1) fundamentally the same as above.

A fourth characteristic of true cellulose may be illustrated by the "cellulose thiocarbonates". When an alkali cellulose such as mercerized cotton is treated with carbon disulphide, the so-called thiocarbonate reaction takes place, which may be represented by the following equation, the details of which have been worked out by Cross and Bevan (2):-



The resulting compound is sometimes called alkali-cellulose-xanthate. During the reaction, according to Cross and Bevan, the mercerized fiber undergoes further swelling and "a gradual conversion into a gelatinous transparent mass, which dissolves to a homogenous solution on treatment with water" (3).

Some attention has been given to certain microchemical reactions to which cellulose is supposed to respond (4). It is true that a few of the so-called plant cell constituents take a characteristic stain under certain conditions. The propriety is

(1) Bray, M. W. and Andrews, T. M. "An Improved Method for the Determination of Alpha, Beta, and Gamma Cellulose". Jour. Ind. and Eng. Chem., No.4, (1923) 15; 377.

(2) Loc. cit., 25.

(3) Loc cit., 25.

(4) Haas and Hill, "An Intro. to the Chem. of Plant Prod." (1921) 163.

extremely variable, and experience proves that little or no reliance can be placed upon it. Actual knowledge regarding the structure and constituents of the cell wall is very limited. While it is probable that certain investigators have become expert enough through years of experience to detect microscopically changes and abnormalities in cell walls, evidence based solely upon staining reactions is not at all dependable. A study recently made by Abrams (1) on the effect of chemical reagents upon the micro-structure of wood, introduces the color reactions of cellulose and lignin in wood sections. After reviewing the subjects of incrusting substances and compound celluloses later, it will be obvious that there probably exists very little color resemblance among the celluloses present in the cell walls of varying degrees of lignification, suberization, and pigmentation. The cellulose is presumably ... invaded and infiltrated by non-cellulose incrustants, each capable perhaps of producing its own color reaction. Early workers on the formation of the cell wall were obliged to resort to staining reactions, and inasmuch as they worked largely with cells free from incrustation, their efforts were satisfactory in associating a definite color reaction with cellulose.

(c) The Hemicelluloses.

The reserve food of the plant known as the hemicelluloses may be laid down adjacent to the cell wall, or as Jost describes

(1) Abrams, A. "The Effect of Chemical Reagents on the Micro-structure of Wood". Jour. Ind. and Eng. Chem., (1921) 13; 786.

it, "thickenings on the walls" (1). The hemicelluloses include a variety of substances whose properties and reactions differ considerably. They are generally classified as hexosans or pentosans according as hexose or pentose sugars are formed by hydrolysis. Gum arabic and wood gum representing two pentosans called arabin and xylan respectively, are said to yield arabinose and xylose upon hydrolysis. The hexosans have been further subdivided into mannans and galactans, the former hydrolyzing to mannose and the latter to galactose.

Schulze detected various sugars resulting, as he affirms, from the hydrolysis of the hemicelluloses in the cell walls of certain seeds and other plant parts (2):-

No.	Versuchsobjekte	Bezeichnung der bei der Hydrolyse erhaltenen Glukosen.
1.	Samen der gelben Lupine (<i>Lupinus luteus</i>)	Galaktose und eine Pentose (Arabinose?)
2.	Samen der blauen Lupine (<i>Lupinus angustifolius</i>)	Galaktose und Arabinose
3.	Samen der behaarten Lupine (<i>Lupinus hirsutus</i>)	Galaktose und Arabinose
4.	Samen der Erbse (<i>Pisum sativum</i>)	Galaktose.
5.	Samen der Wicke (<i>Vicia sativa</i>)	"
6.	Samen der Ackerbohne (<i>Vicia faba</i>)	"
7.	Samen der Sojabohne (<i>Soja hispida</i>)	"
8.	Samen des Kaffeebaums (<i>Coffea indica</i>)	Galaktose und Mannose
9.	Samen der Kapuzinerkresse (<i>Tropaeolum majus</i>)	Galaktose, Dextrose, und Xylose
10.	Samen der Pfingstrose (<i>Paonia officinalis</i>)	Galaktose und eine Pentose

(1) Jost's Plant Physiology, Trans. by Gibson, (1907) 163.

(2) Schulze, E. "Über die zur Gruppe der stickstofffreien Extraktstoffe Gehörenden Pflanzenbestandteile". Jour. f. Landwirtschaft, (1904) 52; 1, 20.

No.	Versuchsobjekte	Bezeichnung der bei der Hydrolyse erhaltenen Glukosen
11.	Samen der Dattel (<i>Phönix dactylifera</i>)	Galaktose und Mannose
12.	Kokosnusskuchen (<i>Cocos nucifera</i>)	Galaktose (und Mannose?)
13.	Palmkernkuchen (<i>Elais guinensis</i>)	Galaktose und Mannose.
14.	Sesamkuchen (<i>Sesamum indicum</i>)	Mannose und Arabinose
15.	Weizenkleie (<i>Triticum vulgare</i>)	Arabinose und Xylose
16.	Roggenkleie (<i>Secale cereale</i>)	" "
17.	Maiskleie (<i>Zea Mais</i>)	Xylose
18.	Unterstes Internodium des Halmes des Besenrieds (<i>Molinia coererulea</i>)	Dextrose, Lävuiose, unx Xylose.
19.	Rot-kleefpflanzen (<i>Trifolium pratense</i>)	Galaktose.
20.	Luzernepflanzen (<i>Medicago sativa</i>)	"

Müller found in various Cryptogams the following cell wall constituents (1):-

No.	Name der Kryptogamen	Hexosen	Hemizellulosen Pentosen	Wahre Zellulose	Andere Bestandteile
1.	<i>Cladophora glomerata</i>	---	Xylan	Glukose Zellulose	----
2.	<i>Cladonia rangiferina</i>	Galaktan	Pentosane	Glukose Zellulose	Chitin
3.	<i>Cetraria islandica</i>	Galaktan Glukosan	Pentosane	Glukose Zellulose	Lichenin
4.	<i>Evernia prunastri</i>	Galaktan	----	Glukose Zellulose	Everniin Chitin
5.	<i>Lecosgyphus Taylori</i> (Hook)	----	Xylan Araban Methyl-pentosan	Glukose	----
6.	<i>Mastigobryum trilobatum</i>	----	Xylan Araban Methyl-pentosan	Glukose Zellulose	----
7.	<i>Sphagnum cuspidatum</i>	---	Xylan	Glukose Zellulose	----
8.	<i>Polytrichum commune</i>	----	Pentosane	Glukose Zellulose	----

(1) Müller, K. "Die Chemische Zusammensetzung der Zellmembranen bei Verschiedenen Kryptogamen". Zeitschr. physiol. Chem. (1905) 45; 264.

(d) The Incrusting Substances.

The Incrusting substances are probably products of the protoplasm (1), and may begin the process of incrustation early in the life of the plant. The incrustants engage the cell membrane in more or less intimate combinations (2) changing materially its nature and properties. The true cellulose of the young cell wall, therefore, becomes infiltrated, at various stages of maturity, with these invading substances, which results in the formation of the compound celluloses to be taken up later. The nature of the incrustation depends of course upon the particular plant, and the extent to which the properties and reactions of the cell wall are affected will be governed by the degree of penetration of the incrustants (1). Thus, the futility of stressing micro-chemical reactions in distinguishing plant cell constituents will be readily seen.

The above conception of the process of incrustation receives support from Tappeiner (3), who considers that young cell walls consist almost exclusively of cellulose, but that as the plant matures, changes take place such as lignification, suberization, and cuticularization which alter the cell wall in various ways according to the stage of development. Esselen considers the process of lignification to depend largely on adsorption (4).

(1) Haas and Hill, loc. cit.

(2) König, J. "Die Zellmembran und ihre Bestandteile in Chemischer und Physiologischer Hinsicht". Die landw. Versuchs-Stationen, (1907) LXV; 59.

(3) Tappeiner, H. "Untersuchungen über die Gärung der Cellulose, Insbesondere über deren Lösung im Darmkanale". Zeitschrift für Biologie, (1884) n.f., 2; 52.

(4) Loc. cit., 628.

Lignin is composed, according to this author, of hydrosols of high molecular weight which are "colloidally dissolved in the formative or cambial sap and which are deposited by adsorption on the surface of the cellulose fibers". Esselen makes the following statement:-

"The variability of the lignin, which is held responsible for the differences in physical properties of the various kinds of wood, is attributed to the variability of the sap. It has been found that the amount of adsorbable material in the formative juices may vary from 6 to 30 per cent. with the season of the year, and the maximum colloidal content coincides with the maximum lignification" (1).

Although the terms used in describing the nature of incrustation usually imply a physical process in the main, yet, from a study of the character of incrustated products it is probable that reactions of a more nearly chemical nature take place. These reactions result, according to Esselen, in dehydration, toughening and aging (1). The process of incrustation is well described by König (2).

(1) Loc. cit., 628.

(2) Loc. cit. Die Zellmembran besteht in der ersten Entwicklungszeit der Pflanzen durchweg aus reiner Zellulose, mit fortschreitender Entwicklung werden jedoch noch andere Stoffe, die Inkrusten, in die Zellmembran abgelagert bzw. aus ihr gebildet. Zu diesen Einlagerungen bzw. Inkrusten werden in den unverholzten Membranen gerechnet die Bitterstoffe, Farbstoffe, Gerbstoffe und besonders die Pektinverbindungen ----- ebenso werden die gummi- harz- und schleimgebenden Stoffe als Bestandteile der Zellmembran zu den Inkrusten gerechnet. Zu diesen Stoffen treten in den verholzten Membranen die nie fehlenden aromatischen Stoffe des Holzes, das Hadromal, Koniferin, und Vanillin, ferner das Suberin oder der Korkstoff. Der Menge nach zu den wichtigsten Inkrusten der Zellmembran gehört aber das Lignin, worunter man allgemein im chemischen Sinne denjenigen Bestandteil der Zellmembran versteht, der ein höheren Kohlenstoffe als Zellulose besitzt und mit der Zellulose eng verbunden ist, d.h. dieselbe entweder durchringt oder umhüllt".

König enumerates the cell membrane constituents as follows:- (1)

"1. Die Hemicellulosen, Hexosane wie Pentosane, werden durch verdünnte Mineralsäuren (auch zum Teil durch organische Säuren) hydrolysiert und gelöst.

2. Von den Inkrusten sind:

a) die Bitterstoffe, Gerbstoffe, Farbstoffe, die Pektinverbindungen, die gummi- und schleimgebenden Stoffe, die aromatischen Aldehyde (Hadromal, Coniferin, und Vanillin) ebenfalls in Säuren oder in verdünntem Alkali löslich;

b) die esterartigen Verbindungen Cutin und Suberin löslich in Alkali, dagegen unlöslich in Kupferoxydammoniak;

c) die Lignine zum Teil auch löslich in Säuren und Alkali, vorwiegend aber oxydierbar durch schwache Oxydationsmittel und auf diese Weise trennbar von der wahren Cellulose.

3. Die wahre Cellulose (bzw. die Cellulosen) unlöslich in verdünnten Säuren und Alkalien, unoxydierbar durch schwache Oxydationsmittel, dagegen löslich in konzentrierten Mineralsäuren und Kupferoxydammoniak (oder auch in einer Lösung von Zinkchlorid in ^{der} /Zweifachen Gewichtsmenge von Essigsäurehydrid)".

(1) König, J. "Chemie der Menschlichen Nahrungs - und Genussmittel", (1910) 3; 451.

"In the early stages of development of the plant the cell membrane consists throughout of pure cellulose; with progressive development, however, other materials, the incrusting substances, are deposited in the cell membrane or formed from it. To these depositions or incrustations in the unligified membranes may be added the bitter principle, pigments, tannin, and especially the pectin compounds - even the gummy - resiniferous - and slime-forming materials are classed with the incrustants. Entering the lignified membrane in addition are the never failing aromatic substances of wood, hadromal, conifer, and vanilla; later, cork or corky material. Lignin is one of the most important incrustants of the cell membrane and is generally understood to include those constituents which have a higher carbon content than cellulose and with which cellulose is intimately associated, that is to say, either penetrated or enveloped".

Translation of footnote (2) page 26.

König enumerates the cell membrane constituents as follows:

"1. The hemicelluloses, hexosans as well as pentosans, are hydrolyzed and dissolved by dilute mineral acids (also in part by organic acids).

2. The incrusting substances consist of:

- a) The bitter principle, tannin, pigments, pectin compounds, the gummy and mucilaginous materials, the aromatic aldehyde (hadromal, conifer, and vanilla) likewise soluble in acids or in dilute alkalis;
- b) The ester-like compounds cutin and suberin, soluble in alkali, but insoluble in copper-ammonium;
- c) Lignin, partly soluble in acids and alkali, chiefly oxidizable, however, by weak oxidizing agents and in this way separable from true cellulose.

3. True cellulose or celluloses, insoluble in dilute acids and alkalis, unoxidizable by weak oxidizing agents, but soluble in concentrated mineral acids and copper-ammonium (also in a solution of zinc chloride in two parts by weight of acetic anhydride)".

Translation of page 27.

(e) The Compound Celluloses.

So intimate is the combination of true cellulose and the non-cellulose incrusting substances that in certain cases it would seem as though a new substance had been formed. These so-called compound celluloses have been classified according as the incrustant is ligneous as in wood or jute; pectic or gummy as in flax; or fatty or waxy as in cork. The classification according to Haas and Hill is as follows (1):-

Lignocellulose.

Pectocellulose.

Adipo - or Cutocellulose.

Most of the incrusting matters are readily extracted by hydrolytic treatments. In the case of lignin, however, so intimate is the combination known as lignocellulose that in the separation of lignin from cellulose in woody tissue, the latter is invariably attacked. Regarding the lignocelluloses, Haas and Hill make the following statement (2):-

"The lignocelluloses are considered by most authorities to consist of cellulose combined with at least two other non-cellulose constituents. One of these, A, appears to contain an aromatic nucleus; and the other, B, contains a furfural-yielding complex, and is possibly a pentosane".

The following diagramatic structure for lignocellulose as exemplified by jute fiber has been worked out by Cross and Bevan (3).

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- (1) Loc. cit., 149; also Cross and Bevan, "Cellulose", (1916) 89, 214, 225.
(2) Loc. cit., 155.
(3) Loc. cit., 94.

Lignocellulose

Cellulose		Lignone (non-cellulose)
Cellulose a	Cellulose b	Furfural-yielding Keto R. hexene
containing	containing	complex group
oxidized	O.CH ₃	and secondary constituents
groups	groups	O.CH ₃ groups and CH ₂ .CO residue.

Numerous attempts have been made to determine the composition of lignin or lignone. The carbon content seems to be higher than in cellulose, and in this contention authorities apparently agree. Dietrich and König (1) carried on extensive work on the carbon content of the non-cellulose portion of meadow hay fiber, and of the corresponding amount as excrement. They found a percentage of carbon present varying between 55 and 56. Work with "Bohnstrohfaser" yielded results - 55.5 to 57 per cent. C. Schulze (2) calculated in an indirect way an average composition for lignin:-

C	55.55%
H	5.83%
O	38.62%

which corresponds closely to the formula C₁₈ H₂₄ O₁₀.

(f) The Cellulose Group.

Apart from the fact that certain plant cell walls probably consist largely of hemi- or reserve cellulose - a subject to be considered later - it may be inferred from the above discussion that originally plant cell walls are composed of one true cellulose. This may or may not be the case. It is possible that there may

- (1) Dietrich, Th. and König, J. "Ueber die Zusammensetzung und Verdaulichkeit der Neben der Cellulose in der Rohfaser des Wiesenheus Vorkommenden Substanz." Die landw. Versuchs-Stationen, (1871) 13; 222.
- (2) Schulze, F. "Beitrag zur Kenntnis des Lignins". Chem. Zentralblatt, (1857); 321.

exist various true celluloses quite distinct from the reserve and compound celluloses. This is mere speculation, however, and until a method is devised for isolating true cellulose from plant tissue without altering in any way its natural condition, the problem will probably remain unsolved. The cellulose residues obtained by extracting the incrusting substances from plant material using the drastic chemical processes to be reviewed later, are not, of course, natural celluloses. Cross and Dorée suggest that cellulose is constitutionally changed by any and every treatment to which it is exposed(1) Hence, no two masses of cellulose are constitutionally identical (1). Cross and Bevan (2) have attempted to group the cellulose of the plant world, "so far as they have been investigated from the point of view of chemical constitution":

Group (a): Those of maximum resistance to hydrolytic action, and containing no directly active CO groups.

Group (b): Those of lesser resistance to hydrolytic action, and containing active CO groups.

Group (c): Those of low resistance to hydrolysis, i.e., more or less soluble in alkaline solutions and easily resolved by acids, with the formation of carbohydrates of low molecular weight.

Group (a) includes cotton cellulose and various fibrous celluloses such as those of flax, hemp, and China grass, which have

(1) Loc. cit., 4, 12.

(2) Loc. cit., 78.

been purified by processes of alkaline hydrolysis and oxidation. According to Cross and Bevan the purified celluloses of this group may be taken as chemically identical with cotton cellulose in spite of certain differences in morphological characteristics and external properties (1). Its members have in common certain properties and reactions such as their relationships to special solvents and their resistance to hydrolysis and oxidation.

Group (b) includes celluloses from woods and lignified tissue generally and celluloses from cereal straws, esparto grass, etc.

Group (c) consists of the so-called hemicelluloses, the reserve food of the plant, to be treated later more in detail.

Cross and Bevan define them as follows:-

"Substances closely resembling in appearance the true celluloses, but easily resolved into simple carbohydrates by the hydrolytic action of enzymes, or of the dilute acids and alkalies" (2).

Whether or not there is any relationship between the hemicelluloses and true cellulose is uncertain. The former seem to constitute a fairly definite group, quite unlike the typical celluloses represented by cotton and filter paper. This view is evidently held by Cross and Bevan in regard to group (c) to which they apply the term Pseudo- or Hemicelluloses (2). They state:-

"It appears, therefore, generally that a large number of plant constituents which have been denominated by

(1) Ibid.

(2) Loc. cit., 87.

the physiologists as 'cellulose' have little more title to be considered as such than has starch".

Investigations have shown that the ultimate product of hydrolysis of true cellulose is glucose, quantitatively determined by several workers. Products resulting from the hydrolysis of hemicelluloses, however, as previously mentioned, are numerous, including galactose, mannose, fructose, xylose, and arabinose (1). In view of these facts, the hemicelluloses would seem to be only remotely related to true cellulose, resembling the latter in appearance, according to Cross and Bevan. A more intimate consideration of the hemicelluloses will be taken up later, but for the present group (c) may be eliminated from this particular discussion inasmuch as the problem is to discover a cellulose group among the true celluloses.

Returning to Cross and Bevan's classification, group (a) is the cotton cellulose group, and its members include various fibrous celluloses (which constitute the best of exogenous flowering annuals) (2). According to these authors, the bast fiber is a pectocellulose (2), that is, cellulose incrustated by pectic substances resulting in the compound cellulose known as pectocellulose, quite distinct from those pectic bodies external to the cells of the fiber which may have a binding or cementing function. The members of this group mentioned above, flax, hemp, China grass, and even raw cotton, are referred to by Cross and Bevan as non-lignified fibers, "containing pectic bodies associated with the

(1) See pp.23-24.

(2) Loc. cit., 79, 80.

cellulose which are hydrolyzed and dissolved by treatment with boiling alkalies" (1). On the other hand group (b) consists of celluloses from woods, including lignified tissues generally, from cereal straws and esparto grass, all of which, according to the authors, belong to the lignocellulose group (1).

Apart from Cross and Bevan's classification, lignin would seem to be the most significant of the incrusting substances from the point of view of the chemist because of the difficulties encountered in obtaining a cellulose even approaching purity from lignified tissue. Furthermore, in view of the fact that there are cell walls among the higher plants representing varying degrees of incrustation, it would stand to reason that in isolating true cellulose the highly incrustated material such as wood, would require a much more drastic treatment than jute fiber, also a lignocellulose. Jute appears to be more highly incrustated than hemp and flax, both pectocelluloses, and these in turn than certain plants to be mentioned later whose cell walls are composed largely of hemicelluloses. Would it not seem reasonable, therefore, to conclude that the cellulose present originally in the plant as true cellulose, probably undergoes change or alteration to a greater or lesser degree depending upon the nature and extent of incrustation, and the severity of the treatment employed in removing these incrustants? If divergencies among the cellulose residues are accounted for on the basis of the mildness or rigorousness of the processes employed, occasioned by the requirements of the particular plant material from which the cellulose is to be isolated, the result might be a much larger group of celluloses than Cross and Bevan suggest.

(1) Ibid., 79, 163, 220.

(g) The Determination of Crude Fiber and Cellulose.

In view of the foregoing discussion of the characteristics of cellulose, the effect of chemical reagents, and the probable relationships existing among cell wall constituents, it would seem exceedingly difficult to effect an isolation of cellulose in a form even approaching purity. Many different chemical processes have been tried in the past in an effort to purify true cellulose. Obviously the most satisfactory material for this purpose would be cellulose which is largely devoid of incrusting substances, such as raw cotton, and unligified fibers such as flax. The highly incrustated tissues would require a more drastic treatment for the removal of lignin, suberin, cutin, etc., thereby endangering to a greater degree the true cellulose. The ability of this original cellulose to withstand the drastic effects of these chemical processes unaltered, is uncertain. The more extreme measures unquestionably effect the nature of the cellulose, while the milder ones may remove only a portion of the non-cellulose constituency. The product of the latter treatment is known as crude fiber, and is composed largely of lignin and pentosans in addition to true cellulose (1).

One of the earliest and probably one of the most important methods for obtaining crude fiber was the Weende method of Henneberg (2). This was for many years the standard process (3) for determining the nutritive value of foodstuffs, and serves even

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- (1) Kellner, O. "Die Ernährung die Landwirtschaftlichen Nutztiere". (1909) 14, 15.
(2) König, J. "Chemie der Menschlichen Nahrungs-und Genussmittel". (1910) 3; 457.
(3) Tollens, B. "On Plant Materials, Especially the Pentosans in Feedstuffs, Their Determination and Properties". Jour. für Landw., (1896) 44; 171.

to-day as the basis for modern methods. Proteins, water-soluble carbohydrates, and fats are more or less satisfactorily separated from the crude fiber by this method (1). In the Weende process the dry, finely ground substance is treated with dilute sulphuric acid followed by heating for one-half hour. After decantation and further heating with water the process is repeated using dilute caustic potash (2).

Another method for estimating crude fiber is that of König (3). The process involves treatment with glycerine and concentrated sulphic acid, with subsequent heating on a return condenser or in an autoclave at 137° . This method followed out in detail yields "aschenfreie Rohfaser", and upon further treatment according to the method of König to be mentioned later, gives "Rein-cellulose".

In 1857 Fr. Schulze (4) found that by treating the plant material with alcohol, ether, and water or dilute acids and alkalies, followed by digestion with dilute nitric acid and calcium chlorate at room temperature, and subsequently heating the residue with dilute ammonia liquor, it was possible to bring about a partial separation of the cellulose and the non-cellulose constituents of plants, and furnished an approximate estimate of the cellulose content. Foreign matters were not entirely eliminated from combination with the cellulose, however.

(1) Cross and Bevan, "Cellulose", 165.

(2) König, J. Loc. cit.

(3) Loc cit.

(4) Chem. Zentralblatt, (1857) 321.

As early as 1873 the danger of cellulose itself being attacked during the process was recognized. In that year, König (1) studied the effect of chlorine water and hypochlorite of calcium upon various forms of cellulose such as Swedish filter paper, rye and bean straw. He found that the cellulose was more or less attacked by these reagents. He proceeded further in an attempt to find an indirect method of estimating cellulose, basing his contentions upon the theory that lignin possessed a very similar chemical composition as a constituent of various kinds of crude fiber. As a matter of fact, previous investigations on the composition of lignin had reported uniform results particularly regarding the carbon content, which averaged 55 per cent. Subsequent work, however, especially by Krauch and w. v. d. Becke (2), proved that lignin not only possessed a different composition in single plants, but that the carbon content showed marked fluctuations, varying from 51.50% to 69.64%.

In 1890, Lange introduced his "Schmelzprozess" for the quantitative estimation of cellulose (3). This process, he claimed, did not attack the cellulose, consequently could be relied upon to yield accurate results. According to the Schmelzprozess, the substance is heated at 180° for one hour with caustic alkali and 30 to 40 c.c. of water in a closed retort on

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- (1) König, J. "Die Bestimmung der Cellulose und Ihre Mängel". Die landw. Versuchs- Stationen, (1873) 16; 415.
(2) Krauch, C. and Becke, w. v. d. "Über die Holzfaserbestimmung und ihre Mängel". Die Landwirtschaftlichen Versuch- Stationen (1882) 27; 387.
(3) Lange, G. "Zur Quantitativen Bestimmung der Cellulose". Zeitschr. physiol. Chem., (1890) 14; 283.

an oil bath. After cooling to 80°, the contents is treated with hot water and washed into a beaker with hot and finally cold water. The cellulose is precipitated with sulphuric acid and the contents of the beaker is made weakly alkaline with dilute caustic soda solution so that all precipitated substances except cellulose dissolve. The residue is washed with hot water, digested in alcohol, washed with ether, dried and weighed. The time consumed by the process was only five to six hours.

A method suggested by Hoffmeister (1) yielded a cellulose approaching purity. After the removal of the fat with ether, the cellulose material was treated with hydrochloric acid and potassium chlorate, followed by digestion with dilute ammonia. Pursuing the work further (2), Hoffmeister investigated the possibility of obtaining a cellulose apparently unchanged by the chemical reagents used in its purification. In the experiments he employed pine and guaiacum woods, ivory nuts, "palmkuchen", and filter paper. The procedure first involved treatment with ether, alcohol, and water in flowing steam followed by extractions with dilute ammonia and sodium hydroxide. In the case of the woods, the process was continued using "Chlorgemisch" and ammonia. After a single treatment the pine was soluble in ammoniacal cupric oxide. With the guaiacum, however, greater difficulty was encountered in getting the cellulose to dissolve. The author

(1) Hoffmeister, W. "Der Rohfaser und Einige Formen der Cellulose". Landw. Jahrb., (1888) XVII; 239.

(2) "Die Cellulose und ihre Formen. Das Cellulosegummi". Die landw. Versuchs-Stationen, (1891) 39; 461.

concludes that the treatment with "Chlorgemisch" on guaiacum is not energetic enough to make all the cellulose soluble in ammoniacal cupric oxide. Hoffmeister did not, according to his own statement, succeed in obtaining a cellulose unaltered by the chemical reagents used, but declared his method quantitative and capable of yielding a pure product.

Kleiber modified Hoffmeister's method, and applied it in the estimation of the cellulose content of roots, grasses and seeds (1). The modification consisted in boiling the material with dilute acid early in the process. For comparison, Kleiber used the weende method on similar materials. He reports the following results:-

Raw Material	Crude Fiber Weende method	Pure Cellulose	
		Hoffmeister method	Hoffmeister method modified
Oat grass	30.35	34.9	31.5
Lucerne	25.25	28.7	20.5
Leaves of Ash	13.05	15.4	13.8
Roots of Melic	21.60	29.1	21.4
Coffee beans	18.30	35.1	23.3
Bran	8.2	19.3	9.3

A new method for cellulose estimation in food and feces was developed by Simon and Lohrlich in 1904 (2). Based upon the Lange method which employed caustic potash solution with apparent

- (1) Kleiber, A. "Versuche zur Bestimmung des Gehalts einiger Pflanzen und Pflanzenteile an Zellwandbestandteilen an Hemicellulosen und an Cellulose". Die landw. Versuchs-Stationen, (1900) LIV; 161.
- (2) Simon, O. and Lohrlich, H. "Eine Neue Methode der Quantitativen Cellulosebestimmung in Nahrungsmitteln und Faeces". Hoppe-Seyler's Zeitschr. physiol. Chem., (1904) 42; 55.

safety, the authors bring the lignin and pectin substances into solution with 50 per cent. caustic potash solution and hydrogen peroxide on a boiling water bath. According to the nature of the cellulose, a portion goes into solution. For instances, the greater part of cellulose dissolves, still less of bread cellulose, while in the case of filter paper or pulp many hours of boiling does not cause the smallest part to dissolve. The dissolved cellulose precipitates upon the addition of alcohol. It was found necessary to add a small quantity of acetic acid in some of the experiments to insure a uniform mixture of the solutions. The precipitate settles, and is finally filtered through a weighed filter, washed with water, then dilute acetic acid to remove the phosphate. After treatment with alcohol and ether, the white cellulose powder is dried and weighed. Appreciating the difficulties involved in cellulose estimation, Simon and Lohrlich sought mainly a pure cellulose, sacrificing accuracy, if need be, by the use of too drastic measures to eliminate the incrusting substances. The shortness of the process was a decided advantage in view of its use in physiological investigations, including work with human beings. Some of Lohrlich's results follow:-

Kartoffelcellulose (Trockensubstanz)

	1.	2.
1.	1.35%	1.41%
2.	1.50%	1.49%
3.	1.38%	1.38%

Brot

	1.	2.	3.	
1.	0.38%	0.34%	0.34%	0.05% N.
2.	0.2 %	0.23%	0.23%	2. % asche

Rademann's Cellulosebrot (trocken)

3.22%	3.26%
-------	-------

Menschliche Faeces.

1.	2.		
1. 3.93%	4.01%	20	% Nach Abzug von
2. 2.31%	2.41%	41	%
3. 1.85%	1.85%	41.6	% asche
4. 1.41%	1.47%		
5. 3.26%	3.29%		
6. 1.97%	1.96%		
7. 3.97%	4.07%		
8. 1.61%	1.55%		

Gemüse, Pflanzen.

1.	2.	
1. Kohlrabi, roh	0.725%	0.732%
2. Mohren, roh	0.73 %	0.756%
3. Hafermehl	0.282%	0.208%
4. weissekraut, roh	0.77 %	0.805%
5. Agar-agar	0.534%	0.602%

A general method for cellulose determination is suggested by König (1), a continuation of the process used by him in the estimation of crude fiber previously described. The further steps in the process include treatments with hydrogen peroxide and ammonia, until the crude fiber becomes white, then a dissolution of the cellulose in ammoniacal cupric oxide followed by filtration and a precipitation of the cellulose.

The chlorination process of Cross and Bevan is of particular significance because of its more recent satisfactory application to the estimation of cellulose in wood. A number of investigators have worked on this subject within the last few years, the majority of them using this process as a basis. Although originally used by Cross and Bevan in their work with jute fiber, the chlorination process has proved to be the best method available for the determination of cellulose in woods.

(1) König, J. "Chemie der Menschlichen Nahrungs- und Genussmittel" (1910) 3; 456.

Of the several processes employed by Cross and Bevan (1), chlorination gave the maximum yield. According to this method, the fibers are boiled for thirty minutes with a dilute solution of sodium hydrate (2). After washing, the fiber is exposed to an atmosphere of chlorine gas. The R. hexene constituent of the lignone group (3) reacts with the chlorine gas, in the presence of water, to form a quinone chloride, without at the same time affecting its union with the furfural-yielding complex. The chlorinated fiber is removed, washed, and placed in a solution of sodium sulphite. The solution is gradually raised to the boiling point, and a small quantity of caustic soda added. The cellulose is now thrown upon a cloth filter and washed with hot water. To remove the last residues of the non-cellulose constituents, it may be bleached by immersion in a dilute solution of hypochlorite or treated with dilute permanganate solution. The authors confined their attention for the most part to jute fiber.

(h). The Isolation of Cellulose from Wood.

The woody stem presents an exceedingly complex chemical structure. In comparison with the tender shoots of the young plant which are probably composed of a relatively pure cellulose (4), quite free from incrustations, the woody stem is highly incrustated. According to Haas and Hill (4), wood represents the extreme limit of this change. From the foregoing consideration

(1) Cross and Bevan, 596-598.

(2) Ibid., 94.

(3) See Diagram, p.29. Ring with hexene radical C_6H_{11} - reacts with Cl gas in presence of water to form a quinone chloride.

(4) Loc. cit.

of the incrusting substances, the difficulties attending a separation of cellulose from lignified tissues without altering the cellulose during the process, may well be appreciated. The basis of most of the methods used has been the chlorination process of Cross and Bevan described above.

Renker in 1910 (1) considered the preliminary alkali treatment employed by Cross and Bevan unessential to obtain a residue free from lignin. This conclusion was confirmed by Schorger (2) who analyzed certain woods for their cellulose contents using preliminary treatments before chlorination. The following statement is made by the author:-

"In all cases the preliminary digestion with alkali was found to assist in the removal of the lignin, the time of chlorinating being perceptibly shortened. As a general rule, however, the treatment results in an appreciable reduction in the yield of cellulose".

Schorger defines cellulose as the residue remaining after alternate treatment with chlorine gas and sodium sulphite, up to a point where the chlorine-sulphite color reaction or the Mäule reaction ceases (3). Some of Schorger's results are as follows:-

Soft Woods	per cent. cellulose	Hard Woods	per cent. cellulose
Western Larch 66.40)	Sugar Maple 63.43)mean
Long Leaf Pine	... 67.20)mean	Yellow Birch 64.38)64.26
Douglas Fir 66.30)65.92	Basswood 64.97)
White Spruce 63.79)		

(1) Renker, M. "Bestimmungsmethoden der Cellulose". Berlin, (1910). Jour. Soc. Chem. Ind. (1909) 28; 1269.

(2) Schorger, A. W. "The Chemistry of Wood". Jour. Ind. and Eng. Chem. (1917) 9; 556.

(3) Loc. cit.

Johnsen and Hovey (1) in 1918 used the chlorination process, but subjected the material to a preliminary acid hydrolysis, with acetic acid and glycerol at 135° to 140° C. They claimed a removal of 22 to 25 per cent. of the furfural-yielding substances from the fiber by the acid hydrolysis, substances which would not be removed by chlorination or oxidation. This method has been severely criticized upon the basis of the drastic treatment involved.

Mahood comments as follows (2):-

"Since Johnsen and Hovey's method gives a residue more identical with the pulps obtainable in the commercial processes, it may be assumed in the absence of experimental data to the contrary that it, too, destroys some of the cellulose".

According to Schorger, the various methods of "manufacturing chemical pulp destroys from 25 to 35 per cent. of the cellulose when based on the cellulose content as found by laboratory analyses" (3).

Using the chlorination process, Ritter and Fleck have determined the percentages of cellulose in hard and soft woods (4):-

	Per cent cellulose
western Yellow Pine	57.41
" White "	59.71
Yellow Cedar	53.86
Incense Cedar	41.60
Redwood	48.45
Tanbark Oak	58.03
Eucalyptus	57.62
Mesquite	45.48
Balsa	54.15
Hickory	56.02

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- (1) Johnsen, B. and Hovey, R.W. "The Determination of Cellulose in Wood". Jour.Soc.Chem.Ind., (1918) 37; 132. "The Estimation of Cellulose in wood". Jour.Ind.and Eng.Chem. (1921) 13; 358; also "Paper" (1918) 21; 36.
- (2) Mahood, S. A. "Some Observations on the Determination of Cellulose in Woods". Jour. Ind. and Eng. Chem., (1920) 12; 873, (1921) 13; 361.
- (3) Schorger, A. W. loc. cit.
- (4) Ritter, G. J. and Fleck, L. C. "Chemistry of wood". Jour. Ind. and Eng. Chem., No.11, (1922) 14; 1050.

An interesting comparison of methods has recently been made by Uyeda (1), who applied the original Cross and Bevan method, Johnsen and Hovey method, and Renker's modification to Korean hemp fiber. By means of the mercerization test which he adopted as a standard of purity, Uyeda determined alpha cellulose from total cellulose, using the three methods.

Alpha Cellulose from Total Cellulose Obtained by Three Methods.

	Total Cellulose indiv.	Cellulose av.	Alpha Cellulose indiv.	Alpha Cellulose av.	Ratio Alpha Cellulose Total Cellulose
Renker's Method	71.08 70.44	70.76	63.98 62.44	63.21	0.89
Original Cross & Bevan Method	64.66 65.99	65.32	59.79 61.22	60.50	0.92
Johnsen & Hovey's Method	70.02 69.54	69.78	61.30 62.68	61.99	0.88

Uyeda makes the following conclusion:-

"If the yield of alpha cellulose from the total cellulose be considered as a basis of standardization of the cellulose obtained, it will be seen that the cellulose obtained by the original Cross and Bevan method shows the highest purity of the three and the other two indicate about the same degree".

The author considers the alkali treatment necessary for a satisfactory removal of the pectin substance in the fiber, and this removal to some extent can be obtained only by the original Cross and Bevan method. From the standpoint of analytical chemistry, however,

(1) Loc. cit.

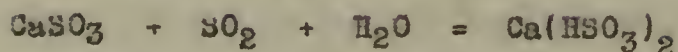
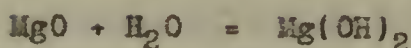
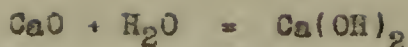
better results were obtained in the absence of hydrolysis before chlorination as shown by the following table:-

Weights of Residue by Various Hydrolytic Processes and Comparison of Cellulose Obtained by Three Methods.
(Percentage on Air Dry Basis - 8.83 Per Cent. Moisture)

Treatment	Residue after Hydrolysis		Cellulose	
	indiv.	av.	indiv.	av.
1. Renker's modification of Cross and Bevan's method, no hydrolysis			71.08	
			71.14	
			70.80	
		88.05	70.44	70.81
2. Original Cross and Bevan method treated for 30 min. with 1% NaOH	67.92		63.28	
	68.97		64.66	
	70.90		65.82	
	69.80	69.39	65.99	64.93
3. Johnson and Hovey's method, heated for 4 hours with acetic acid and glycerol	76.42		67.60	
	75.34		67.16	
	77.80		70.02	
	77.02	76.64	69.54	68.58

In paper-making, the incrusting substances are removed in the manufacture of pulp by the sulphite, soda, and sulphate processes (1). The pioneer work on the sulphite process was carried on by several investigators including Tilghman, Ekman, and Mitscherlich (1866-1875) (2). A bisulphite liquor known as "cooking acid" is prepared according to the "milk of lime" or "Tower" methods. In the milk of lime system sulphur dioxide gas is absorbed by milk of lime solution containing magnesia, and forming finally calcium bisulphite and magnesium bisulphite.

- (1) Stephenson, J. N. "The Manufacture of Pulp and Paper". (1922) 3; Sections 4, 5, 6.
(2) Ibid., Sec. 4, 1.
Miller, R. N. and Swanson, W. N. "Chemistry of the Sulphite Process". Jour. Ind. and Eng. Chem. (1925) 17; 843.



In the Tower system, limestone or dolomite are used. The cooking acid is fed into the digester along with the chips, followed by the introduction of steam. This is termed the "cooking process".

In the soda process, caustic soda solution is employed. This solution may be prepared with soda ash, slaked lime, and water, or by electrolysis. The caustic soda liquor goes into the digester with the chips, and the digester head or cover is bolted on. The contents are cooked by admitting steam.

The sulphate process is a modification of the soda process, and was introduced by C. F. Dahl in 1884 to reduce the manufacturing cost by substituting sodium sulphate for soda ash. The active components of the liquor are sodium hydrate and sodium sulphide. The process is performed under pressure in the digester.

In concluding this phase of the subject, the following paragraphs are of the nature of a summary of the status of true cellulose.

From the viewpoint of those investigating the decomposition of cellulose in nature, the particular form of cellulose used is of great importance. The desire of these workers is to obtain a pure cellulose and at the same time one which is as nearly as possible identical with the cellulose of the plant cell wall. The nature of this original true cellulose is uncertain. The nearest approach to it is probably raw cotton. There may, however, be

numerous true celluloses differing only slightly from each other. True cellulose or celluloses differ considerably, apparently, from that particular part of the reserve food of the plant, the hemicelluloses.

The association of true cellulose with the incrusting substances is probably a physical union from which the former may be freed in a pure if not unaltered form. If, however, the combination be of a chemical character, the problem of isolation becomes much more involved. It is obvious from the above review that the forms of cellulose which may be isolated from plant materials vary in constituency and purity. As a result of studies made of cell walls and of certain substances generally held to be the purest forms of cellulose available, such as cotton and Swedish filter paper, numerous characteristics are now applied to true cellulose. By common consent, therefore, cotton and filter paper have been widely employed as sources of true or pure cellulose in investigations of cellulose fermentation. The limitations of such forms of cellulose, however, should be recognized. One of the great demands of microbiological cellulose research is a natural, standard, unaltered, cellulose - as nearly as possible like cell wall substance or substances of young, unincrustated, plant tissue. At present the purest forms of cellulose available must suffice.

The various methods for purifying the cellulose content of plant material have been reviewed at some length for the purpose of revealing as much as possible the physico-chemical properties and characteristics of true cellulose; the real difficulties in the way of obtaining a pure unaltered product; and the drastic procedures necessary to free the cellulose from the exceedingly intimate associations with non-cellulose in crustants in the cell membrane of plants. These considerations are of importance, for in the investigational work to follow, both matters of technique and interpretation of results require such a review for a full and adequate explanation.

2. Biological Aspect of Cellulose.

The subject of cellulose decomposition has undergone investigation since the middle of the last century. The general uncertainty surrounding the exact nature of cellulose has, no doubt, tended to lessen the number of investigations and hence retard effective advance. Nevertheless with various sources of cellulose available, such as Swedish filter paper, raw cotton and other plant fibers, and with the process of cellulose destruction constantly in progress, evidences of which are abundant in nature, much may be accomplished toward a solution of the problems attending this universally important process.

(a) Cyto-hydrolytic Enzymes.

Although the existence of a cellulose-dissolving enzyme is generally admitted, attempts to isolate it have been only partially successful. Certain micro-organisms acting upon vegetables are supposed to secrete such an enzyme. De Bary (1886) cultivated Pezizas of the Sclerotinia group on the pulp of carrots and turnips. The tissues became softened, ^{and} the mycelium destroyed the cell walls of pith and cortex, according to the author (1). The juice from the affected pulp was found to possess this lytic property, but lost it when boiled. In 1888, Ward found that a "fungus of the kind often called Botrytis or Polyactis" penetrated the cell walls of Lilium candidum, causing disease (2). He held

(1) Bary, A. de. "Ueber Einige Sclerotinien und Sclerotien krankheiten". Bot. Zeit., (1886) 44; 377.

(2) Ward, H. M. "A Lily-Disease". Ann. Bot., (1888-1889) 2; 319.

that a cellulose-dissolving ferment was secreted by the fungus at the tips of the hyphae. This contention receives support from Brown (1) who worked with Botrytis cinerea in his "Studies in the Physiology of Parasitism". He concludes:-

"The elaboration of cytase in the fungal hypha would appear to be a process bound up with the protoplasmic activity associated with growth in the hyphal tip. The view that enzyme formation is confined to the growing apex is further supported by the results obtained in extract-hyphae which have ceased growing. The reduced activity of the older cultures is thus to be set down in this case, not to a reduced number of hyphal tips in the extracted mass, but reduction in the amount of enzyme in each hyphal tip, this reduction being correlated with the fact that these hyphal tips have ceased growth on account of the development of stale conditions in the culture".

The presence within the cell of the indefinite group of substances known as hemi- or reserve celluloses, complicates somewhat the study of cyto-hydrolytic enzymes. Sorauer (2) suggests that certain cell walls may be composed largely of hemi-celluloses. According to Schellenberg (3) true cellulose is an unimportant constituent of cell walls of various plants, whereas hemicelluloses are found in large amounts. If both true cellulose and hemicellulose are present, microscopical observations of the

(1) Brown, W. "Studies in the Physiology of Parasitism".
Ann. Bot., (1917) 31; 489.

(2) Sorauer, P. Handbuch der Pflanzenkrankheiten, (1921) 1;
939.

(3) Schellenberg, H. C. "Untersuchungen über das Verhalten
Einiger Pilze gegen Hemizellulosen". Flora (1908) 98;
261.

dissolution of one or the other becomes difficult. It would seem necessary, therefore, in work of this sort, to differentiate, one way or another, true cellulose from hemicellulose. Newcomb (1), in his work on "Cellulose Enzymes", is dealing presumably with a dissolution of reserve cellulose, but reports a "melting away" of the cell wall. The work of Schellenberg offers a somewhat more satisfactory answer to questions regarding the true cellulose - hemicellulose relationship. Sorauer's conclusion is supported by this author who found that cell walls are not dissolved uniformly by fungi; that in certain cases the true cellulose is dissolved and in others the hemicelluloses, implying a specialization among the fungi toward one or the other. As sources of true cellulose, Schellenberg used cotton, flax fiber, and Swedish filter paper in his investigations. He chose as sources of hemicellulose several plants whose cell walls were known to consist largely of hemicelluloses. Sections were prepared from the material under examination and these in hanging drop cultures, inoculated with the fungus, revealed microscopic pictures which enabled the author to observe the dissolution of the cell wall. A brief tabular summary of some of Schellenberg's results follows:-

(1) Newcombe, F. C. "Cellulose Enzymes". Ann. Bot., (1899)
13; 49.

	Echte Zellulose	Hemizellulosen von				
		Molinia	Lupinus	Ruscus	Phoenix	Impatiens
<i>Mucor racemosus</i>	-	+	-	-	-	-
" <i>neglectus</i>	-	-	+	-	-	-
" <i>piriforme</i>	-	-	+	-	-	+
" <i>globosus</i>	-	+	+	-	-	+
<i>Thamneidium</i>						
<i>elegans</i>	-	-	+	-	-	+
<i>Rhizopus nigricans</i>	-	-	+	-	-	+
<i>Penicillium</i>						
<i>glaucum</i> 1	-	-	-	-	-	+
" " 11	-	-	+	-	-	-
<i>Sclerotinia fruc-</i>						
<i>tigena</i>	-	-	+	-	-	-
" <i>cinerea</i>	-	-	+	-	-	-
<i>Botrytis cinerea</i>	-	-	+	-	-	+
" <i>vulgaris</i>	-	+	+	-	-	+
<i>Hectria cinnabarina</i>	-	+	+	-	-	-
<i>Cladosporium</i>						
<i>herbarum</i>	-	+	+	-	-	+
<i>Colletotrichum</i>						
<i>lindemuthianum</i>	-	+	+	-	-	+
<i>Trichothecium</i>						
<i>roseum</i>	-	+	+	+	+	+

Newcombe (1) does not differentiate between true cellulose and hemicellulose but observes microscopically the gradual "melting away" of cell walls being acted upon by the ferment. He found this ability possessed by extracts of barley malt, Aspergillus oryzae, and of seedlings from Lupinus albus and Phoenix dactylifera. The cell walls of sections from the barley endosperm, both starch-bearing cells and aleurone layer, from lupine cotyledons, and from the endosperm of the date, were dissolved by ferments according to the author. Each extract was found to exert a solvent action on starch, but in the cases of lupine and date seedlings, so feebly, that Newcombe definitely considers them cyto-hydrolysts. It is probable that in these experiments as

(1) Loc. cit.

in Schellenberg's investigation, the dissolution is largely of reserve cellulose. The diastatic action reported by Newcombe may have been due to the presence of other enzymes in the extracts.

Brown and Morris (1) discovered a cyto-hydrolyst in the germinating barley grain. Experiments with the malt extract showed its activity as a cyto-hydrolyst to be somewhat limited. The cellulose of certain cell walls was dissolved, while in the case of others no solvent action was apparent. In view of the evident uncertainty regarding the character of the celluloses of cell walls, whether present as reserve material, true cellulose, or both, the limitations of such an enzyme is not at all inconceivable. Brown and Morris state that the enzyme was not prepared free from diastase. If, as Schellenberg states, certain cell walls are composed largely of hemicellulose, and others of true cellulose, then the existence of two enzymes, cellulase acting upon true cellulose, and cytase acting upon hemicellulose, a distinction brought out by Euler (2), would be admissible. Schmitz and Zeller (3) refer to cellulase and hemicellulase in their studies of enzyme action in wood-rotting fungi, while other authors give the name "seminase" (4) to the hemicellulose-dissolving enzyme. Since, however, in the opinion of Schellenberg, fungi exhibit such specialization in their ability to dissolve hemicelluloses, the existence of at least four different

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- (1) Brown, H. J. and Morris, G. H. "Ein Celluloselösendes Enzym in d. Würzelchen d. Embryo v. keimender Gerste". Jour. Chem Soc., (1890) LVII; 497, (1892) 57; 503.
(2) Euler, H. "Allgemeine Chemie der Enzyme", (1910). "General Chem. of the enzymes", Euler, (Trans. by Pope) (1912) 6.
(3) Schmitz, H. and Zeller, S. M. "Studies in the Physiology of the Fungi". Ann. Miss. Bot. Gard., No.3, (1919) 6; 193.
(4) Flora, (1908) 98; 297.

ferments for this group must be assumed (1).

In the light of this review of the investigations dealing with cyto-hydrolytic enzymes, it would seem advisable to differentiate, at the outset, between true cellulose, the structural material of the plant cell, and hemicellulose, the reserve food, laid down in certain of the cells for the use of the plants. It is possible that in nature cyto-hydrolytic enzymes are engaged in dissolving both of these substances. In all probability a splitting of true cellulose takes place through the agency of enzymes secreted by such micro-organisms as the wood-destroying fungi. A discussion of the activity of these micro-organisms follows in the succeeding chapters. The rôle of cyto-hydrolysts or cellulose-digestive enzymes in the animal body will be taken up later under the title: "The Fate of Cellulose in the Animal Body".

(b) Cellulose Decomposition by Micro-organisms.

Pioneer investigations dealing with micro-organisms as the agency of cellulose destruction probably relied almost exclusively upon microscopical observations of decomposing plant tissue, attributing the phenomenon to the existing microbial flora (2).

(1) Moliniacytase. Sie löst die aus Hemizellulose bestehende Wandverdickung des Speicherinternodiums von Molinia coerulea Mönch.

Lupinuscytase.

Phoenixcytase.

Impatienscytase. Sie löst die Hemizellulose in den Kotyledonen von Impatiens balsamina.

Flora (1903) 98; 299.

(2) Lafar, F. "Handbuch der Technischen Mykologie" (1904-06) 3; 248.

Mitscherlich (1), Trécul (2), Van Tieghem (3) and Prazmowski (4) are prominent among these early workers. An anaerobic genus known as Amylobacter, so named because its members stained blue with iodine, occupies a conspicuous position among the early attempts to determine the agency of cellulose decomposition. They were described by Trécul in 1865 and studied further by Van Tieghem. According to the latter, Amylobacter exerted a solvent action upon young and tender tissues, but did not extend their activities to fibers which were lignified or suberized (5). This latter fact has associated Amylobacter with the complex fermentation known as the retting of fibers (6). This process presumably frees the fiber from pectic substances and does not concern itself, as far as visible evidence goes, with the cellulose content of the fiber (7).

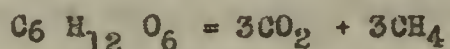
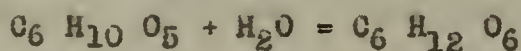
Observations of cellulose destruction in nature reveal the fact that agents of tremendous significance are rapidly splitting and destroying cellulose materials. In stagnant water, septic

- (1) Mitscherlich, E. "Zusammensetzung der Wand der Pflanzenzelle". Bericht über die zur Bekanntmachung Geeigneten Verhandlungen d. Kgl. preuss. Akad. d. Wissensch. zu Berlin, (1850) 102.
- (2) Trécul, A. "Production de plantules amylofères dans les cellules végétales pendant la putréfaction. Chlorophylle cristallisée". Comptes Rendus de l'Acad. des Sci., Paris, (1865) 61; 432, (1867) 65; 513.
- (3) Van Tieghem, P. E. L. "Sur la fermentation de la cellulose". Ibid (1879) 88; 205, 89; 5, 1102.
"Sur le Bacillus amylobacter et son rôle dans la putréfaction des tissus végétaux". Bull. de la Soc. Bot. de France (1877) 24; 128.
- (4) Prazmowski, A. "Untersuchungen über die Entwicklungsgeschichte und Fermentwirkung einiger Bakterien-arten, Leipzig, (1880) 58.
- (5) Loc. cit.
- (6) Tanner, F. W. "Microbiology of Flax Retting". Bot. Gaz., No. 2, (1922) 74; 174.
- (7) Omelianski, W. "Die Histologischen und Chemischen Veränderungen der Leinstengel unter Einwirkung der Mikroben der Pektin- und Cellulosegärung". Cent. f. Bakt., etc. (1902) 8; 195, (1904) 12; 33.

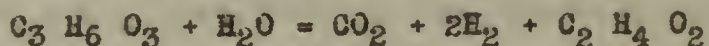
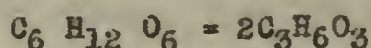
tank, sludge deposits, compost heaps, and manure piles, evidences of the rapidity and completeness of this process are particularly apparent. Very little is actually known concerning the agents involved, and as yet no method seems to have been devised whereby they may be more thoroughly investigated in the laboratory while exercising or capable of exercising such powers of cellulose dissolution as they may possess in the natural habitat. Investigations along this order, therefore, would require a more or less intimate acquaintance with the conditions existing in nature, under which the physiological efficiency of cellulose-decomposing micro-organisms is maintained. It is significant that the varied examples of cellulose decomposition mentioned above as remarkably rapid and complete, are essentially anaerobic. That is, the most rapid changes seem to take place where air is largely excluded. For example, it is the interior of a compost heap which reveals these changes, where due to such factors as compactness and moisture, anaerobic conditions prevail. In the septic tank also, bacterial action is largely the work of anaerobic species (1). This is particularly true of the "settling chamber" where most of the decomposition occurs. No general statement can be made placing anaerobic cellulose^{destroyers}/first in importance among micro-organisms carrying on cellulose destruction. It is merely significant that rapid and efficient decomposition should be so frequently associated with anaerobic conditions.

(1) Jones, D. H. "Bacterial Action in the Septic Tank System of Sewage Disposal". O.A.C. Bull. 267.

Literature dealing with the anaerobic process often makes reference to the formation of combustible gases, particularly methane. The distinction of being the first to discover this gas is given to Volta in 1776 (1), who described "entzündbaren Luft", which, he claimed, was formed in ponds, marshes, wells, and soils containing organic matter. Hoppe-Seyler obtained methane and carbon dioxide during fermentations of cellulose in the form of filter paper, by sludge (1). Methane was produced in considerable quantities. Hoppe-Seyler represents this fermentation by the following equations:



If a typical carbohydrate fermentation were to take place, however, numerous acids would probably be formed. In this case, Hoppe-Seyler would revise his final equation as follows:-



As a result of further investigation (2), he concluded that in the presence of oxygen, plant materials undergoing fermentation with the production of acetic acid, are converted into carbon dioxide and water, while in the absence of oxygen, ferric oxide, and gypsum, the ultimate products are carbon dioxide and methane.

The classic investigation of Omelianski, started in 1894, was one of the first contributions of moment in the field of

(1) Hoppe-Seyler, F. "Über Gärung der Cellulose mit Bildung von Methan und Kohlensäure". Zeitschr. physiol. Chem., (1886) 10; 201, 401.

(2) "D. Methangärung d. Essigsäure". Zeitschr. physiol. Chem., (1887) 11, 561.

cellulose fermentation, establishing the importance of the anaerobic process. His use of a sterile nutrient medium was distinctly an innovation, marking one of the first attempts to eliminate contaminating strains (1). The composition of the medium was as follows:-

Potassium phosphate	1 g.
Magnesium sulphate	0.5 g.
Ammonium sulphate or phosphate	1 g.
Sodium chlorate	Trace
Water (distilled)	1000 c.c.
Chalk	
Filter paper.	

In place of ammonium salts, 0.5% asparagin or 0.1% peptone were occasionally employed. At times, a 0.5% meat extract solution or manure decoction served as a nutrient medium. Filter paper, parchment paper, and oat straw were among the various sources of cellulose used. The fermentations were carried on in long necked flasks, sometimes in Pasteur flasks, under anaerobic conditions in either case. The inoculating materials consisted of manure and river mud. According to Omelianski's statement he soon became convinced that there were two types of cellulose fermentation, the hydrogen and methane fermentations. In the isolation of the particular micro-organisms concerned he used the so-called "elective culture method". He found that by using slime or manure as the inoculum, the methane fermentation set in. By heating the inoculum for fifteen minutes at 75°, conditions were created which were favorable for the hydrogen fermentation of cellulose. This fact enabled Omelianski to

(1) Omelianski, w. "Ueber die Gärung der Cellulose". Cent. f. Bakt. etc., (1902) 8; 225.

effect a separation of these two species of bacteria (1). The products of decomposition besides methane and hydrogen included carbon dioxide, acetic and butyric acids.

Orla-Jensen (2) proposes a genus Cellulobacillus composed of these anaerobic bacteria which ferment cellulose. The type species is Cellulobacillus methanigenes (3). The genus is included under the family Butyribacteriaceae.

It is probable that under certain conditions, thermophilic bacteria may actively decompose cellulose. This group has been investigated by Macfayden and Blaxall (4), Kroulik (5), and Fringsheim (6). A study of this type of fermentation is being carried on by Fred (7), who finds the products to consist largely of acetic acid and ethyl alcohol.

Typically aerobic decompositions of cellulose also take place in nature. The extent of this process is uncertain and the relative significance of aerobic micro-organisms as agents of cellulose destruction is not well established. On the other

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- (1) Omelianski, W. "Ueber die Trennung der Wasserstoff-und Methan-gärung der Cellulose". Cent. f. Bakt. etc.; (1904) 11; 369.
- (2) Orla-Jensen, S. "Die Hauptlinien des Natürlichen Bakterien-systems". Cent. f. Bakt. etc., (1909) 22; 305.
"Vorschlag zu einer neuen Bakteriologischen Nomenklatur". Ibid. 24; 477.
- (3) Buchanan, R. E. General Systematic Bacteriology, (1925) 1; 247, Bact.
- (4) Macfayden, A. and Blaxall, F. R. "Thermophilic Bacteria". Trans. Jenner Inst. of Prev. Med., ser.2, (1899), 162.
- (5) Kroulik, A. "Über Thermophile Zellulosevergärer". Cent. f. Bakt. etc., (1912) 36; 339.
- (6) Fringsheim, H. "Über Vergärung der Zellulose durch Thermophile Bakterien". Ibid., (1913) 38; 513.
- (7) Fred, E. B., Peterson, W. H. and Viljoen, J. W. "The Fermentation of Cellulose by Thermophilic Bacteria". abs. Bact., (1924) 8; 11.

surfaces of compost heaps and in decaying wood aerobic organisms are apparently active. Van Iterson (1), McBeth (2), and Sack (3) have isolated numerous aerobic bacteria which they claim will attack cellulose. Groenewege (4) has investigated the aerobic process and describes the association of organisms in cellulose denitrification.

In the recent classification of Bergey (5), the aerobic cellulose-decomposing bacteria are listed under the genus Cellulomonas. Thirty-one species are described, and comprise mostly soil forms. The type species is Cellulomonas biazotus (Kellerman).

The fungi as destroyers of cellulose have been studied by Van Iterson (6), Daszewska (7), Kellerman, McBeth and Scales (8), and Otto (9). Krainsky (10) and Waksman (11) have investigated actinomycetes.

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- (1) Van Iterson Jr., C. "Die Zersetzung von Cellulose durch Aerobe Mikroorganismen". Cent. f. Bakt. etc., (1904) 11; 689.
 - (2) McBeth, I. G. "Studies on the Decomposition of Cellulose in Soils". Soil Sci., (1916) 1; 437.
 - (3) Sack, J. "Zellulose Angreifende Reinkultur der Zellulosebakterien". Cent. f. Bakt. etc., (1924) 62; 77.
 - (4) Groenewege, J. "Untersuchungen über die Zersetzung der Zellulose durch aërobe Bakterien". Bull. du Jardin Bot. de Buitenzorg. Ser. IV, T.2, fasc.3 (1920) 261. Cent. f. Bakt. etc., (1921) 53; 414.
 - (5) Bergey's Manual of Determinative Bacteriology, (1923) 154. Bact.
 - (6) Loc. cit.
 - (7) Daszewska, W. Bull. soc. Bot., Geneve, 2 ser., No.7, (1912), 255.
 - (8) Kellerman, K. F. and McBeth, I. G. "The Fermentation of Cellulose". Cent. f. Bakt., etc., (1912) 34; 485.
Scales, F. M. "Some Filamentous Fungi Tested for Cellulose-Destroying Power". Bot. Gaz. (1915) 60; 149.
McBeth, I. G. and Scales, F. M. "The Destruction of Cellulose by Bacteria and Filamentous Fungi". U.S.D.A. Bur. of Plant Ind., Bull.266, (1913).
 - (9) Loc. cit., 190.
 - (10) Krainsky, A. Die Aktinomycceten und Ihre Bedeutung in der Natur. Cent. f. Bakt. (1914) 41; 649.
 - (11) Waksman, S. A. "Cultural Studies of Species of Actinomyces." Soil Sci., (1919) 8; 87.

(c) Cellulose Decomposition in the Soil.

As a medium for microbial activity, soil offers exceedingly complex physical, chemical, and biological factors which influence biological processes and hence the availability of plant food. In order to determine the physiological activity of specific soil micro-organisms it is necessary to control the environmental factors or the external conditions of microbial life as far as possible. Obviously the factors operating in soil are difficult to control in the field and to reproduce in the laboratory. To be sure, such factors as micro-organisms, temperature, moisture, and soil structure may be controlled fairly effectively. Chemical analysis may yield some information, although in this regard it is probably true that no two samples of soil will be identical. In spite of every effort to reduce unknown factors to a minimum, there are chemical and biological substances present in soil which seem to exert a considerable influence upon microbial activity but whose effects are difficult to estimate or determine. This significant factor will be discussed later under the subject: "Essential Food Substances". At present it is sufficient to cite a few cases where the stimulating effect has been particularly marked. Experiments carried on at Macdonald College (1) indicate that the essential food factor of green manure exerts a stimulating influence upon Azotobacter. Other workers have found that organic acids, carbohydrates, and other organic compounds in the soil exert

(1) Zoond, A. "The Influence of Green Manures upon the Growth and Physiological Efficiency of Azotobacter chroococcum". M.Sc. thesis, McGill Univ., Oct. 1925.

and its significance does not appear to be adequately appreciated. In soil processes - ammonification, nitrogen-fixation, and cellulose decomposition, for example - it is probable that microbial associations play a significant role.

The importance of association upon such processes has been suggested by several investigators, including Pringsheim (1), Bottomley and Mockeridge (2), and Hutchinson and Clayton (3).

Hutchinson and Clayton have shown a very definite relationship between cellulose decomposition and nitrogen-fixation. In the association of cellulose destroyers with azotobacter the breakdown products of the former process seemed to aid considerably in the assimilation of atmospheric nitrogen. They calculated that the fixation of nitrogen per gram of mannite supplied was equal to 3.18 mgms., while that per gram of cellulose actually decomposed was no less than 19.3 mgms. Results obtained by Pringsheim were very similar. He showed that certain cleavage products resulting from the methane and hydrogen fermentations of cellulose, served as sources of energy for nitrogen assimilation by the anaerobic nitrogen-fixing bacteria of the genus Clostridium. He also used Azotobacter in a similar association. Some of Pringsheim's results are presented in the following table.

- (1) Pringsheim, H. Loc. cit., Cent. f. Bakt. etc. (1909) 23; 300.
"weiteres über die Verwendung von Cellulose
als Energiequelle zur Assimilation des Luftstickstoffs".
Ibid., (1910) 26; 222.
- (2) Bottomley, W. B. "The Effect of Nitrogen-fixing Organisms
and Nucleic Acid Derivatives on Plant Growth". Proc. Roy.
Soc. Lond., B., (1919) 91; 83.
Mockeridge, F. A. Loc. cit.
- (3) Hutchinson, H. B. and Clayton, J. "On the Decomposition of
Cellulose by an Aerobic Organism (Spirochaera cytophaga, n.sp.)"
Jour. Agr. Sci. (1919) 9; 143.

TABLE I.

Ausnutzung des Energiematerials in verschiedenen Konzentrationen in Milligramm pro lg vergorenes Material.

Trauben- zucker	Rohr- zucker	Stärke	Milch- zucker	Mannit	Cellulose		
					Methan- gärung plus Clos- tridium	Wasser- stoff- gärung plus Clos- tridium	Methan- gärung plus Azoto- bacter
0.25%	3.7						
0.5 %	3.2	4.2			10.4	8.3	4.5
1.0 %	2.85	(1.4) ?	2.3	3.7	3.2	5.4	7.7
2.0 %	2.0	2.8	1.7	3.1	1.7	3.4	
4.0 %	1.2	1.8					

According to Fringsheim, the association of Clostridium and cellulose-destroyers permits a better utilization of the energy-yielding substances produced during the decomposition of cellulose than that of Clostridium alone upon the various other carbohydrates investigated. It will be apparent that from the standpoint of nitrogen-fixation there is a greater stimulation in physiological efficiency in the presence of the smaller concentrations of carbohydrate which again suggests the possibility that association may partake of the nature of essential food factors, in which essential substances are rendered available for utilization by the organism, or if not actually utilized, act as accessory or stimulating agents through some means not yet sufficiently recognized. This subject will be treated more in detail later under the heading: "Essential Food Substances".

In view of the uncertainty surrounding the use of soil as a medium in which to study microbial activity, and the wide variations even in adjacent soils, the elemental approach would seem to

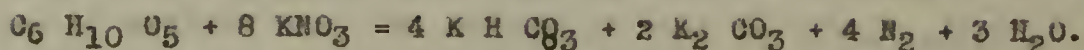
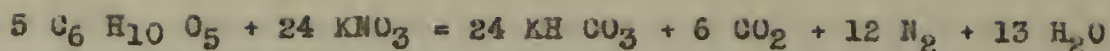
be the only safe and accurate procedure. For example, pure, quartz sand sterile, and free from organic matter would aid in providing proper physical conditions. Chemically pure mineral nutrients may be added, also sterile water to insure appropriate moisture conditions. By approaching the problem in this way, controlling the factors as far as possible, the real activity of a specific micro-organism may be investigated. In other words, work with only one unknown at a time, and eventually a considerable number of the factors operating will be known.

As far as cellulose decomposition is concerned, green and animal manures, plant roots, and other plant residues are decomposed in the soil, involving complex biological processes of transformation and cleavage, in which celluloses, starch, sugars, and nitrogen compounds, play important roles. The decomposition of cellulose, influenced probably by these other processes, as well as by the physical and chemical conditions of the soil, proceeds with greater or lesser rapidity yielding organic acids and carbon dioxide (1), which may, in turn, act upon such insoluble minerals as calcium phosphate or magnesium carbonate (1). Thus, an influence may be exerted upon the availability of plant food.

It is probably true that the anaerobic destruction of cellulose proceeds actively in the soil. Van Iterson found that with insufficient admission of air, and in the presence of nitrates, cellulose underwent decomposition by denitrifying bacteria (2).

(1) Rahn, O. "Soil and Soil Problems", 13th Report, Mich. Acad. of Science, (1911).

(2) Loc. cit., 689.



Löhnis states that in the soil as in manure, acid formation during the decomposition may be attributed chiefly to the anaerobic methane and hydrogen bacteria (1).

The factors operating in the decomposition of cellulose in nature, therefore, are of a physical, chemical, and biological character. The influence of the growth-accessory factor and association, which are to be investigated later, may be emphasized.

(d) Cellulose Decomposition in Green and Barnyard Manure.

The fermentation of barnyard manure is exceedingly complex, involving, in all probability, both aerobic and anaerobic processes. These are in turn greatly affected by factors which determine to a large extent the fertilizer value of the manure. Some of the factors are enumerated in the Farmer's Bulletin on Barnyard Manure (2), as follows:-

- Temperature,
- Supply of air, as determined by compactness of heap,
- Moisture,
- The composition of the manure,
- The nature of the preservatives added.

On the outer surface of the heap, where air finds ready access, the prevailing decomposition is, without doubt, aerobic. The anaerobic process is confined mainly to the interior of the heap depending to a large extent upon the above factors in operation. The problem increases in complexity when the varying composition of the excrement, and the different decompositions that set in, are considered. The quantity of cellulose in manure is also

(1) Löhnis, F. "Handbuch der Landwirtschaftlichen Bakteriologie", Berlin, (1910) 528.

(2) U.S.D.A., Farmer's Bulletin No.192, (1915).

variable, depending chiefly upon the amount and kind of food, the kind of animal, and the amount and kind of litter added. Stoklasa (1) found that the dry matter of stable manure may contain 30 to 40 per cent. of cellulose and 20 to 30 per cent. of pentosans. The manure pile has been studied by Dehérain (2) and Schloessing (3), who found that methane was the predominating gas produced.

An investigation leading to the preparation of artificial farmyard manure was started by Hutchinson and Clayton in 1919 (4). They isolated an aerobic organism, Spirochaeta cytophaga, which they claimed dissolved cellulose. Two years later, Hutchinson and Richards (5) devised a method for converting straw into manure by the subjugation of the straw to the action of Spirochaeta cytophaga, mentioned above, thereby eliminating the participation of live stock in the process. The most rapid and complete breakdown occurred when the following conditions were controlled: air supply, suitable temperature, presence of available or indirectly available nitrogen, and a neutral or slightly alkaline reaction of the medium. Suitable concentrations of urine, urea, ammonium carbonate, or peptone, as sources of nitrogen, caused the most rapid decompositions.

The decomposition of green manure is another process of

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- (1) Stoklasa, J. "Ueber die Wirkung des Stallmistes". Cent. f. Bakt. etc., (1908) 20; 301.
- (2) Dehérain, P. "Recherches sur les fermentations du fumier der ferme". Annales Agronomiques (1884) 10; 385-409.
- (3) Schloessing, T. "Contribution à l'étude des fermentations du fumier". Ibid., (1892) 18; 5-18.
"Sur la fermentation forménique du fumier". Comptes Rendus, Paris, (1889) 109; 835.
- (4) Hutchinson, H. B. and Clayton, J. "On the Decomposition of Cellulose by an Aerobic Organism (Spirochaeta cytophaga, n.sp.) Jour. Agr. Sci. (1919) 9; 143.
- (5) Hutchinson, H. B. and Richards, E. H. "Artificial Farmyard Manure". Jour. Min. Agr., London No. 5, (1921) 28; 398-411.

tremendous significance to agriculture. As in the case of barnyard manure, many factors operate, the influence of which are sometimes difficult to determine. Some of the factors influencing the decomposition of green manure are as follows:-

- Kind and age of crop;
- Physical and chemical conditions existing in soil, such as:-
 - Temperature,
 - Moisture,
 - CO₂ in soil air;
- Meteorological conditions;
- Microbial content
- Other biological influences, such as "growth accessory substances".

Russell and Appleyard (1) have sought to determine some of these factors and their relative importance. Changes in bacterial numbers, nitrate content of soil, and CO₂ content of soil air were ascertained at regular intervals during several seasons. Sufficient resemblance was apparent in the curves plotted to indicate that the phenomena were related. Systematic determinations of certain factors were made, and insofar as the curves for the nitrate, CO₂, and bacterial numbers agreed with the curves of any one of these factors "over a sufficient period to eliminate accidental coincidences", that factor was taken as dominating the situation. The authors found that temperature, moisture conditions, and dissolved oxygen of the rain, were probably important factors in determining the rate of biochemical decomposition in the soil. They state:-

(1) Russell, E. J. and Appleyard, A. "The Influence of Soil Conditions on the Decomposition of Organic Matter in the Soil". Jour. Agr. Sci., (1917) 8; 385.

"Temperature is the chief factor in determining the extent of the biochemical changes in the soil. Until the temperature exceeds 5° C., change is only very slow; from November to March the reactions we are considering seem to be almost at a standstill. As soon as the temperature begins to rise above 5° C. biochemical activity sets in; bacterial numbers, CO₂, and nitrates all increase, the curves agree so closely with those for temperature, that we are justified in regarding the temperature rise as the determining factor. The increased activity is not always equally sustained, nor does it always quite coincide with the rise in temperature; occasionally it follows later. But this agreement soon ceases, and after a short period the activity begins to fall off notwithstanding the continuance of the favorable temperature. This is not a result of the sustained higher temperature because it is not obtained in the laboratory experiments where soils are kept at different constant temperatures, all other conditions being alike. The result seems to be due to lack of moisture, because the curves now begin to resemble the moisture curves. But the moisture curve does not fit very well, and therefore it is not the only factor concerned. The rainfall curve fits better. We conclude, then, that under favorable temperature conditions, rainfall becomes the dominating factor, but that rainfall does something else besides supplying water, and search was therefore made for this new factor. . . . our observations suggest that the dissolved oxygen of the rain is the important factor, renewing the oxygen in the dissolved atmosphere of the soil, and thus giving the organisms a new lease of activity".

In this last regard, Richards states that rainwater is very nearly saturated with oxygen when its temperature as collected is below 15° C. This is the case the majority of the year under the climatic conditions of England where the work was done (1). It is possible that the factors mentioned

(1) Richards, E. H. "Dissolved Oxygen in Rainwater". Jour. Agr. Sci. (1917) 8; 331.

above may operate conspicuously in the decomposition of green manure. There are probably other factors, however, which also play an important part. The physical nature of the soil, for example, together with certain moisture conditions might favor the anaerobic process. Of course the element of uncertainty accompanying the use of soil in field or laboratory experiments makes generalizations difficult and often misleading.

Regarding the rates of decomposition of various kinds of green manure, Potter and Snyder (1) state that clover decomposes faster than oats, and that stable manure used with green seems to increase the rate of decomposition of the latter. Whiting and Schoonover (2), studying green and cured red clover, found that curing retarded the rate of decomposition as measured by ammonification, nitrification, and loss of carbon in both laboratory and greenhouse experiments. Martin (3) discovered that the greater the succulency of the green manure the faster the decay. The stage of maximum succulency he

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- (1) Potter, R. S. and Snyder, R. S. "Decomposition of Green and Stable Manures in Soil". Jour. Agr. Res., (1917) 11; 677.
 - (2) Whiting, A. E. and Schoonover, W. R. "The Comparative Rate of Decomposition of Green and Cured Clover Tops in Soil". Soil Sci., (1920) 9; 137.
 - (3) Martin, T. L. "Decomposition of Green Manures at Different Stages of Growth". Agr. Exp. Sta., Cornell Univ., (1921), Bul. 406.

found to be when the crops were about half grown. The question might be raised here how one is to determine when a crop has reached this half grown stage. The green manures investigated by Martin were rye, oats and buckwheat.

These problems are, of course, of fundamental importance to soil fertility in maintaining proper physical conditions and in bringing about chemical transformations of great benefit to the plant. The value of green manure depends upon the rapidity of its conversion by decomposition into a form which will furnish these beneficial physical and chemical influences.

Besides these physico-chemical factors the presence of the so-called growth-accessory or essential food substances in plant tissue appears to be a significant factor in effecting a stimulation in biological activity. According to Mockeridge and others (1), this factor is liberated from soil organic matter by microbial action and stimulates soil micro-organisms in growth and physiological activity (2).

Eastcott (3) finds that the growth-accessory factors are widely distributed in the plant kingdom, and it is probably true in the decomposition of green manures by micro-organisms

(1) Loc. cit., also Mockeridge, F. A. "The Formation of Plant Growth-Promoting Substances by Micro-organisms". Ann. Bot., (1924) 36; 724.

(2) Loc. cit.

(3) Eastcott, E. V. "The Natural Distribution of 'Bios I' and 'Bios II'", Roy. Soc. Can., (1923) 17; 157.

substances are liberated which function as essential food substances for certain groups of organisms. This subject will be discussed later more in detail.

(e) The Decay of Wood.

The decay of wood is another process of which little is actually known. It is considered to be essentially aerobic, fungi playing a leading rôle. The significance of fungi in wood decay has received a great deal of emphasis in recent years, probably because of the often striking visible evidence in decaying building timbers and trees (1). It is possible, however, that bacteria may constitute a significant agency in the process. Schmitz, in his "Studies in the Decay of Wood", concludes that cellulose-dissolving bacteria ^{do not} play an important part in the decay under natural conditions, but that the rate of decay may be materially increased by the presence of the ordinary saprophytic bacteria (2). This statement is open to dispute inasmuch as the extent of the activity of bacteria in wood decay has probably never been definitely determined.

Wood has an exceedingly complex structure. As enumerated by König the constituents of the cell membrane during its progressive development include the bitter principle, pigment, tannin, and pectin groups; the gum, resin, and mucilaginous materials; lignin; the aromatic compounds of wood, (hadromal, conifer and vanilla); suberin and cutin (3). Besides these incrusting

(1) Snell, W. H. "Studies of Certain Fungi of Economic Importance in the Decay of Building Timbers". U.S.D.A. Bur. of Plant Ind. (1922) Bull. 1053.

(2) Schmitz, H. "Studies in the Physiology of the Fungi". Ann. Missouri Bot. Gard. (1919) 6; 93.

(3) Loc. cit.

substances, wood contains cellulose and other carbohydrates. It seems highly probable that the process of wood decay might include several complex changes involving various of these constituents. Bray and Staidl state that the action of wood-destroyers is selective, and that cellulose is attacked to a larger extent than the non-cellulose materials (1). This is shown by the relative weights of these substances in sound and decayed wood.

Enzymes elaborated by wood-rotting fungi have been reported (2) whose proclivities show a wide variation. Some of these are cytase, cellulase, amylase, hadromase, laccase, tyrosinase, lipase, rennetase, coagulase, and certain glucoside-splitting enzymes such as emulsin. Hadromase is supposed to split the hadromal-cellulose combination, hadromal being one of the aromatic aldehydes, and to whose presence the lignified membrane owes its ligneous reaction, according to König (3). The existence of a delignifying enzyme has been claimed by several authors (4). Under the influence of the fungus secreting this enzyme, the lignified walls become converted into cellulose in the process of decay (4). Species of fungi considered to be prominent in wood decay are as follows: Merulius lacrymans, Coniophora cerebella, Poria vaporaria, Polyporus squamosus, Lenzites sepiaria, L. trabea, Trametes serialis, Pomes roseus, and Lentinus lepideus.

(1) Bray, M. W. and Staidl, J. A. "The Chemical Changes Involved during Infection and Decay of wood and wood Pulp". Jour. Ind. and Eng. Chem., No.1, (1922) 14; 35.

(2) Lafar. "Handbuch der Tech. Myk" 3; 291.

Buller, A. H. R. "Enzymes of Polyporus squamosus". Ann. Bot., (1906) 20; 49.

(3) Loc. cit.

(4) Potter, M. C. "On the Occurrence of Cellulose in the Xylem of woody Stems". Ann. Bot., (1904) 18; 121.

(f) The Fate of Cellulose in the Animal Body.

The fibrous portion of vegetable material representing crude fiber, was for a long time considered to be quite indigestible (1). The experiments of Haubner (1855) (2) and Henneberg and Stohmann (3) established the fact that only a portion of the crude fiber taken into the animal body is excreted as such. The remainder is converted into another form, and possibly digested and utilized by the animal. The cellulose material consumed such as hay, green fodder, and vegetables, is probably incrustated to a greater or lesser extent, affecting to some degree the digestibility. Hence, in crude fiber digestion, besides true cellulose and hemicellulose, lignified and cutinized cell walls must be considered. Regarding the digestibility of lignified cell walls, Haberlandt (4) states that such cell walls are much more digestible than has hitherto been supposed. The "Libriformzellen" of wood often undergo remarkable local corrosions ("Korrosionen") in the digestive canal of human beings, dogs and sheep, or dissolutions of the tender "Fibrillenbündel". On the other hand cutinized walls, according to Haberlandt, are wholly indigestible. He states that the digestibility of cell walls depends upon the chemical structure, and that cell walls most

(1) Tappeiner, H. "Untersuchungen über die Gärung der Cellulose, insbesondere über deren Lösung im Darmkanale". Zeitschr. für Biologie, (1884) 2: 52.

(2) Haubner, K. "Amts- und Anzeigebblatt für die landwirtschaftlichen Vereine des Königreiche, Sachsen", (1854).
Berichte über das Veterinärwesen im Königreich, Sachsen, (1859) 104.

(3) Henneberg, J. W. J. and Stohmann, F. Beiträge zur Begründung einer Rationellen Fütterung der Wiederkäuer, (1860-64), Heft 1, 100 u. 227, Heft 2, 342.

"Ueber die Bedeutung der Cellulose-Gärung für die Ernährung der Thiere". Zeitsch. für Biologie, (1885) 21; 613.

(4) Loc. cit.

easily dissolved or altered are those composed of pure or true cellulose and hemicellulose.

As Tappeiner points out (1), it would be reasonable to expect the presence of a cellulose or cell wall dissolving enzyme within the animal body, which besides dissolving the cell wall would convert the cellulose into a more easily digested form, possibly resembling sugar. Such an enzyme might easily fulfil both functions.

According to Wille (2), the animal body secretes cytase or hemicellulase. Regarding the dissolution of true cellulose by digestive enzymes, however, there seems to be a decided difference of opinion. Referring to the cell wall or "cellulosic membrane" of cereal grains, Hall makes the following statement (3):-

"There does not appear to be any provision in the digestive tract of the herbivora for the secretion of an enzyme capable of attacking this investing membrane, the dissolution of which under ordinary conditions is brought about in the stomach by an enzyme pre-existent in the grain" (3).

The authority for this view is found in the work of Brown (4):

"On the Search for a Cellulose-dissolving (Cytolytic) Enzyme in the Digestive Tract of certain Grain-feeding animals". Brown does not regard the dissolution of the cell walls of the Grasses of great importance from a nutritive standpoint, but by this removal

(1) Loc. cit. 58.

(2) Wille, F. "Beiträge zur Kenntnis der Hemizellulose-Verdauung bei höheren Tieren und über das Vorkommen einer Hemizellulase in Tierischen Drüsen, Nebst Einigen Ergänzungen zur Anatomie der Weizenkleie". Landw. Jahrb. (1918) 52; 411.

(3) Hall, A. D. "Rothamsted Experiments", (1919) 265.

(4) Brown, H. T. "On the Search for a Cytolytic Enzyme in the Digestive Tract of Certain Grain-feeding animals". Jour. Chem. Soc. Trans., (1892) LXL; 352.

the contained starch granules and protein are brought "within the sphere of action of the amylolytic and proteolytic enzymes of the digestive tract" (1). The pancreas of pig, horse, ox, and sheep, were examined for the presence of a cellulose-dissolving enzyme. Extracts of the pancreas were made and their action upon sections of the endosperm of barley ascertained. According to the author there was no evidence of any action on the cell walls. The search was now directed toward the stomach as the breaking down of the cell walls had already taken place before the stomach contents passed through the pylorus. Experiments were carried on to determine the effect of the churning and propulsive movements of the stomach during digestion, the action of the acids of the stomach, the presence of an enzyme secreted by the stomach, and the possible activity of micro-organisms, as agencies in causing the disappearance of cell walls of oats and barley meal in the stomachs of the horse and pig respectively. Each of these agencies was eliminated from the discussion as a result of the experiments, and the conclusion arrived at by Brown was that the cell membrane is destroyed in the stomach by a "cyto-hydrolyst existent in the grain before ingestion". Rye, oats, and barley were found to contain this cyto-hydrolyst.

According to Khouvine (2), who has studied the digestion

(1) Loc. cit., 353.

(2) Khouvine, Y. "Digestion de la cellulose par la flore intestinale de l'homme". *Ann. de l'Inst. Pasteur*, No. 8, (1923) 37; 741.

of cellulose by the intestinal flora of man, cellulose is not acted upon by digestive ferments, nor does the food of herbivorous animals contain cellulase (1). This author concludes that the digestion of cellulose is carried on by micro-organisms. It is possible, however, that while human beings particularly may depend largely upon micro-organisms for the destruction of cellulose material taken into the body, certain animals might logically possess an enzyme capable of bringing about the process of dissolution. Haberlandt (2) evidently holds this view, for he states that mammals including human beings are dependent upon the activity of cellulose-dissolving enzymes, cytases (cellulases) (Zytasen, Zellulasen) contained in the digestive juices of the animal, as well as upon microbial digestion.

It is probably true that apart from the consideration of a digestive enzyme, the rôle played by micro-organisms in decomposing cellulose within the animal body is of primary importance. Whether digestive enzymes act upon the cellulose first, converting it into a form readily attacked by micro-organisms, or whether microbial activity alone breaks down the cell walls, is uncertain. It is possible that digestive enzymes may convert cellulose into products resembling sugars, which are partly absorbed and partly attacked by micro-organisms resulting in the formation of gaseous and acid products so characteristic of microbial fermentations of cellulose in the intestines. On

(1) Loc. cit.

(2) Loc. cit.

the other hand, as suggested above, the action of micro-organisms may convert the cellulose directly into gases and acids. Hence, the nutritive value of cellulose probably depends considerably upon the nature of the fermentation which it undergoes. The "transition stages" mentioned by Henneberg and Stohmann (1), during which the cellulose is converted into a soluble body which may be absorbed directly, brings out the same idea as that expressed above. They state that the assumption of a not yet known transition stage is highly probable, which lies between the insoluble cellulose and the fermentation products. The cellulose is converted into a soluble body which bears to the former a similar relationship as maltose does to starch. The authors state that if such bodies exist and are capable of absorption, it will be uncertain what part of the cellulose is absorbed and how much is left for the fermentation process. The soluble body referred to above is probably the disaccharose cellobiose previously mentioned as one of the products obtained in the conversion of cellulose to glucose.

Various attempts have been made to isolate specific cellulose-destroying micro-organisms from the animal body. Distaso (2) isolated Bacillus cellulosaes desagregans from the intestinal flora of the hen, which, he claims, is capable of dissolving cellulose in the form of Berzelius paper. Hopffe (3) investigated the normal intestinal flora of cattle (Rinder),

(1) Zeitschr. für Biologie, (1885) 3; 624.

(2) Distaso, A. "Sur un microbe qui désagrége la cellulose".
Comptes Rendus, (1911) 70; 995.

(3) Hopffe, A. "Bakteriologische Untersuchungen über die
Cellulose-Verdauung". Cent. f. Bakt. etc., (1919)
83; 374, Originale.

isolating the micro-organisms in pure culture. Several exerted a solvent action upon filter paper in the majority of cases. These were B. megatherium, B. ellenbachensis, B. butyricus, B. mycoides, B. mesentericus vulgatus, and Bt. fluorescens. This ability was soon lost, however, during subsequent cultivations. An organism capable of destroying cellulose has recently been isolated from the intestinal flora of man by Khouvine (1). This organism, B. cellulosa dissolvens, n. sp., is strictly anaerobic, forms spores, and decomposes cellulose with gas and acid production. The author concludes as follows:-

"Among the products of the decomposition of cellulose we have found carbon dioxide, hydrogen, ethyl alcohol, acetic and butyric acids, and a yellow pigment, which represents only about sixty per cent. of the cellulose in fermentation. We have also been able to detect traces of lactic acid and products of hydrolysis precipitable by the alcohol".

Khouvine used cellulose in the form of Berzelius paper or precipitated cellulose in a nutrient medium composed of certain salts, peptone, and some of the fecal extract. Regarding the use of the latter, the author claims that B. cellulosa dissolvens prefers as a source of nitrogen, products of degradation found in the fecal extract.

The exhaustive studies of Tappeiner (1) have proved trustworthy. He showed that the process of cellulose destruction

(1) Loc. cit.

(2) Zeitschr. für Biologie, (1884) 20; 52, 215.

in the animal body is one of fermentation in which micro-organisms are concerned. This process takes place in the large intestines, (caecum and colon) of herbivorous animals, and in the first stomach of ruminants. The products appeared to consist chiefly of gases and acids (methane, hydrogen, carbon dioxide, butyric and acetic acids). In the paunch of cattle traces of formic acid were found, also small amounts of aldehyde and propionic acid; acetic and normal butyric acids and another called iso-butyric acid, were present in large amounts. No attempt was made to isolate the micro-organisms concerned, in pure culture, but portions of the paunch contents representing a mixed culture served as the inoculum. The activity of this culture in a nutrient medium containing cellulose was determined. The medium consisted of a one per cent. meat extract solution. Sources of cellulose employed were vellum paper (Velin papier) or cotton (Bruns'sche Watte). After inoculation, analyses of the gases were made at intervals during the fermentation which proceeded from one to four weeks.

Am ersten Tage		Am zweiten Tage		Gegen Ende der Gärung	
CO ₂)		CO ₂)		CO ₂)	
) 55.19%) 85.48%) 76.98%	
SH ₂)		SH ₂)		SH ₂)	
H	0.18	H	0.03		
CH ₄	37.08	CH ₄	11.86	CH ₄	23.01
N	7.56	N	2.73		
Verhältnis von CO ₂ : CH ₄ = 7.2:1 CO ₂ : CH ₄ = 3.4:1					

The control experiments without cellulose revealed a very small gas production - negligible compared to the amounts recorded above. Tappeiner found that in the case of the horse, the fermentation of cellulose started in the stomach, in all probability, with the production of hydrogen. The gases formed were as follows:-

Beginning of gas production		End of gas production	
CO ₂)	85.23%	CO ₂)	71.16%
SH ₂)		SH ₂)	
H	14.08	H	28.76
CH ₄	0.06		
N	0.62	N	0.07

With herbivorous animals the food consists largely of carbohydrates, particularly cellulose. The question arises, however, might not these gases arise from certain protein constituents. In answer to this, Tappeiner carried on experiments similar to those described above, using plant protein and peptone instead of cellulose, in an effort to arrive at the fermentative power of the contents of the paunch and caecum of the ox (des Rindes). The gases obtained were as follows:-

Paunch		Protein Fermentation		Caecum	
CO ₂)	89.15%			CO ₂)	92.88%
SH ₂)				SH ₂)	
H	7.60			H	6.85
CH ₄	0.79			CH ₄	0.25
N	2.46			N	0.06

Man's diet of meat and vegetables presents a different problem from the carbohydrate-rich food of cattle. Fries (1) has investigated the intestinal gases of man resulting from a simple diet of the following composition:-

Breakfast Oatmeal, Graham bread, butter and coffee.

Dinner Soup and crackers, roast beef, potatoes, boiled rice and milk, Graham bread, butter and coffee.

Supper A small piece of beefsteak, stewed potatoes, bread, butter and cocoa.

The man was doing no muscular work. The figures given by Fries for the intestinal gases produced are as follows:-

CO ₂	10.3%	by volume
O ₂	0.7%	" "
CH ₄	29.6%	" "
N	59.4%	" "

The large quantity of free nitrogen is not claimed to be a decomposition product, by the writer, but rather: "atmospheric nitrogen swallowed as air with the liquid and solid foods and with saliva, the oxygen having been absorbed into the blood, or has to some extent served to supply living organisms in the alimentary canal with oxygen". Fries states that for a healthy medium-sized man on very plain food such as mentioned above, one liter of practically odorless intestinal gas a day as rectal discharge can be considered a none too high average.

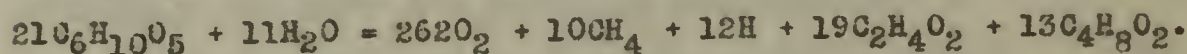
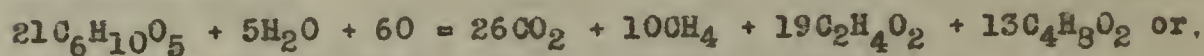
Returning to animals, granting that the gaseous products are all lost to the body, Tappeiner accounts for the remainder of the products by stating that about sixty parts appear as volatile fatty acids.

(1) Fries, J. A. "Intestinal Gases of Man". Amer. Hour. Physiology, (1906) 16: 468.

A part of this acid is excreted and is thereby lost to the body, according to this author, some is absorbed, but a fairly considerable part of this leaves the animal body in the urine, itself unchanged (1). Neuberg (2) claims that the urine contains only small amounts of these volatile fatty acids. Henneberg and Stohmann state that with the exception of hippuric acid, the amounts of organic acids in the urine are negligible in importance. They further establish energy values for cellulose and its products. Guided in part by Tappeiner's results, they obtained the following quantitative analyses of 100 grams of cellulose (Papierbrei oder Baumwolle).

100 g. Cellulose liefern an Gärungsproducten			
33.5 g. Kohlensäure mit	9.14 g. Kohlenstoff		
4.7 g. Sumpfgas	"	3.52 g.	"
33.6 g. Essigsäure	"	13.44 g.	"
33.6 g. Buttersäure	"	18.33 g.	"
-----		-----	
105.4 g. Gärungsproducte	mit 44.43 g. Kohlenstoff.		

The following fermentation equations are offered to explain this relationship:-



According to this equation 100 grams of cellulose with the assumption of 5.82 g. of water would yield:

(1) Loc. cit.

(2) Neuberg, C. "Der Harn, etc.". (1911) 1 Teil, 222.

33.63 g. Kohlensäure
4.70 g. Sumpfgas
0.35 g. Wasserstoff
33.51 g. Essigsäure
33.63 g. Buttersäure

105.82 g. Gärungsprodukte.

The carbonic acid and marsh gas were discharged from the stomach and intestines of the herbivora, and in the present work were considered non-utilizable by the animal. The calorific value of cellulose is determined by Henneberg and Stohmann. From 100 grams of cellulose (100 - 4146) ... 414,600 cal. are produced.

33.5 g. Kohlensäure	0
4.7 g. Sumpfgas 4.7 13344	62717
33.6 g. Essigsäure ... 33.6 3505	117768
33.6 g. Buttersäure .. 33.6 5647	189739
Gärungswärme	44376

	414,600 cal.

In spite of the loss of energy occasioned by the elimination of methane, the authors consider cellulose a substance of high nutritive value. These results are in accord with the conclusions of Kellner who makes the following statement (1):-

"Since of the organic acids produced in great quantities in the stomach of plant-eating animals, only insignificant amounts go into feces and urine (2), these materials must be burned in the tissues yielding energy to the organism".

(1) Kellner, O. "Die Ernährung der landwirtschaftlichen Nutztiere" (1909) 93.

(2) Wilsing, H. Zeitschr. für Biologie, (1895) 21; 625.
V. Knierrum, W. Ebenda, 139.

Attention thus far has been centered chiefly upon herbivorous animals because of the quantities of cellulose consumed by them. One additional characteristic of the herbivora must be mentioned, important from the standpoint of intestinal motility, ^{and} distinguishing this group from ^{the} carnivorous animals.

Thomson in his "Treatise on Clinical Medicine" (1) states:-

"It should be borne in mind that the only natural remedy for constipation is cellulose, so largely present in all vegetables. This is illustrated in nature by the difference between carnivora, who do not have loose movements, and herbivora, who are never long without semi-fluid passages".

The following tables compiled by Lohrsh give a synopsis of the investigations dealing with the utilization of the crude fiber in various foods by herbivorous and carnivorous animals, and also birds (2):

Beim Pflanzenfresser

Citat	Tier	Futter	Ausnutzung der Rohfaser in Prozent
Haubner- Sussdorf	Kuh	Wiesenheu	61
		Wiesenheu + Gerstenstroh	61

Haubner- Sussdorf	Kuh	Wiesenheu	78

Henneberg and Stohmann	Ochse	Haferstroh	55
		Wiesenstroh	52
		Bohnenstroh	36
		Kleeheu	39
		Wiesenheu	60

(1) Thomson, W. H. "A Treatise on Clinical Medicine". (1918). 374.

(2) Lohrsh, H. "Über die Bedeutung der Cellulose im Haushalte Menschen". Hoppe-Seyler's Zeitschr. für physiol. Chemie. (1906) 47; 227.

Citat	Tier	Futter	Ausnutzung der Rohfaser in Prozent
V. Hofmeister	Hammel	Wiesenheu	53.9
Derselbe	Pferd	Hafer + Heu + Strohhacksel	20.04 (für das Pferd zum erstenmal nachgewiesen)
Kühn, Aronstein, und Schulze	Ochse	Kleeheu	53.6
		Wiesenheu	64.8
		Haferstroh	63.8
Stohmann	Ziege	Wiesenheu	58
V. Hofmeister	Schaf	Wiesenheu + Haferstroh + Ruben	40
Kühn und Fleischer	Kuh	Wiesenheu	60
Henneberg	Hammel	Wiesenheu	59.7
Weiske	Schwein	Wicken + Hafer	48.87
Derselbe	Hammel	Bieheln	62.24
Wilsing	Ziege	Wiesenheu	60
V. Knieriem	Kaninchen	Schnittkohl	25.36
		Heu	52.47
		Filtrierpapier	54.3
		Filtrierpapier	28.08
		Möhrenmehl	65.3
		Sägespäne	20.49
		Kohlblätter	77.99
		Nussschalenpulver	5.03
		Kleeheukotrohlfaser (welche bereits einmal den Darmkanal passiert hat)	40.79
		Kleeheukotrohlfaser (welche den Darm bereits zweimal passiert hat)	22.04
		Strohrohlfaser	22.59
Weiske, Schulze und Flehsig	Hammel	Haferstroh	47.48
		Bohnenschrot	52.74
Lehmann	Hammel	Wiesenheu + Erbsen	62.2
Henneberg und Pfeiffer	Hammel	Wiesenheu + Gerstenschrot	55.8 60.6
Lehmann und Vogel	Hammel	Wiesenheu	60.71
		Reisfuttermehl	34.37
		Steckrüben	100
		Gerstenschrot	100

Studies on the utilization of cellulose by human beings were made by Lohrlich in 1906 including experiments conducted under normal and diseased conditions. The following table gives a resume of previous work.

Citat	Nr.	Dauer des Ver- suchs	Nahrung	Rohfaser- gehalt der Nahrung	Rohfas- er-gehalt des Kotes	Ausnut- zung der Rohfaser in Prozent
Weiske	1	3 Tage	Möhrengemüse Selleriesalat Kohlgemüse	37.48	13.963	62.7
	2	3 Tage	Dieselbe Nahrung	31.057	16.372	47.3
V. Knieriem	1	1 Tag	Gekochte Schwarzwurzel	3.3675	3.2196	4.4
	2	1 Tag	Kopfsalat roh ohne Mittelrippen	1.263	0.9433	25.32
Wicke	1	3 Tage	Brot aus geschältem Roggen	20.24	14.72	27.3
	2	3 Tage	Brot aus ungeschäl- tem Roggen	27.9	25.9	7.1
Rubner- Wicke	1		Brot 1	0.59	0.3	29.2
			Brot 2 (stärker cellulosehaltig)	2.04	0.96	53
			Brot 3 (viel cellu- lose enthaltend)	8.27	7.96	4.1
Mann	1		Feines Weizenbrot			ca 50
Barany	1	4 Tage	Rademann's Cellulose- brot, daneben Fleisch, Eier, Butter, Rahm, Zucker	137.7	80.1	58.2
	2	4 Tage	Dieselbe Nahrung	96.4	72.2	74.9

These investigations show that human beings are able to utilize crude fiber in a similar manner ^{to} as the herbivora, and that the actual amount is dependent upon the nature of the cellulose and the individual ability of the organism to dissolve it. Lohrlich used

Simon's method (1) in the experiments. His analyses of a few foods and plants to be used in the investigation, follow:-

Cellulosegehalt in %

Fischfertiger Spinat	0.364
Junger zarter Staudensalat, ohne Rippen, roh	0.479
Junger zarter Kohlrabi, roh	0.728
Junge Möhren, roh	0.743
Junges zartes Weisskraut, roh	0.787
Gurke, roh, ohne Kerne und Schale	0.114
Kartoffel, trocken	1.22
" gekocht	0.25
" roh	0.21
Linsen, roh	3.39
Hafermehl, trocken	0.245
Hoggenbrot, frisch	0.15
Grahambrot, frisch	0.94
Rademann's Cellulosebrot (Trocken- substanz)	3.24
Kakaopulver, trocken	2.29
Pulverisiertes Heu, trocken	17.5
Agar-Agar, trocken	0.618

The "Probediät" of Schmidt-Strasburger (2) was employed as a basic diet. To this the various foods under examination were added. The cellulose content of food and feces was determined in each case.

(1) Loc. cit.

(2) Schmidt und Strasburger, "Die Faeces des Menschen", Berlin, (1905) 191.

Morgens: 0.5L Milch, dazu 50 g. Zwieback.

Vormittags: 0.5L Hafererschleim (aus 40 g. Hafergrütze, 10 g. Butter, 200 g. Milch, 300 g. Wasser und 1 Ei bereitet).

Mittags: 125 g. gehacktes Rindfleisch (Rohgewicht) mit 20 g. Butter, leicht übergebraten, 250 g. Kartoffelbrüi (aus 190 g. gemahlenden Kartoffeln, 100 g. Milch und 10 g. Butter bereitet).

Nachmittags: Wie morgens.

Abends: Wie vormittags.

Nr.	Name Geschlecht	Zahl der Versuch- stage	Nahrung	Cellulosegehalt der Nahrung	Cellulosegehalt der Feces	Ausnutzung der Cellu- lose in Prozent
H.						
1.	Frau	1	Probediät	0.8916	0.4947	44.5
2.	"	1	Probediät Weisskraut	2.8591	0.3514	87.7
3.	"	1	Probediät Kohlrabi	3.0756	0.95	69.2
4.	"	1	Probediät Spinat	1.9836	0.59	70.3
5.	"	1	Probediät Brot	1.8666	0.6080	67.4
6.	"	1	Probediät Linsen	4.9576	2.608	47.4

J.						
7.	Mann	1	Probediät	0.8916	0.4531	49.2
8.	"	2	Probediät Weisskraut	6.1132	0.936	84.7

G.						
9.	Mann	1	Probediät	0.8916	0.327	63.4
10.	"	1	Probediät Möhren	2.7491	0.3318	87.8

Lohr'sch's experiments show that the digestive system of normal human beings is able to utilize more or less cellulose according to the physical nature of the latter, its age, and its origin. The utilization of cellulose under diseased conditions of the stomach and intestines constituted an important part of the investigation. For these experiments he chose the test diet previously employed because of its suitable composition and its easy digestibility. Each experiment lasted three days.

Nr.	Diagnose	Name	Kot-trocken-substanz (3 Tage)	Cellulosegehalt der Nahrung	des Kotes	Ausnutzung der cellulose in Prozent.
1.		Sch.	51.6	2.6748	0.9424	64.8
2.		Ho.	51	2.6748	1.4841	44.5
3.	Normale	J.	69	2.6748	1.3593	49.2
4.		G.	40	2.6748	0.98	63.4
5.		P.	55	2.6748	0.869	67.5
Mittelwerte			53.3	2.6748	1.1269	57.9
6.		Gr.	37	2.6748	0.4716	82.4
7.	Chronische	M.	30.8	2.4548	0.3316	86.5
8.		Lo.	30	2.6748	0.421	84.3
9.	habituelle	H.	38	2.6748	0.684	74.4
10.		Fr.	31	2.6748	0.5766	78.4
11.	Obstipation	Gu.	33	2.6748	0.4752	82.2
Mittelwerte			33.3	2.6381	0.4934	81.4
12.	Gärungs-	F.	123	2.6748	1.4514	46.2
13.	dyspepsie	Gl.	105	2.6748	1.89	29.3
Mittelwerte			114	2.6748	1.6707	37.8
14.	Gastrogene	Ja.	112.2	3.5908	2.4381	32.1
	Diarrhöe bei			probediät mit		
				Kakao		
15.	Achylia gastrica	Lu.	85.6	2.6748	1.9688	26.9
Mittelwerte			98.9	3.1328	2.2035	29.5
16.	Fettstuhl bei Ikterus	V.	202	2.6748	1.93	27.8
17.	Fettstuhl bei Pankreas-erkrankung	B.	147	2.6748	2.1168	20.9

It becomes evident, in studying the situation with regard to the fate of cellulose in the animal body, that the subject is largely in its infancy. Experimental evidence indicates that cellulose materials undergo certain changes during the process of digestion. Not only are the readily assimilated carbohydrates and

proteins made use of, but the cellulose itself is probably converted into another form, so that it does not appear as crude fiber in the excrement. work thus far seems to point to micro-organisms as the significant agency in this conversion.

Authorities apparently differ regarding the possibility of true cellulose dissolution by digestive enzymes secreted or elaborated by organs, glands, or fluids of the body. The existence of such an enzyme is entirely within the range of possibility, particularly in the case of animals which consume quantities of cellulose. a great deal of careful work is necessary along this line of investigation.

It is probably generally admitted that the animal body secretes a hemicellulose-dissolving enzyme which aids materially in the digestion of the reserve substances of the plant. These substances are, on the whole, easily broken down, and yield definite sugars on hydrolysis. True cellulose, however, does not, in all probability, come within the sphere of activity of this enzyme.

Granting that micro-organisms play a leading rôle in the dissolution of cellulose in the animal body, it is probable that the process is carried on in the large intestines, (caecum and colon) of herbivorous animals and in the first stomach of ruminants. The products appear to consist largely of gases and acids, but in this connection, too, further work is necessary, for cellobiose and glucose are becoming more and more prominent

as intermediate products. The presence of alcohol has also been detected.

The possibility of the utilization of cellulose as a food nutrient from which the animal may derive energy must be investigated further. Several factors are concerned in this problem of which little is actually known. One may determine with a fair degree of accuracy the amount of crude fiber excreted as such by the animal. It is exceedingly difficult, on the other hand, to ascertain the fate of the portion that has been altered, converted into intermediate products, or more completely broken down to simpler compounds.

The question may be asked legitimately whether true cellulose is, after all, a food nutrient. Might not its function in the body be primarily physical in nature, in the maintenance, for example, of intestinal motility. As far as the dissolution of true cellulose is concerned, it is possible that in rendering accessible the food nutrients of the cell to the digestive fluids of the body, the agencies at work may be fulfilling their real function.

S U M M A R Y

From the review of literature it is possible to draw certain conclusions which should focus upon a specific plan of investigation.

Cellulose, in nature, does not usually occur free from foreign substances. The forms in which it is found represent stages of incrustation and deposition by divers non-cellulose materials. Attempts to purify the true cellulose content by eliminating foreign matters, though successful from the standpoint of industrial and chemical research, have failed to furnish the investigator of cellulose fermentation with a satisfactory product. He desires the material in a natural, unaltered form. Most of the chemical reactions involved in the purification of plant tissue exert such drastic effects that the final products reveal newly acquired properties, obviously quite different from the properties of the natural cellulose.

The best solution of the problem, therefore, would probably be to select a natural cellulose as free from incrustation as possible. Raw cotton or seed fibers of milkweed should prove suitable. Unfortunately most of the investigators of cellulose fermentation have employed the altered forms of cellulose and the results obtained have usually been far less efficient than the results observed in nature.

In the critical review above rather indefinite comparisons of natural processes of cellulose fermentation with artificial laboratory processes, constantly recur. Those who studied the natural processes, though not attempting to control conditions, demonstrated the the rapidity and efficiency attained by nature. Laboratory investigations of the decompositions, while attempting to control conditions, demonstrated

neither rapidity nor efficiency. As a rule, the greater the control over the conditions of the experiment, the less efficient were the results. These experiments usually employed purified filter paper in a nutrient medium inoculated with cultures of micro-organisms. If pure cultures were used the activity was almost always negligible. With mixed cultures, direct from nature and without isolation, better results were obtained. In this case, however, the conditions were not controlled and the experiment approached the indeterminate demonstrations of fermentative activity in nature, without actually establishing the true rôle of any one factor. Similarly, those who used soil, sludge, or intestinal contents as a medium, merely demonstrated the process of natural cellulose fermentation, for the unknown factors in such experiments are legion.

In the present investigation the study of nature at work has a place. A piece of cotton or filter paper deposited in soil or compost pile and closely observed, will furnish many suggestions regarding the ways of nature. Such methods do not solve the secret of rapidity and efficiency. They merely suggest possible factors in operation and plans of procedure. It is for the laboratory worker to recognize these hints and suggestions and then to utilize them in the investigation of the secrets of the effective and efficient fermentation process.

P A R T I I .

INTRODUCTION AND PURPOSE.

A review of the literature on cellulose fermentation, supplemented by extensive observations of plant materials undergoing natural decomposition would seem to indicate that the secrets of rapidity and efficiency in the process still rest largely with nature. The results of laboratory experiments appear to have fallen far short of this standard. For example, consider the decomposition of leaves. In laboratory experiments, in order to investigate the action of pure cultures, leaves are sterilized, usually by heat, resulting in very perceptible changes in the nature of the substratum. So great are these changes that, upon inoculation with species of cellulose-decomposing organisms, the leaves prove extremely resistant to attack. In nature, on a leaf compost pile, the leaves undergo rapid decomposition. Further investigation shows that a growth-accessory factor or some essential food substance is operating in the leaf compost pile. This factor, though thermolabile and hence destroyed during sterilization by heat, is readily obtained by filtration of a leaf compost extract through a sterile porcelain filter. The filtrate is found to exert a marked influence upon the efficiency of cellulose-destroying organisms in the laboratory process where the conditions are under control. In this instance

the natural process was more effective than the laboratory process. Observation of nature at work and procedure along these lines revealed one of the secrets of nature.

Another illustration of the greater effectiveness of the natural over the artificial processes of the laboratory is found in the decay of wood. In woods and forests the decay of logs may be attended by a striking combination of factors which often results in relatively rapid decompositions. Such a case has been under the author's observation. Huge logs were lying in a forest and in the midst of a bog, where ^{moist} conditions prevailed continually. Aided, no doubt, by favorable physical conditions, an active decomposition set in. Extremely diversified forms of life developed and from observation it seemed highly probable that association was a leading factor in the process. At any rate in a few months fungus mycelium had penetrated the entire log, eventually reducing the latter to a structureless mass. As the wood-rotting organisms are usually studied in the laboratory, wooden blocks are placed in flasks and sterilized in the autoclave. The blocks are inoculated with the wood-destroying organism or organisms and proper moisture conditions are maintained by adding sterile water. In the experience of the author the latter process is considerably less efficient than that of nature. In the laboratory experiments the organisms often confine their activities to the surface of the blocks, the inner structure being unaffected.

As a final illustration of the efficiency of nature, consider the decomposition of green manure in the soil. The

rapidity of the process in nature is well-known to the farmer who expects a decomposition of certain crops to take place within a month or two. In the laboratory, however, the green manure is "plowed under" using sand or sterile soil. Moisture conditions are under control and the manure is inoculated with cellulose-decomposing organisms. As carried on by the author, the green manure was sterilized using both heat and disinfectants. In none of the experiments was the process of decomposition as effective as that of nature. Even in the case of the heated green manure, which was partly broken down apparently during autoclaving, the decomposition was extremely slow. It is probable that in this process, too, the growth-accessory substance is a leading factor, for in the present investigation it will be shown that this accessory factor is secreted by the growing plant, and elaborated during decomposition, and that the cellulose-decomposing ability of micro-organisms is subsequently increased. This factor has been extracted by the author from seeds, before and after germination, and from the seedling. Any procedure, however, which was sufficiently severe to sterilize the manure seemed also to destroy or inhibit the accessory factor. The results of these observations of the natural versus the laboratory process may be summarized as follows:-

In trying to investigate the secrets of cellulose decomposition in the laboratory, and hence control the factors operating, the results have fallen short of the natural process in efficiency. Certain factors such as moisture, temperature,

microbial flora, and food nutrients, are essentially controllable. Due to such artificial procedures, however, as sterilization by autoclaving or by some disinfectant such as HgCl_2 , considerable change is usually effected in the nature of the substratum. Such procedures are of course necessary in order to control the conditions of the experiment. Unless the factors governing cellulose decomposition are under control, the process is not actually being investigated, and one is dealing merely with the natural process. Apparently the important factors so affected by the technic of the laboratory are the chemical and biological factors which constitute the medium or external conditions for the growth of micro-organisms. These factors are present in nature and seem to aid in the maintenance of the physiological efficiency of micro-organisms. Hence, when plant materials are autoclaved not only are carbohydrates and proteins partly broken down, but the growth-accessory factor, bios, auximone, or vitamine, ^{may} be destroyed. Therefore, the great secret of the efficiency of nature's process seems to reside in the composition of nature's media, the constituents of which are altered or destroyed by the drastic procedures of the laboratory.

The next logical step is to determine the way in which the composition of the medium affects the process of cellulose decomposition. As the cellulose-destroying organisms are most efficient on nature's media, it may be concluded that their physiological efficiency is maintained to a high degree. A particularly active cellulose-destroyer, which decomposed raw cotton rapidly in a nutrient salt solution, was cultivated by the

author upon beef-peptone agar to which the organism gradually acclimatized itself. After almost a year of cultivation on this medium the organism refused to attack cellulose as vigorously as before; in fact, the action was hardly discernible. In this case the organism suffered a loss in physiological efficiency, and the cause was directly traceable to the factors present in the medium. Gray and Chalmers (1) found that the cellulose-decomposing power of the organism with which they were working did not decrease after being sub-cultured for nine months. Here, too, a great deal depends upon the ^{medium} / used during this period, for sub-culturing cellulose destroyers upon a suitable cellulose medium does not usually result in a considerable loss of physiological efficiency. In support of the above statement regarding the loss of physiological efficiency, the conclusions of Hopffe (2) deserve mention. This author found, in studying the digestion of cellulose in the animal body, that the isolated micro-organisms, originally active in destroying cellulose, soon lost this ability after cultivation outside of the body. It is probable that by establishing favorable conditions, embodied for instance in a suitable medium, the physiological efficiency of such an organism would be maintained. This has actually been accomplished. Hutchinson and Richards (3) have succeeded in providing proper conditions for the cellulose-decomposing organism

(1) Gray, F. H. H. and Chalmers, C. H. "On the Stimulative Action of Certain Organic Compounds on Cellulose Decomposition by Means of a New Aerobic Micro-organism that Attacks both Cellulose and Agar". *Ann. Appl. Biol.*, Nos. 3 & 4, (1924) 11; 329.

(2) Hopffe, A. "Bakteriologische Untersuchungen über die Celluloseverdauung". *Cent. f. Bakt. etc.*, (1919) 83; 385.

(3) Hutchinson, H. B. and Richards, E. H. "Artificial Farmyard Manure". *Jour. Min. Agr.*, (1921) 28; 398.

Spirochaeta cytophaga isolated by Hutchinson and Clayton (1), with the result that straw is rapidly and efficiently decomposed on a large scale.

The goal of the present investigation is to obtain an artificial fermentation of cellulose of maximum efficiency.

This is to be accomplished by determining the factors which make for rapidity and efficiency in the process by observing nature's method of procedure, and then applying these factors to the practical problems of agriculture. The investigation will center about external conditions optimum for the functioning of the organism in carrying on the process of cellulose decomposition. Of the various factors operating, attention will be focused mainly upon the growth-accessory and other stimulating factors, which include the influence of association.

Preparatory to a discussion of these factors it seems desirable to present certain considerations which confront the investigator at the outset of the work. The following subjects will be treated:-

1. Organisms Employed.
2. General Considerations of Media.
3. Proofs of Cellulose Decomposition and Indexes of the Process.
4. Nature of Cellulose and Forms selected.

(1) Hutchinson, H. B. and Clayton, J. "On the Decomposition of Cellulose by an Aerobic Organism (Spirochaeta cytophaga, n. sp.) Jour. Agr. Sci., (1919) 9; 143.

PRELIMINARY CONSIDERATIONS.

1. Organisms Employed.

One of the more remarkable of the decompositions observed in nature consisted of a leaf compost pile of several years' standing, in which leaves decomposed each year with evident rapidity. The attention of the author was directed to this compost pile by the owner, Prof. John Tyler of Amherst College. Because of the rapidity of the process, this pile was chosen for more intensive study. From the compost material the cellulose-decomposing micro-organisms to be used in this investigation were isolated. In general, the procedure of isolation was as follows:

Ordinary filter paper in a nutrient solution (1) was readily attacked by the micro-organisms in the compost. Suspensions of these organisms were plated out on various media using high dilutions. Pure cultures were eventually obtained with the aid of certain technic such as shaking the suspension with beads, varying the hydrogen-ion concentration, and the employment of special media. Several of these micro-organisms revealed the ability to attack cellulose and are probably among the active agents of destruction in the compost pile. Of the organisms isolated, twenty to twenty-five per cent. showed this ability.

Each organism in pure culture was cultivated in a nutrient solution containing filter paper, and each attacked cellulose to some extent.

In order to demonstrate the probable

(1) Nutrient solution used by McBeth and Scales, U.S.D.A., Bull. 266, Bur. of Plant Ind., (1912).

importance of each of these pure cultures in nature, a natural medium was prepared the details of which will be taken up later under: "General Considerations of Media". Finally, the organisms were recovered after having decomposed the cellulose under the more or less natural conditions provided. Of the organisms studied in this way, two were selected for use in this investigation. These particular species were chosen because of the greater rapidity manifest by them in attacking and breaking down cellulose. One of these may be classified with the genus Cellulomonas (1) and was used almost exclusively in the present investigation. This was merely for the sake of convenience, inasmuch as this organism grew more readily upon nutrient agar which is to be employed in growth experiments later. The other organism belongs to the genus Actinomyces (1) and is remarkable not only because of its aggressive cellulose-decomposing ability, but also on account of the striking polychromatic properties exhibited accompanying the fermentation of various carbohydrates. As far as the author is able to ascertain, neither of these species has been described. Following is a more detailed description of both organisms.

(1) Bergey's Manual of Determinative Bacteriology. (1923) Balt.

Cellumonas folia, n.sp.

Small rods, (1.0 - 1.5 x 0.8 - 1.0 microns) occurring in soil and active in decomposing leaves in compost, having the property of digesting cellulose.
Motile by means of polar flagella numbering two to four.
Non spore-forming.
Gram negative.
Grows on ordinary culture media.
Gelatine not liquefied.
Non-chromogenic.
Acid and gas in glucose, sucrose, glycerol, and mannite broth; neither in lactose. Medium contained beef extract and peptone.
Starch hydrolyzed.
Facultative anaerobic.
NH₃ produced.
Litmus milk unchanged.
Optimum temperature 25-30°.
Ph range, 5.0 - 9.0 m. beef-peptone broth.
Indol not formed.
Decomposes cellulose in synthetic nutrient media with an increase in the concentration of hydrogen ions.
Acetic and butyric acids are the foremost products.

Actinomyces colorata, n. sp.

Much branched, filamentous mycelium; tendency to break up into segments that function as conidia. Aerobic and non-motile.
Filaments extremely fine and stain readily.
Active cellulose-destroyer, and decomposes leaves with rapidity.
Grows upon ordinary nutrient media, also N-free, potato-dextrose, and cellulose agars; luxurious growth upon all carbohydrate media. Life cycle may be followed successfully in such liquid media as Cohn's solution.
Growth on nutrient agar colorless, becoming white and powdery, then bluish.
Forms definite colonies upon agars mentioned, with white powdery aerial mycelium.
Pigment upon carbohydrate media, pink, blue and purple.
Ferments practically all carbohydrates commonly employed, including pentose and hexose sugars, di and polysaccharoses. Cellulose is decomposed with acid production.
Gelatin is liquefied with dense cream-colored growth.
Litmus milk - no coagulation, peptonization.

2. General Considerations of Media.

In the introduction emphasis was laid upon the optimum conditions existing in nature which result in efficient processes of decomposition. Moisture and temperature conditions, food and oxygen supply, and the presence of certain substances which exert an accessory or stimulating influence, are some of the factors which go to make up favorable environments for biological processes in nature particularly cellulose decomposition. Verworn (1) calls these factors the external conditions of life, and treats under this subject food, water, oxygen, temperature, and pressure. As far as the decomposition of cellulose in nature is concerned, the processes are usually governed by certain of the above factors. For example, in the leaf compost pile mentioned above, oxygen supply and the growth-accessory factor seemed to be significant. The process was essentially aerobic for the compost heap had a loose, open structure. The presence of the growth-accessory factor was found to determine to a large extent the efficiency of the process. An experiment to demonstrate this latter point has been reviewed above. Under anaerobic conditions, the nature of the decomposition of the leaves was materially changed, the process losing in efficiency. Therefore, oxygen supply and the presence of the growth-accessory factor seem to be the limiting factors operating in the compost pile, since, even in the presence of otherwise favorable conditions, the leaves

(1) Verworn, M. "General Physiology", Trans. by Lee, p.274.

are not efficiently decomposed unless these requirements are satisfied. In the case of a pile of weeds and other vegetation undergoing decomposition, the process of destruction was confined in the main to the interior of the heap where compactness, moisture and temperature conditions favored the anaerobic and thermophilic species. Gray and Chalmers (1) find that the rate of decomposition of filter paper by a pure culture of a cellulose destroyer is increased in the presence of small quantities of certain other carbohydrates and related substances. Hutchinson and Richards (2) state that the most essential factors for the production of well-rotted straw are air supply, suitable temperature, and a suitable supply of soluble nitrogen compounds. The external conditions of life, therefore, are apparently of great importance in the processes of cellulose decomposition in nature, such, for example, as food requirements, reaction, growth-accessory and other stimulating factors, oxygen supply, moisture, and temperature. All this means a medium, and the important problem confronting the investigator of cellulose fermentation is the problem of medium, for the factors mentioned above are elements of the medium.

The composition of the medium may depend largely upon the purpose which it is to serve. For instance, for purposes of rapid isolation and cultivation of an organism, in the laboratory, such guides as the natural habitat, hydrogen-ion concentration, or the presence of the so-called growth-accessory substances,

(1) Loc. cit.

(2) Loc. cit.

may insure an abundant growth.

To demonstrate the activity of the organism, the medium generally consists of the natural substratum, governed as far as possible by such conditions as are found existing in nature. For example, the plant pathologist is often confronted with the problem of demonstrating the action of a micro-organism upon some plant or plant part. The medium in this case may consist of root, stem, leaf, flower, or fruit, and it is possible to observe the effect of the organism upon its host. The factors of nutrition in such instances are not, of course, under control, and it is difficult to state definitely what particular chemical substances are involved in the relations between parasite and host.

In physiological investigations where an individual substance is involved, a synthetic medium may be employed. Such a medium permits quantitative methods, and unknown factors may be reduced to a minimum through its use. When the effects of an organism upon sugar, starch, cellulose, peptone, or any other substance is to be determined, it is desirable to know definitely the composition of the medium. If the substance under investigation is not clearly defined chemically, as in the case of cellulose, it is best to use the purest form available, and also several of the existing forms which are considered more or less pure.

In the present investigation the following media were found to be useful in the isolation of cellulose decomposing organisms:

Nutrient agar (pH 7.0-8.0),
Cellulose agar (1),
Potato-dextrose agar,
Nitrogen-free agar (2).

The above media furnished suitable conditions for the isolation and rapid and abundant cultivation of bacteria and molds. Nutrient agar was used because of its wide adaptation to the nutritive requirements of a large number of bacteria. A greater number of species would probably be available through the use of this medium than could be obtained from cellulose agar for example. In the experience of the author the latter supports the growth of a limited number of bacteria, even of cellulose-decomposing bacteria. Certain species of Actinomyces and molds will grow upon cellulose agar. This being a selective medium, most of the micro-organisms isolated from it will attack cellulose to some degree. Other filamentous fungi attacking cellulose but not finding conditions on a cellulose agar plate suited to their needs, grow abundantly upon potato-dextrose agar. In purifying mixed cultures, nitrogen-free agar was found useful in isolating such organisms as the Actinomyces described above. After isolation these organisms were cultivated in nutrient solution containing raw cotton or filter paper, and the fermentative activities of each determined.

Subsequent demonstration of the cellulose-decomposing ability of bacteria and filamentous fungi was effected by means of

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- (1) McBeth, I. G. "Studies on the Decomposition of Cellulose in Soils". Soil Science, (1916) 1: 437.
(2) O.A.C. Medium.

a medium which, by virtue of its physical, chemical, and biological character, approached conditions in nature. The choice of this medium was the result of experimentation upon the influence of media on physiological efficiency. The details of this general study will come later. The present phase of the work was undertaken as a preliminary experiment to try to obtain a laboratory fermentation as efficient and rapid as was observed in the case of the compost pile mentioned above. In an effort to get as close to nature as possible, a very important factor was discovered, one which is to be investigated more in detail later, the growth-accessory factor. This natural medium is a chemical complex and therefore does not allow a control of the factors of nutrition. Its function is merely to demonstrate visibly the ability of micro-organisms to decompose cellulose. Inasmuch as the natural decomposition under investigation was the leaf compost pile, this material was used as a basis for the medium. Several Erlenmeyer flasks (500 c.c.) were prepared, each containing approximately 100 grams of the compost and a piece of filter paper pressed firmly upon the surface. The flasks were sterilized at 15 pounds pressure for fifty minutes. An aqueous extract from the compost was made by shaking some of the fresh material with water, allowing to settle, and then filtering through a sterile porcelain filter. The sterile extract was added to the flasks in 2 c.c. quantities, and undoubtedly aided in establishing natural conditions in the medium. A twenty-four hour nutrient medium

culture (1) of the organism was poured upon the surface of the filter paper. Proper moisture conditions were maintained by the addition of sterile water from time to time. Upon this medium the isolated micro-organisms revealed their ability or inability to decompose cellulose.

In the present physiological study of cellulose decomposition a basic nutrient medium of the following composition was employed throughout the investigation:-

K_2HPO_4	1 gram
$MgSO_4$	1 "
Na_2CO_3	1 "
$(NH_4)_2SO_4$	2 grams
H_2O (dist.)	1000 c.c.
Cellulose	15 grams.

This medium was selected mainly because of its simplicity, and because its buffer index proved to be negligible. While offering most of the elements necessary for the cell structure of many species of bacteria, the medium without cellulose did not support the growth of Cellulomonas folia, an important consideration in this investigation inasmuch as products are to be measured as an index of the process, using hydrogen-ion concentration changes to indicate the rate of cellulose decomposition. In order that this method may be a legitimate criterion it is necessary to determine the buffer action of the medium employed. The above medium was investigated with regard to buffer action (2) which

(1) McBeth's Nutrient Solution.

(2) Brown, J. H. "Hydrogen-Ions, Titration and the Buffer Index of Bacteriological Media". Jour. Bact. (1921) 6; 555.

as mentioned previously, was found to be negligible.

3. Proofs of Cellulose Decomposition and Indexes of the Process.

Quantitative methods for proving cellulose decomposition are few, particularly in work with pure cultures. Such methods, if carefully worked out, may not only furnish proof of the process but actually serve as indexes of the rate of cellulose destruction. Qualitative evidences are, of course, abundant. According to the present interpretation of the process, however, it is impossible to designate quantitatively any one method as an infallible criterion of cellulose decomposition applicable to all cases. For, as will be shown later, the products resulting from the fermentation as well as the physiological efficiency of the organisms are widely variable, depending upon certain factors among which the composition of the medium used in the cultivation of the organism, and the particular form of cellulose provided, are important. The above statement applies to investigations in which pure cultures of micro-organisms are employed and where these variable individual physiological characteristics are encountered and taken into consideration.

Some of the methods that have been used to prove the decomposition of cellulose or to indicate the rate of decomposition quantitatively or qualitatively are mentioned below. In certain cases where the actual procedures are not available, the principles involved will be discussed. For the sake of clearness the methods have been grouped as follows:-

(a) Macroscopical methods.

(b) Microscopical methods.

(c) Chemical methods.

(a) Macroscopical methods.

Christensen (1) attempted to give a quantitative interpretation to the appearance of filter paper during its destruction, by observing five stages of change characterized by the figures 0 to 4. He used 300 c.c. Erlenmeyer flasks containing 50 grams of dried soil which covered about four-fifths of the bottom. By means of a pipette water was introduced upon the uncovered part of the flask bottom to be absorbed by the soil without destroying the soil structure. Two narrow strips of filter paper were pressed against the surface by means of a glass rod so that the paper came into direct contact with the soil. Another criterion which has been adopted as more or less dependable evidence of cellulose decomposition involves the "cleared zone" test which is a purely artificial means of determining the activity of an organism (2). Experience has proven that the presence or absence of a cleared zone around a colony is no indication of the real ability of the organism to dissolve cellulose. The medium used to give this cleared zone is the cellulose agar employed originally

(1) Christensen, H. R. "Ein Verfahren zur Bestimmung der Zellulose-Zersetzenden Fähigkeit des Erdbodens". Cent. f. Bakt., etc. (1910) 27; 449.

(2) Pringsheim, H. and Lichtenstein, S. "Zur Vermeintlichen Reinkultur der Zellulosebakterien". Cent. f. Bakt. etc., (1924) 60; 309.

by Kellerman, McBeth and Scales (1), (2), (3). The method has certain disadvantages quite apart from its purely artificial features. It is, therefore, difficult to draw accurate conclusions. The medium contains an insoluble salt, calcium carbonate, the presence of which is easily confused with the precipitated cellulose, also a constituent. Moreover a homogenous mixture of the latter throughout the medium could not always be insured. To claim a dissolution of cellulose under these conditions would seem hardly sufficient proof, at least for investigational work, inasmuch as the cleared zone might possibly have been caused by a dissolution of the calcium carbonate in the medium by acid produced by the micro-organism. This is probably a legitimate and reasonable conclusion in spite of the assertion to the contrary made by Löhnis and Lochhead (4). These authors experimented with cellulose agar using one per cent. hydrochloric acid as a solvent for the calcium carbonate upon plates containing colonies surrounded by the cleared zone. They claim that the dissolution is one of cellulose and not of calcium carbonate. At best, however, the method is hardly applicable to investigations in which a definite and accurate criterion is desired. Aside from the discussion of the cleared zone, certain other artificial features of this method should be mentioned, features which may apply also to various methods of proving cellulose decomposition. In the experience of the

(1) Soil Science, (1916) 1; 437.

(2) McBeth, I. G. and Scales, F. M. "The Destruction of Cellulose by Bacteria and Filamentous Fungi". U.S.D.A., Bull. 266, Bur. of Plant Ind., (1912).

(3) Kellerman, K. F. and McBeth, I. G. "The Fermentation of Cellulose". Cent. f. Bakt., etc., (1912) 34; 485.

(4) Löhnis, F. and Lochhead, A. G. "Über Zellulose-Zersetzung". Cent. f. Bakt. etc., (1913) 37; 490.

author certain cellulose-decomposing organisms, active under natural conditions, refuse to attack filter paper under the conditions existing in a cellulose agar plate. Yet, filter paper surrounded by natural conditions will undergo destruction. In other words, the organisms find conditions in nature more favorable for physiological activity than under the artificial conditions imposed by man, as in the case of the cellulose agar plate method. Extremely active cellulose-destroyers in nature do not seem to be able to dissolve cellulose at all readily under these artificial conditions. It has also been found that various forms of cellulose differ considerably as to the ease, or difficulty, with which they are attacked. Filter paper in general has proved to be a most resistant cellulose, and the greater the degree of purification, the more resistant it seemed to become. Whether this be due to its altered chemical structure, or to other physico-chemical factors which tend to resist the action of micro-organisms, is uncertain.

Experiments on the decomposition of various forms of cellulose by micro-organisms show that raw cotton, untreated in any way, is one of the forms most readily attacked. In fact, raw cotton probably represents more nearly the natural, unaltered cellulose as it occurs in the cell membrane. It has been found further that active cellulose-fermenting organisms will attack raw cotton rapidly and effectively in pure culture and under favorable moisture conditions. This being true a homogeneous

surface of raw cotton moistened with a nutrient solution in a petri plate, may offer suitable conditions for the development of cellulose-destroyers and observations of their activities. Such a method will be developed later.

Not only are Swedish and English filter papers relatively resistant to microbial attack, but cotton prepared for laboratory purposes proves to be most unsuitable as a source of cellulose for fermentation experiments. It is probably true also that the habituation of the culture to a definite form of cellulose may be a factor of considerable importance in the cleared zone as well as in other similar methods.

(b) Microscopical methods.

Visible proof of the decomposition of cellulose based upon microscopical examination of the cell walls of plant tissue is not, as a rule, a dependable criterion, particularly where staining reactions are involved. It is probably true, however, that after exhaustive examinations and studies of cell walls, certain carefully trained investigators may be able to discern by microscopical observation, evidences of destruction. Thus, Haberlandt (1) and Otto (2) refer to "Korrosionen" of the cell walls revealing the activities of micro-organisms. Haberlandt describes this phenomenon as follows:-

(1) Haberlandt, G. Sitzungsber.d.k.pr.Akad. d. wiss., (1915) XLI; 704. "Beiträge zur Allgemeinen Botanik", (1918) Erster Band, 530, Berlin.

(2) Ibid.

"If the attack on the membrane is local, characteristic corrossions appear which take the form of irregular holes, pockets, channels and hollows".

Schellenberg (1) and Newcombe (2) have also observed microscopically the breaking down of cell walls under the attack of fungi.

Thaysen and Bunker use the microscope in studying the destruction of cellulose fibers by micro-organisms (3). Direct examination of cotton fibers did not reveal marked differences between the attacked and normal fibers. By means of the so-called "swelling test", however, changes in the structure were accentuated. The swelling test consisted of the viscose treatment of Cross and Bevan (4), which includes treatment of the fibers with a mixture of carbon bisulphide and sodium hydrate solution. Thaysen and Bunker were able, after sufficient swelling of the fibers, to distinguish the good from the bad. Solutions of cuprammonium or calcium thiocyanate were also used successfully as swelling agencies.

(c) Chemical methods.

The use of a suitable synthetic medium in experiments dealing with cellulose decomposition permits quantitative determinations of products formed which may be considered definite

(1) Loc. cit.

(2) Loc. cit.

(3) Thaysen, A. C. and Bunker, H. J. "The Destruction of Cellulose Fibers and Fabrics by Micro-organisms, and the Importance of the Microscope in the Study of this Destruction". Jour. Roy. Micros. Soc., (1923) 303.

(4) Cross and Bevan. "Cellulose". (1916) 25, 247, 318.

proof of the process provided pure cultures are used and the individual physiological characteristics of these organisms are known. For example, if an organism should be isolated which would convert cellulose to cellobiose or glucose, a quantitative estimation of the sugar formed might serve as a legitimate criterion of the process. As a matter of fact, the products resulting from the fermentation of cellulose as reported in the literature are somewhat variable. In the thermophilic decomposition the following products have been found: (1), (2), alcohol, carbon dioxide, hydrogen, formic and acetic acids; even slight amounts of lactic acid. As far as gaseous products are concerned, Omelianski (3) found that in the anaerobic decomposition of cellulose, two distinct processes are involved, one in which methane is the characteristic product; the other, hydrogen. Methane seems to be typical of the anaerobic fermentation. Hoppe-Seyler found that in the fermentation of cellulose by sludge, methane and carbon dioxide were given off (4). It would appear from the figures presented that methane was by far the more abundant. According to Van Iterson (5) the products of the anaerobic process in addition to hydrogen, methane and carbon dioxide, may include acetic and butyric acids. These references should be sufficient

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- (1) Fred, E. B., Peterson, W. H., and Viljoen, W. "The Fermentation of Cellulose by Thermophilic Bacteria". Abs. of Bact., (1924) 1; 11.
- (2) Pringsheim, H. "Über Vergärung der Zellulose durch Thermophile Bakterien". Cent. f. Bakt. etc. (1913) 39; 513.
- (3) Omelianski, W. "Ueber die Gärung der Cellulose". Cent. f. Bakt. etc., (1902) 8; 193, 225, 289, 321. (1904) 11; 369.
- (4) Hoppe-Seyler, F. "Über Gärung der Cellulose mit Bildung von Methan und Kohlensäure". Zeitschrift physiol. Chem., (1886) 10; 401.
- (5) Van Iterson, C. "Die Zersetzung von Cellulose durch Aerobe Mikroorganismen". Cent. f. Bakt. etc., (1904) 11; 689.

to indicate the variation among the products of cellulose decomposition. It is obvious that in order to designate any one method in which products are measured as the real criterion or index of the process it would be necessary to know the individual physiological characteristics of the organisms employed. Hence, a measurement of the carbon dioxide evolved or the acid produced might not constitute a legitimate criterion applicable to all cases. If the true physiological characteristics of the organism are known, however, any such quantitative method might serve as the index.

In the present work hydrogen-ion concentration determination was the quantitative method selected. This method was chosen as a measure of the process of cellulose fermentation because of its applicability to the physiological proclivities of the organisms under investigation. Both *C. folia* and *Act. colorata* ferment cellulose with an increase in the concentration of hydrogen ions.

Besides serving as a suitable means of measuring the progress of the fermentation, the P_h change also furnishes an index to the physiological activity of the cellulose destroyer. If, for example, the cellulose-decomposing ability of *C. folia* should decrease, because of some change in environmental conditions, the production of acid would correspondingly decrease; perhaps measurable only with the aid of the electrometric method for H-ion determination. Should *C. folia* be rendered physiologically active, the increase in efficiency may be readily detected by definite increases in P_h during the more

effective decomposition. This statement will be substantiated and the details more thoroughly described in the first experiment to follow.

Regarding the increase of hydrogen-ions, the possibility arises that the acid produced may not result directly from the fermentation of cellulose. For example, the cellulose may furnish energy for the organism to split some inorganic constituent of the medium. In this case the acid production would be indirect, but directly traceable, nevertheless, to the presence of cellulose. This possibility arises with the use of the synthetic medium mentioned above. The source of nitrogen in this medium is ammonium sulphate. A chemical reaction is going on involving several of the constituents, and accompanied by an evolution of ammonia. At the same time there is a gradual but slight increase in the concentration of hydrogen-ions. The presence of C. folia in such a medium does not seem to alter this situation in the least; in fact, the medium does not support the growth of this particular micro-organism. In the presence of cellulose there is a considerably greater increase in the concentration of hydrogen-ions, and the cellulose shows evidence of destruction. The acid may arise directly from the accumulation of the (SO_4) radical due to the further splitting of the ammonium salt (cellulose furnishing the energy), or cellulose may be broken down directly by the organism. Detailed study of the process would seem to indicate the latter as the true explanation. In either case the cellulose is decomposed, and

for that reason H-ion concentration changes produced may serve as an index of the process.

Under certain conditions, weighing experiments may be taken as proof of cellulose decomposition or as an index of the rate of decomposition. Here, however, difficulties may arise which would probably render such a method entirely unsuitable and inaccurate. One possibility would involve direct weighings of cellulose before and after the decomposition. In this case it would seem necessary to control as thoroughly as possible the conditions of the experiment regarding the processes that go on in the medium, to avoid or to take into account factors which might detract from the accuracy of the weights obtained. The simpler the medium, therefore, the more satisfactory the results would prove in all probability. When soil, sand, or beads are used as a substratum, an added difficulty arises in the handling of the partially decomposed cellulose to avoid loss of material and consequent error.

Another procedure involving weighing experiments includes chemical treatment of the cellulose, either dissolving and precipitating or determining the amount of cellulose present according to prescribed methods. In the former case one must rely upon the efficiency of cellulose solvents. At the outset this would appear to be quite unreliable because of the insolubility of the cellulose, the lack of universally recognized standard methods for the preparation of solvents, and the uncertainty surrounding the present conceptions of true cellulose

itself. One of the prominent solvents of cellulose is Schweitzer's reagent, (copper-ammonium-cellulose reaction). The efficiency of Schweitzer's reagent as a quantitative method may legitimately be questioned. In the first place the nature and extent of the action of the reagent upon cellulose is uncertain. According to Heuser (1) if the precipitation is made a short time after solution takes place, the cellulose will suffer practically no chemical change. Upon longer standing, however, the cellulose undergoes oxidation by the copper oxide with the formation of oxycellulose. If this be true, the use of such a process would seem altogether precarious. In a recent investigation at Rothamsted Experimental Station, Schweitzer's reagent has been employed to determine the amount of cellulose consumed in the process of decomposition. The reagent was prepared according to the method advised by Charpentier (2). Whatman's No.41 filter paper served as a source of cellulose. The latter was precipitated by HCl and washed. Under the conditions prevailing during the experiments the loss of cellulose proved to be greater than when handled in a more convenient manner and with less likelihood of error. For example, in the experiments, 500 c.c. Erlenmeyer flasks were filled to a depth of about 2 cms. with small glass beads. The weighed sheet of filter paper was laid upon the surface of the

(1) Loc. cit. 137.

(2) Charpentier, C. A. G. (1921) "Studien über den Einfluss des Rindvieh und Pferdestallmistes auf die Zersetzung der Zellulose in der Ackererde. Inaug. Diss. Tavastehus (Finland). Thesis, Helsingfors.

beads. The amounts of cellulose recovered were as follows

(1):-

Weights in Grammes
Cellulose

No.	Filter paper (at start)	Recovered	Loss	Average
1	1.3410	1.3300	0.0110	0.0100
2	1.3370	1.3280	0.0090	
3	1.3450	1.3350	0.0100	

Charpentier's method of cellulose determination has been used also by Waksman and Henkelekian (2) as an index and measurement of cellulose decomposition in the soil. The amount of residual cellulose is determined by this method, which, subtracted from the amount of cellulose originally present, gives the amount actually decomposed, according to these authors.

Gross and Bevan point out, however, that the cuprammonium reaction may not always be an efficient solvent of cellulose (3). They state:-

"Cotton cellulose does not appear to be hydrolysed by the process of solution, that recovered from the solution by precipitation by acids, etc., having approximately the same weight as that of the fiber originally dissolved. There are celluloses, on the other hand, which are partially hydrolysed, and when precipitated the cellulose recovered is found to be in defect, and the solution to contain dissolved carbohydrates".

- (1) Gray, P. H. H. and Chalmers, C. H. The Annals of Applied Biology, Nos. 3 and 4, (1924), 11; 324.
 (2) Waksman, S. A. and Henkelekian, O. "Microbiological Analyses of Soil as an Index of Soil Fertility: VIII. Decomposition of Cellulose". Soil Science, (1924) 17; 275.
 (3) Loc. cit., 11.

Referring to solvents of cellulose, Crane states (1):

"Cellulose is insoluble in all ordinary solvents and no true solution can be made from which it may be precipitated in unchanged condition".

In a recent publication by Hess (2) it is stated that the Röntgen diagram shows that the cellulose regenerated from cuprammonium solution is identical with the original cotton; no chemical change is detected.

The uncertainty of present knowledge regarding the real action of solvents upon cellulose, applies also to chemical reagents used in the prescribed methods for freeing cellulose materials of foreign and incrusting substances (3). In either case the action may be drastic enough to cause some alteration in the nature of the original cellulose. It is obvious that unless something fairly definite is known regarding the nature and extent of the action, weighing experiments may be quite inaccurate and entirely inadequate in revealing the amounts of cellulose decomposed by micro-organisms.

Furthermore, as long as the exact chemical nature of cellulose is unknown, it would hardly seem advisable to use such methods exclusively as quantitative means of determining amounts of true cellulose actually destroyed. Suppose, for example, that cellulose were found to be, not a single substance, but a representative of a group of similar or related substances. This does not

(1) Crane, J. E. "Cellulose Industries". Indust. Chem., A. Rogers, (1921) 1052.

(2) Hess, K. Z. Angew. Chem. (1924) 37; 993.

(3) Cross and Bevan. "Cellulose".

refer to the hemi- or reserve celluloses (1) which are probably stored by the plant as reserve food, or to the compound celluloses (2) which include combinations of true cellulose with one or more of the so-called incrusting substances, such as lignin, pectin, cutin, suberin, tannin, pigment, etc. If, however, there should prove to be more than one true cellulose forming the structural unit of plant cells, such as the "callose" of Mangin, (3), it is possible that several of the more or less firmly established reactions, as solubility in Schweitzer's reagent and certain micro-chemical tests, might be found untenable, necessitating some knowledge of the specific form of cellulose used. According to certain authorities, for instance, the differences noted in celluloses obtained from various sources are not due to the presence of impurities, but that the impurities so-called are really an "integral part of the cellulose derived from such sources" (4).

Other indexes of cellulose decomposition which might be used would include the increase of cells accompanying rapid processes of destruction, and nitrogen-fixation determination, for it has been known for some time that cellulose breakdown products increase nitrogen-fixation (5).

(1) Loc. cit.

(2) König, J. "Chemie der Menschlichen Nahrungs-und Genussmittel". (1910) 3; 451. Haas and Hill, An Intro. to the Chem. of Plant Prod., (1921) 1; 148.

(3) Mangin, L. "Sur la callose, nouvelle substance fondamentale existant dans la membrane". Comptes Rendus, Paris, (1890) CX; 24. Jour. of Mycology, (1894) 7; 140.

(4) Esselen, G. J. "Colloidal Behavior", Bogue, Chap. 27, (1924); 2; 629.

(5) Hutchinson, H. B., and Clayton, J. Jour. Agr. Sci., (1919) 9; 170. Pringsheim, loc. cit.

The decision of the author to employ hydrogen-ion concentration determination as the quantitative method in this investigation, has been stated and commented upon above. This method will serve as a measure of the process of cellulose fermentation and as an index to the state of physiological efficiency of the cellulose destroyer.

4. Forms of Cellulose Selected.

From a review of the literature on the chemistry of cellulose and the numerous procedures adopted for the isolation of true cellulose, it would seem desirable to select the forms of cellulose to be used in investigational work with great care and with an understanding of the probable limitations of each. Forms of cellulose available for work on cellulose decomposition may be divided into two groups, natural and artificial celluloses, each form approximating, more or less closely, the substance known as true cellulose. The former group consists largely of the so-called compound celluloses, that is, true cellulose incrustated to a greater or lesser degree by foreign matters. Provided the material is sufficiently rich in true cellulose with a minimum of incrustation, as is true in the case of raw cotton, such a source of cellulose would be of value because of its freedom from drastic chemical treatment and hence its close adherence to nature. The following table taken from Bowman (1), gives the approximate composition of cotton fiber from various sources.

Source of Cotton	Surat Per cent.	American Per cent.	Egyptian Per cent.
Cellulose	91.35	91.00	90.8
Wax, oil and fat40	.35	.42
Protoplasm and derivatives (Pectose)	.53	.53	.68
Mineral matter, i.e., salts of K, Na, Ca, Mg, Fe, and Al,22	.12	.25
Water	7.50	8.00	7.85

(1) Haas and Hill. "An Intro. to the Chem. of Plant Prod." (1921) 1; 150. "The Structure of the Cotton Fiber", Bowman, London, (1908) 147.

Highly incrustated celluloses such as wood with its comparatively large non-cellulose content (1), would not ordinarily serve as desirable forms of cellulose for investigations in which a true cellulose is to be employed.

The artificial celluloses may be represented by the various kinds of filter paper available. The treatment involved in the production of each kind probably differed somewhat in severity. It is possible, therefore, that provided the true cellulose is affected by such reagents as chlorine gas, bromine water, and caustic alkali, which seems likely, some differences will exist among the final products. For example, the cellulose obtained from wood would probably differ more or less considerably from that obtained from raw cotton. Besides filter paper, prepared cotton, linen, parchment paper, and other products derived from chemical treatments of plant material, may be employed as sources of cellulose. In the present investigation the following sources were chosen:-

Raw cotton (2),
Berzelius paper (Swedish),
Ordinary English filter paper,
Prepared cotton,
Leaves and straw.

(1) "Cellulose", Cross and Bevan, (1916) 176.

(2) Obtained from the Agronomy Department of the University of Arkansas. Not ginned and chemically untreated.

P A R T I I I .

SITUATION REVIEWED.

The goal of the present investigation is to obtain a cellulose fermentation of maximum efficiency. Previous investigations reveal the fact that some factor or factors necessary for maximum rapidity and efficiency in the process have been overlooked or neglected for the results of laboratory experimentation have fallen far short of the natural process in efficiency. Investigators invariably report loss of ability to decompose cellulose on the part of the organisms, under the artificial conditions of the laboratory. In other words, the physiological efficiency of the cellulose-destroyer is decreased or lost. When first isolated, C. folia decomposed cellulose with striking rapidity, accompanied by a decided increase in the concentration of hydrogen-ions. At this time, the organism grew scantily upon laboratory media, nutrient agar, for example. With prolonged cultivation upon nutrient agar, however, the organism gradually became accustomed to the beef-peptone medium and was able to grow rapidly and abundantly upon it. C. folia now refused to attack cellulose in any form, and had, therefore, suffered a loss in physiological efficiency. This phenomenon may be viewed as a variation in fermenting power, which, according to Gurney-Dixon (1), may be brought about by the conditions of growth, such as modifications in the composition of the medium. Loss of

(1) Gurney-Dixon, S. "The Transmutation of Bacteria". (1919)
Cambridge, 54.

physiological efficiency and virulence are encountered constantly in laboratory investigation, and present one of the most important problems which the worker is called upon to face. Gurney-Dixon makes the following statement:-

"Practically every organism becomes less virulent when cultivated for any length of time outside the body, that is to say, on artificial media, even under the most favorable conditions.

"Possibly some of the factors responsible for this change are those just mentioned, namely, differences in temperature, the presence of oxygen, exposure to sunlight, the increased acidity of the medium. Other contributing factors are found, no doubt, in the nature of the medium itself - both as regards its chemical composition and its physical properties.

"The difference in chemical composition between the body fluids and laboratory media must necessarily profoundly influence the metabolism of organisms transferred one to the other"
(1).

In the above quotation, the author refers, of course, to virulence and microbial invasion of the animal body. In general the same principle applies to the physiological efficiency of organisms operating outside of the animal body, as in the great transformation processes of nature. Loss of physiological

(1) Ibid. 77.

efficiency in such processes probably involves different combinations of factors from those concerned in the loss of virulence on the part of a pathogenic micro-organism.

However, whether the phenomenon be one of virulence or physiological efficiency the problem invariably comes down to one of media, the general conditions of life, so appropriately and effectively fulfilled by nature.

How can the physiological efficiency of a cellulose-destroyer be restored? Or, to put the question in another way: how can its physiological efficiency be maintained; not necessarily as it is maintained under natural conditions, but maintained at a maximum efficiency, even exceeding, if possible, the results observed in nature.

The cellulose-decomposing organisms to be employed, C. folia and Act. colorata, were chosen because of their particular adaptability to the plan, purpose, and technique of this investigation. Both species ferment cellulose in pure culture with great rapidity, accompanied by a decided increase in the concentration of hydrogen-ions.

Temperature, moisture conditions, reaction, oxygen supply, and food nutrients, will be known factors in the experiments to follow. These are merely contributory factors, however, for unless the cellulose-destroyer is physiologically efficient no decomposition of cellulose will take place, even though these factors are operating favorably. For example, after the loss

in cellulose-decomposing ability on the part of C. folia brought about by artificial cultivation, this organism will not ferment raw cotton in the basic nutrient solution under favorable conditions of temperature, moisture, reaction, etc.

The important and controlling factors of physiological efficiency in cellulose decomposition are the so-called "essential food substances", those stimulating and accessory factors of growth and physiological activity. Under the term "essential food substances", are included the influence of the known and unknown physical, chemical, and biological agents of protoplasmic activity, and the stimulating effect of radiant or other forms of energy upon the chemical reactions of the cell.

ESSENTIAL FOOD SUBSTANCES.

Verworn (1) calls attention to the conception of conditions of life; conditions that must be fulfilled if an organism is to exist. He divides these into the special and general conditions. The former are manifold, and, in the words of the author, "to describe them is to describe the natural history of every organism". The general conditions of life, however, must be fulfilled for all organisms, and these Verworn again divides into the external and internal conditions. The latter "are inherent in the composition of the organism" including special peculiarities of structure and chemical composition, for example, the association of nucleus and

(1) Verworn, M. "General Physiology". (1899), trans. by Lee, London, 272.

protoplasm in the cell (1), and the presence of a certain chromosomal complex (2).

The external conditions are afforded by the medium, and include the factors previously referred to, food requirements, temperature, moisture, oxygen supply, H-ion concentration, light, gravity, electricity, sometimes certain mechanical conditions; and finally the factor under investigation in the present work, the essential food substances. These are all factors of the environment, and each is capable of acting as a stimulus to the living cell resulting in definite responses. Lillie states (3):-

"A stimulated gland cell secretes, a muscle cell contracts, a protozoön passes through an often complex sequence of motor reactions; a stimulated plant organ accelerates or otherwise changes its state of growth. But notwithstanding this diversity of detail, all show one fundamental property in common, namely, modifiability of the characteristic physiological activities under comparatively slight changes of external conditions".

In other words, there are factors, i.e., chemical substances, mechanical shock, radiant energy, electric currents, and thermal changes, which are capable of producing a transformation of the molecules of protoplasm from the "reactive and unstable forms, to the forms containing less energy. - the

(1) Loc. cit.

(2) Cowdry, E. V. "General Cytology". (1924), 181.

(3) Ibid., p.169.

unreactive and stable forms", that is to say, they cause the "discharge of energy" (1). Living protoplasm has a high energy content, and its atoms and molecules, in the words of Bovie (2), are "maintained in a chemically active condition". Under the influence of a stimulus, ultra-violet light, for example, the internal condition of the protoplasm is altered, and the velocity of chemical reactions is increased. The metabolic rate is therefore increased as evidenced by increased growth, increased mechanical activity, and increased respiration, that is, increase in its consumption of oxygen and liberation of carbon dioxide. It is evident, therefore, that both katabolic or energy-yielding reactions and synthetic anabolic reactions may be influenced in this way. Bovie (2) exposes ameba to ultra-violet light causing cytolysis. Under the influence of ultra-violet energy, the atoms and molecules of protoplasm become more chemically active, and in the word of the author, "chemical reactions occur at unnatural and uncontrolled velocities. The organization of the protoplasm of the cytolized ameba is destroyed and a radiation environment is produced in which products of cytolyses may play an important role. At any rate, other amebas in the vicinity which had not been exposed to the light at all, reveal definite stimulation, and draw nearer to the cytolized ameba."as the living ameba arrives, a food-cup is formed, the edges of which creep along the surface of the radiated organism" (3).

(1) quotations from Cowdry, p.25.

(2) Bovie, W. T. "Relation between Physiological Dominance and the Biological Effects of Rays". Read at 25th Annual Meeting, American Roentgen Ray Society, (1924).

(3) Ibid.

Quotation from Barr, C. E. "A Stimulating Effect of the Photocytolytic Products of Protoplasm". Jour. Med. Res., (1923) 44; 79.

Bovie also observes an acceleration of cell division on the part of Paramecium exposed to ultra-violet light (1).

The possible rôle of ultra-violet light in chemically activating living tissue and in bringing about proper physiological functioning will be taken up later. At present, confronted by examples of definite stimulation, it may be well to proceed upon the theory that a photo-chemical substance is produced by the action of ultra-violet light upon protoplasm in the cases cited above, and discuss, more in detail, the essential food substances.

There are chemical substances in nature which seem to cause a definite stimulation of cellular metabolism, and function as essential factors in physiological activity.

The rôles of iron, iodine, and phosphorus in the metabolic processes of cells are little understood, but they belong without doubt to the essential substances. According to Warburg, the high velocity of oxidation of foodstuffs in the cell is based upon a catalysis on surfaces containing iron (2). This element is necessary for the formation of chlorophyll and hemoglobin. Phosphates play an important part in the activity of muscle cells and as a significant factor in respiration (3). In the presence of phosphates, the hexose diphosphoric acid, important in fermentation and muscle metabolism, is formed.

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- (1) Bovie, W. T. and Hughes, D. M. "The Effect of Quartz Ultra-Violet Light on the Rate of Division of Paramecium caudatum". Jour. Med. Res. (1918) 39; 231.
- (2) Warburg, O. "Beiträge zur Physiologie der Zelle, insbesondere über Oxydations-geschwindigkeit in Zellen". Ergebn. d. Physiol. (1914) 14; 253.
- Meyerhof, O. "Chemical Dynamics of Life Phenomena". (1924) Phila.
- (3) Ibid.

Potassium, manganese, and boron should be included in the list of essential food substances, for the action of these elements in certain concentrations is one of stimulation, particularly upon the plant cell (1).

Other essential food substances will be mentioned later in connection with the activity of micro-organisms, for it is true that these unicellular forms receive stimulation through the agency of certain chemical factors of the surrounding medium - the essential food substances. Sometimes these substances are more or less well-known chemically as lignin, xylose, amino-acids, and colloidal iron; in certain cases, however, the true nature of the substance is not yet clearly understood, and such terms as "vitamines", "bios", "auximones", "factors X and V", "X-substance", and "growth hormone" are found in the literature to indicate the unknown agent of stimulation.

One characteristic possessed by the essential food or growth accessory substances in common, is the ability to cause marked effect with a minimum quantity of substance being involved. Sometimes this stimulation results in increased growth and multiplication on the part of the cell. For example, certain forms of organic nitrogen cause a remarkable increase in growth of the genus Rhizobium, though usually accompanied by a decided loss of nitrogen-fixing ability. Another illustration of accelerated multiplication is found in the work of Robertson (2)

(1) Cowdry, loc. cit., p.55.

Thatcher, R. w. "The Chem. of Plant Life", (1921), New York, p.13.

(2) Robertson, T. B. "Experimental Studies on Cellular Multiplication". Biochem. Jour. (1921) 15; 595, 612.

on infusoria. He demonstrated the existence of "autogenous catalysors of cellular multiplication". Contiguous cells, Robertson found, are able to catalyze each others reproductive rate by virtue of the presence of a 'soluble substance in the culture medium ('X-substance') which is produced by bacteria and which is requisite for the manufacture of the autocatalyst by the infusoria themselves".

In some cases physiological activities apart from multiplication, and functions essential to propagation, such as accompany cellular metabolism, receive the stimulus. The fundamental rôles of phosphate and iron mentioned above are examples of this type of stimulation. In the case of micro-organisms both multiplication and cellular metabolism have to be considered, and therefore stimulation is manifested through either or both of these activities. The genus Azotobacter offers an illustration of this influence. Itano (1) has shown that vitamine B (?) (2) causes a stimulation of Azotobacter both in the multiplication of cells and in the ability to fix atmospheric nitrogen.

The above classification of cell function into growth and multiplication and cellular metabolism is not an entirely adequate and satisfactory differentiation. Yet, for the purposes of the present investigation it offers a favorable and effective means of demonstrating the influence of essential food substances upon cell activity. A similar division of these functions has been

(1) Itano, A. "Physiological Study of Azotobacter chroococcum, I" Jour. of Bact. (1923) 8; 483.

(2) "Yeast Vitamine". Bull. No.14, Vitamine-Harris, The Harris Laboratories, Tuckahoe, New York.

by Leuf (1), who distinguishes those functions relating to maintenance and reproduction, as in amebas from the so-called "special work" not necessarily essential to existence or propagation such as is carried on by cells of the brain or liver. "Special work", used in this sense, refers more particularly to the higher animals and man. Such functions in the lower forms are included under the term "cellular metabolism" mentioned above.

A resumé of some of the important investigations relating to the rôle of essential food substances in plant and microbial nutrition follows.

work upon the growth-accessory, or essential food factor, dates back to Pasteur's investigation of yeast (2). According to wildiers (3) some substance was necessary for the vigorous development of yeast in addition to the medium used by Pasteur. He therefore modified this medium to include "bios", an organic substance present in wort, extract of meat, and commercial peptones. wildiers' contention was confirmed by Williams and Bachman (4) (5). As far as the culture of yeast is concerned, excellent results may be obtained from the use of wort (6). According to Miller (7), none of the sugar-salt solutions recommended in

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- (1) Leuf, A. N. P. "The Medical World". (1956) 14; 13.
(2) Pasteur, L. "Mémoire sur la fermentation alcoolique". Ann. de Chimie et de Physique, (1860) 58; 323.
(3) wildiers, K. "Nouvelle substance indispensable au développement de la levûre". La Cellule, (1901) 18; 313.
(4) Williams, R. J. "The Vitamine Requirement of Yeast". Jour. Bio. Chem. (1919) 38; 465.
(5) Bachman, F. M. "Vitamine Requirements of Certain Yeasts". Jour. Bio. Chem. (1919) 39; 235.
(6) Clark, N. A. "The Rate of Formation and Yield of Yeast in wort". Jour. Phys. Chem. (1922) 26; 42.
(7) Miller, W. Lash. "Further developments in the Study of wildiers' Bios". Can. Chem. Metallurgy, (1924) 8; 57.

the literature "gave anything like the growth of yeast obtainable with wort". Miller and his associates (1) have been endeavoring to extract this essential food substance from wort and malt combings, and have succeeded in revealing certain of its physico-chemical characteristics and properties. It was found that bios is not a single substance, but two; "Bios I" carried down by baryta, but not by charcoal, nor removed from aqueous solution by shaking with yeast; and "Bios II", taken up by charcoal, and removed from solution by shaking with yeast. The rate of reproduction of yeast suspended in culture fluids in a "rocker tube" served as a convenient method for the determination of bios. Bios II has recently been fractionated, so that Bios III is a third constituent of Wildiers' Bios.

The growth-promoting power of these substances is reported by Miller as follows (2):-

"By adding 0.1 mg. Bios I and 0.3 mg. Bios II to 10 c.c. of a solution containing sugar, and 180 mg. dry salts, the crop after 24 hours at 25° C. is raised from 3 to 4 mg. to 50 mg. of moist yeast containing 6 mg. nitrogen".

Hastcott (3) shows that bios is widely distributed in nature.

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- (1) Miller, W. L. "Wildiers' Bios". Science, (1924), 59; 197.
 "The Effect on the Growth of Yeast of an Unknown Constituent of Malt". Roy. Soc. Can. (1921) 15; 47.
 Sims, H. des B. "The Preparation and Purification of Bios I". Ibid., (1924) 18; 116.
 Hastcott, E. V. "The Formation of Bios in Infusions". Ibid. 117.
- (2) Loc. cit.
- (3) Hastcott, E. V. "The natural distribution of Bios I and Bios II". Roy. Soc. Can., (1923) 17; 157.

In contrast to the line of approach described above, another view of the situation regarding the essential food substances is being taken by several investigators. These workers hold that bios, or vitamine, is not essential for the growth of micro-organisms. It is undoubtedly true that certain micro-organisms, such as yeast, may grow in a medium which is deficient in this factor. At the same time the addition of a little wort causes great stimulation in the growth and fermentative activity of yeast. Miller reports that one-fifth per cent. of wort could be detected by fermentation tube or agar plate (1). Also there is evidence that certain salts, such as phosphates and compounds of magnesium, are indispensable in the nutrition of yeasts (2). It is therefore quite safe to conclude that yeast is not only capable of receiving stimulation, but that in a good growth of, or fermentation by, yeast, some essential food substance is operating, be it bios, vitamine B, phosphate, or halide.

It has been shown, too, that organisms may synthesize the growth-accessory substance.

In support of this view the investigations of Robertson (3), Fulmer and his collaborators (4), Heller (5), MacDonald (6),

(1) Loc. cit.

(2) Guilliermond, A. "The Yeasts", Trans. by Tanner, (1920), New York, p.68.

(3) Robertson, K. C. "Food Accessory Factors (vitamines) in Bacterial Growth". Jour. Infect. Dis., (1924) 34, 395; 35, 311.

(4) Fulmer, E. I., Nelson, V. E., & Sherwood, F. F. "The Nutritional Requirement of Yeast". Jour. Amer. Chem. Soc. (1921) 43; 186.

Fulmer, E. I., Nelson, V. E., & White, A. "The Growth of Yeast on a Medium of Wholly Synthetic Origin". Jour. Bio. Chem. (1923) 57; 397.

Fulmer, E. I. and Nelson, V. E. "Studies on Yeast". Jour. Infect. Dis. (1923) 33; 130.

Nelson, V. E., Fulmer, E. I., and Cessna, R. "Nutritional Requirements of Yeast". Jour. Bio. Chem., (1921) 46; 77.

(5) Heller, V. G. "Vitamine B in Yeast". Jour. Bio. Chem. (1923) 55; 385.

(6) MacDonald, M. B. "Synthesis of 'Bios' by Yeast Grown in a Solution of Purified Nutrients". Jour. Bio. Chem. (1923) 56; 489.

MacDonald, M. B. and McCollum, E. V. The Cultivation of Yeast in Solutions of Purified Nutrients. Ibid., (1921) 45; 307.

Hunter (1), and Pacini and Russell (2) may be cited. An important phase of this work, carried on by Fulmer and his associates, is the cultivation of the organism upon a purely synthetic medium. One of the most satisfactory of the media studied is the one used by Fulmer, Nelson and White for the growth of yeast (3).

Besides the inorganic constituents the medium contains "methose", a synthetic fermentable product, instead of cane sugar as a source of carbon and energy. The use of this material would eliminate the general criticism that bios was supplied by substances of natural origin, even in spite of meticulous purification. These investigators sub-cultured Sacch. cerevisiae on this medium. As suggested by Funk and Dubin (4), however, different strains of yeast probably behave differently as regards vitamine requirement.

Kijkman, van Hoogenhuijze and Derks (5) have shown that yeast cultivated in vitamine-free media fail in curative effect on polyneuritic fowls, and that yeast takes its antineuritic factor as such from the culture medium, but is not "capable of synthesizing the vitamine unless the medium contains at least the products of decomposition of the vitamine by heating". These authors conclude that the antineuritic factor and the growth-promoting water-soluble B substance are not identical.

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- (1) Hunter, O. W. "Production of Growth-Promoting Substance by Azotobacter". Jour. Agr. Research, (1923) 23; 825.
(2) Pacini, A. J. P. and Russell, D. W. "The Presence of a Growth-Producing Substance in Cultures of Typhoid Bacilli". Jour. Bio. Chem. (1918) 34; 43.
(3) Ibid.
(4) Funk, C. and Dubin, H. E. "The Vitamine Requirements of Certain Yeasts and Bacteria". Jour. Bio. Chem. (1921) 48; 437.
(5) Kijkman, C., van Hoogenhuijze, C. J. C., and Derks, T. J. G. "The Vitamine Content of Microorganisms in Relation to the Composition of the Culture Medium". Jour. Bio. Chem. (1922) 50; 311.

Yeast is generally recognized as a rich source of vitamins, and the results obtained by the use of yeast, or extracts and other preparations from yeast, in experiments upon nutrition, cellular metabolism, multiplication of cells, and therapeutics, are remarkable (1).

Hunter (2) showed that azotobacter is capable of synthesizing a growth-accessory substance similar to vitamin B. He also showed that azotobacter exhibits growth-promoting properties and curative effects in experiments with rats and pigeons. Bottomley and Mockeridge have shown that azotobacter and Rhizobium radicicolum elaborate products which have a growth-promoting effect upon green plants (3).

The investigations of Bottomley and Mockeridge of "bacterized peat" showed that this material contained certain organic substances (auximones) which, when supplied even in small quantities to Lemna plants growing in complete mineral culture solutions, have a great stimulating effect upon their growth. In the absence of these auximones, however, normal growth and multiplication was not maintained (4). These substances influence the nitrogen-fixing

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- (1) Bailey, E. M. "The Potency of some Commercial Vitamin Preparations as Compared with that of Dry Brewers' Yeast". Conn. Agr. Exp. Sta., (1922) Bull. 240.
Funk, C. "The Vitamines". (1922) Balt.
Ellis, C. and Macleod, A. L. "Vital Factors of Foods". (1922) New York.
Hawk, P. B., Knowles, C. F., Rehfuess, M. H., and Clarke, J. A. Jour. Am. Med. Assn., 69 (1917).
- (2) Loc. cit.
- (3) Mockeridge, F. A. "The Formation of Plant Growth-Promoting Substances by Micro-organisms". Ann. of Bot., (1924) 38: 723.
Bottomley, W. B. "The Effect of Nitrogen-Fixing Organisms and Nucleic Acid Derivatives on Plant Growth". Proc. Roy. Soc. Lond., B., (1919) 91: 83.
- (4) Bottomley, W. B. "Some Effects of Organic Growth-Promoting Substances (Auximones) on the Growth of Lemna minor in Mineral Culture Solutions". Proc. Roy. Soc. Lond., B., (1917) 89: 102, 481.

genera Azotobacter and Rhizobium, increasing nitrogen-fixation and also the rate of nitrification by nitrifying soil bacteria (1). The auximones, according to Bottomley, are decomposition products of bacterial action upon peat. The peat is treated with a mixed culture of soil organisms and incubated for two weeks at 26° (2). Mockeridge obtained plant growth-promoting substances from "all the well-known and frequently used organic fertilizing materials and the greater the degree of bacterial decomposition which the material has undergone the greater was the proportion, as measured by their effect", of auximones (3). According to Mockeridge soil bacteria liberate from the soil organic matter the growth-accessory factor necessary for plant growth (4). Some of the growth-accessory substances, therefore, present in bacterized peat and extracts from organic manures may be products of bacterial metabolism. The significance of this possibility is shown by Mockeridge who proved that Azotobacter and Rhizobium have a stimulating effect upon plant growth in experiments where sterilized cultures of these organisms were added to the culture solutions in which the green plants were growing (5). She found, too, that the addition of yeast to the culture medium consisting

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- (1) Mockeridge, F. A. "Some Effects of Organic Growth-Promoting Substances (Auximones) on the Soil Organisms Concerned in the Nitrogen Cycle". Ibid., 508.
(2) Bottomley, W. B. "Some Accessory Factors in Plant Growth and Nutrition". Proc. Roy. Soc. Lond., (1914) 88; 237.
(3) Loc. cit.
(4) Loc. cit., also.
Mockeridge, F. A. "The Occurrence and Nature of the Plant Growth-Promoting Substances in Various Manurial Composts". Biochem. Jour., (1920) 14; 432.
(5) Loc. cit., also Bottomley.

of inorganic ingredients only was as effective as a similar addition of nitrogen-fixing bacteria (1). Autoclaved yeast caused a greater stimulation than autolyzed yeast.

Investigating further the stimulation in plant growth and multiplication exhibited by the extracts of organic manures and bacterized peat, also the "auximonic" effect of Azotobacter, Rhizobium, and yeast, Bottomley and Mockeridge found at least a partial explanation of this phenomenon in a study of nucleic acid and its derivatives. Schreiner and Skinner (2) name nucleic acid, hypoxanthine, xanthine, and guanine, among the nitrogenous soil constituents beneficial to plant growth.

The investigation of crude nucleic acid derivatives by Mockeridge reveal a stimulation in plant growth, an effect approximately proportional to the amount of material added (3). It was shown further that all the extracts of organic manures and peat which had a growth-promoting effect contained also the nucleic acid derivatives (4). Appreciable quantities of nucleic acid and derivatives in varying stages of decomposition were found in well-manured soil, leaf mold, fresh and well-rotted stables manures, and sphagnum (4). Bottomley and Mockeridge conclude that the greater the decomposition of the material, the greater is the resolution of the original nucleic acid into its free bases. The latter investigator presents the following table:-

(1) Loc. cit.

(2) Schreiner, O. and Skinner, J. J. "Nitrogenous Soil Constituents and Their Bearing on Soil Fertility". U.S.D.A., Bur. of Soils, (1912) Bull.87.

Schreiner, O., Reed, H. S., and Skinner, J. J. "Certain Organic Constituents of Soils in Relation to Soil Fertility". U.S.D.A., Bur. of Soils, (1907), Bull.47.

(3) Loc. cit.

(4) Loc. cit.

Compost	Nucleic Acid	Dinucleotide	Free Nitrogen Bases
Sphagnum	Fair amount	Small quantity	Fair quantity of adenine, guanine, cytosine, and uracil.
Surface peat	Less than above	More than above	More than above.
Deep peat	None	Less than surface peat	More than surface peat
Bacterised peat	Trace	None	Still more than above.
Leaf-mould	Very little	Small quantity	Fairly large amounts of four bases as above
Fresh stable manure	Small quantity	Practically none	Fair amounts of adenine, guanine, cytosine and uracil; small amounts of xanthine and hypoxanthine
Well-rotted stable manure	Practically none	Very small quantity	Rather more adenine and guanine than above; less cytosine and uracil; small amounts of xanthine and hypoxanthine.
Soil	Small quantity	Small quantity	Small amount of adenine; less guanine, xanthine, and hypoxanthine; still less cytosine and uracil.

Mockeridge states (1):-

"It would therefore appear, assuming that the whole effect is due to nucleic acid or its derivatives, that the free bases, individually or collectively, are of more value as growth-promoting substances than the nucleic acid".

This view is supported by the findings of Mockeridge with

(1) Loc. cit.

regard to the relative effects of autolyzed and autoclaved yeast. It is well known that yeast contains nucleic acid (1). In the case of the autoclaved yeast the nucleic acid had undoubtedly become very largely resolved into its derivatives, and because of the resistant nature of the nucleic acid the hydrolysis would probably have taken place in the autoclaved rather than in the autolyzed yeast, as in the former process the yeast was maintained at a temperature of 140° for fairly long periods, and in the latter at 35° for 72 hours. If this be so, then the autoclaved yeast should be the more effective agent of stimulation, and, as mentioned above, such proved to be the case.

Mockeridge also showed that purine and pyrimidine bases, phosphoric acid, and a carbohydrate, are all present in the *azotobacter* cell; all the necessary radicals, therefore, for the formation of nucleic acid. The growth-promoting effect of *Azotobacter*, *Rhizobium* and yeast, may be due to one or more of the nucleic acid radicals present, as all the materials investigated by Mockeridge having this effect on plant growth, also contained purine and pyrimidine bases. The effect of *Azotobacter* upon plant growth, contrary to the effect of the crude nucleic acid derivatives, was by no means commensurate with the quantity added, suggesting the possibility that the nucleic acid materials may function as actual nutrients, while the active principle of *Azotobacter* may partake of the nature of auximone. It is quite probable that the nucleic acid derivatives exert a decided

(1) Guilliermond, A. "The Yeasts". (1920); Trans. by Tanner, 57.

influence upon the plant, as indicated by the observations of Mockeridge of the effect upon the cell nuclei. Itano found that nucleic acid had a stimulating influence upon Azotobacter both in growth and nitrogen-fixing ability(1), and suggests that this accessory action would appear to be quite apart from the provision of a small quantity of food substance.

Nucleic acid and its derivatives, then, may be considered as essential food substances, for in their absence and provided with organic nutrients only, the plants "failed to maintain their original size and vigour, the new shoots successively formed becoming progressively smaller during the course of the experiment". It is possible that the "auximonic" effect may be attributed to certain of these nucleic acid substances.

Auximones have been considered vitamine-like largely for two reasons. First, because the effect, though increasing with the supplied, is not proportional to it; second, because the Azotobacter growth, presumably containing the true auximone, gives the blue color-reaction with the Folin-Macallum phosphotungstic acid preparation (2). This reaction has been used as a test for growth-accessory substances (3). According to Williams and Seidell (3) this test indicates the absence, and with less assurance the presence, of vitamine. These authors found, in attempting to purify the antineuritic substance or vitamine factor of yeast by recrystallization, that its

(1) Loc. cit.

(2) Folin, O. and Macallum, A. B. "On the Blue Color Reaction of Phosphotungstic Acid (?) with Uric acid and other Substances". Jour. Bio. Chem. (1912) 11; 265.

(3) Williams, R. R. and Seidell, A. "The Chemical Nature of the 'Vitamines'". Jour. Bio. Chem. (1916) 26; 431.

antineuritic properties were lost and the product proved to be identical with adenine. By suitable treatment these non-curative crystals acquire antineuritic properties and the ability to react positively to the Folin-Macallum test. In view of this consideration Mockeridge concludes that in the case of Azotobacter and Rhizobium, both of which give the blue color, the reaction may be due to the purine bases or to "some closely allied substance". In the light of this evidence, and also the relative thermostability of the substance, it would seem legitimate at present to consider auximones as essential food substances.

The work of Warington (1) suggests that boron may have a special function in the nutrition and development of plants. He found a small amount of boron necessary for the healthy growth of certain species of bean and clover.

Warington states:-

"The action of boron is presumably of a specific nature, since it appears to function in a different manner in different plants, possibly being in some cases an essential element, and in others of comparatively little importance. The relationship of boron to plant life is somewhat suggestive of that between vitamins and animals, and in various ways the resemblance appears to be very close. The main lines of agreement are:-

1. The comparatively small quantity of the substance required.
2. The unhealthy condition resulting from a deficiency of the substance.

(1) Warington, K. "The Effect of Boric Acid and Borax on the Broad Bean and Certain Other Plants". Ann. Bot., (1923) 37; 629.

3. The prevention of, or recovery from, the unhealthy condition by the addition of the substance.
4. The need for the supply of the substance to be maintained throughout life".

Warington would classify such substances as boron having a stimulating influence upon the plant as "accessory plant foods".

Puri notes the stimulating effect of ethyl and methyl alcohols upon plants at certain stages of growth (1).

Besides the micro-organisms mentioned above, yeast, Azotobacter, and Rhizobium, having a more or less definite relationship to the growth-accessory substances, there are other organisms which have received a great deal of attention from the standpoint of the accessory factor of media. These are the so-called hemophilic or "delicate" organisms. It has been known for some time that blood is necessary for the continued growth of certain bacteria (2), and that hemoglobin is the essential constituent in the blood on which growth depends. Work upon the growth accessory substances in blood has been done by several investigators, among whom Cole and Lloyd (3) (4), Davis (5) and Thj8tta and Avery (6) may be mentioned.

Lloyd found that blood contained an accessory factor or

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- (1) Puri, Amar Nath. "Effect of Methyl and Ethyl Alcohol on the Growth of Barley Plants". Ann. Bot. (1924) 38; 745.
 - (2) Pfeiffer. Zeitschr. f. Hyg. u. Infektionskrankh. (1893) 13; 357.
 - (3) Cole, S. W. and Lloyd, D. J. "The Preparation of Solid and Liquid Media for the Cultivation of the Gonococcus". Jour. Path. Bact. (1917) 21; 267.
 - (4) Lloyd, D. J. "On Vitamines, Amino Acids, and Other Chemical Factors Involved in the Growth of the Meningococcus". Jour. Path. Bact. (1916) 21; 113.
 - (5) Davis, D. J. "Food Accessory Factors (Vitamins) in Bacterial Culture with Especial Reference to Hemophilic Bacilli" I: Jour. Infect. Dis. (1917) 21; 392. II: Ibid., (1918) 23; 248.
 - (6) Thj8tta, T. and Avery, O. T. "Studies on Bacterial Nutrition". Jour. Exp. Med. (1921) 34; 97.

factors, the addition of which to the ordinary media is essential for the growth of the meningococcus. These substances were found to be present also in serum, milk and other animal fluids; and also in vegetable tissue.

Cole and Lloyd showed that in the cultivation of the gonococcus three factors are concerned:-

1. The concentration of hydrogen-ions.
2. The concentration of amino acids.
3. The presence of certain growth hormones or "vitamines" furnished by blood.

The important rôle played by blood, as well as other animal tissue and fluids, in the cultivation of these organisms is reflected in the so-called "hemoglobin" and "hormone" media which have been employed to furnish the accessory factor (1). At the outset there appeared to be two distinct factors operating in blood causing the stimulation in growth (2). One, the hemoglobin factor, seemed to be associated with the iron content and was thermostable. Hemoglobin alone would not support growth, however, but the growth of these organisms on hemoglobin media proved to be more abundant if mixed with other organisms. For example, large colonies of the influenza bacilli appeared when grown on a hemoglobin medium with Staphylococci, Streptococci, pneumococci, meningococci, diphtheria bacilli, chromogens,

(1) Hunteon, F. H. "Hormone Medium". Jour. Infect. Dis. (1918) 23; 169.

Bailey, S. F. "Hormone Mediums". Ibid. (1925). 36; 340.

(2) Davis, D. J. Loc. cit.

Cole and Lloyd. Loc. cit.

blastomycetes, sporotricha, and yeasts. This second factor, the tissue factor, was furnished by kidney, liver, spleen, brain, myocardium, testicle, lung and muscle of rabbit and guinea pig; also by tissue of potato and carrot. The tissue factor is thermolabile and alone does not support the growth of influenza bacilli.

Further investigation of the rôle of growth-accessory substances in the cultivation of hemophilic bacilli was carried on by Thjötta and Avery. They found that the accessory substances in blood essential to the growth of hemophilic bacilli are not destroyed by exposure to 100° C. for 10 minutes, but that after 30 minutes of autoclaving at 120°, the blood extracts are no longer capable of supporting the growth of the organisms. The substance or substances present in blood which are destroyed by heat can be replaced, according to these authors, by the addition of the growth accessory factor extractable from yeast. Neither the autoclaved blood extracts nor the yeast extract factor by themselves were capable of supporting the growth of B. influenzae. This led to the obvious conclusion that at least two factors of stimulation are present in blood, one thermostable, the other thermolabile. Thjötta and Avery call these factors "X" and "V" respectively. "X" substance is associated with the cell fraction of blood, and is absorbable from solutions of crystalline hemoglobin by bone charcoal. "V" factor appears to be vitamine-like, and is contained in blood corpuscles, yeast and vegetable cells. It is absorbed by charcoal and is required in greater concentration than "X" factor. Sterile, raw potato furnished both "V" factor and "X" substance

requisite for the growth of B. influenzae.

Ayers and Mudge (1) have shown that small amounts of fats and oils, (vegetable, animal and mineral) stimulate the growth of a pathogenic streptococcus. Other stimulating substances discovered by these authors are cabbage extract, glucose, and autolyzed yeast.

Davidsohn (2) has conducted a detailed investigation of the water-soluble growth-promoting factors, particularly with regard to a quantitative measure of the stimulation. He used as sources of the growth-accessory factor, orange juice, lemon juice, and the expressed juices of white cabbage (Weisskohl), rhubarb (Rhabarber), carrot, and tomato. According to Davidsohn, not all bacteria require the growth-accessory substances to the same degree, as the following results of stimulation in growth reveal:-

Stark bei	Mässig bei	Fehlend bei
Pyocyaneus	Diphtheriae	Geflügelcholera
Typhus	Staphylococcus	Crassus
Coli	Streptococcus	Cholera
Flexner	Friedländer	Tetragenus
Shiga		Melitensis
Gärtner		Milzbrand
Metschnikoff		Pneumococcus
Prodigiosus		Subtilis
		Pertussis
		Rotlauf

- (1) Ayers, S. H. and Mudge, C. S. "The Relation of Vitamines to the Growth of a Streptococcus". Jour. Bact. (1922) 7: 449.
 (2) Davidsohn, H. "Vitaminstudien". Biochem. Zeitschr. (1924) 150: 304.

Observation of the final results were made from ten to twenty-four hours at room temperature. with Serratia marcescens the maximum acceleration came at the end of forty-eight hours. The methods used to measure the stimulation in bacterial growth and activity were as follows:-

Plate method.

Counting chamber method (Zählkammermethode).

Volumetric method (centrifuged bacterial sediment).

Turbidity "

Reduction test.

In the opinion of the author the physiological activity of the organism is also influenced.

Gray and Chalmers (1) have shown that xylose and lignin, in certain concentrations, exert a marked stimulating effect upon the process of cellulose fermentation. They isolated a new aerobic cellulose-destroyer, Msp. agar-liquefaciens, which also attacks agar, and through a series of weighing experiments, showed that these substances produced a very considerable increase in the amount of cellulose broken down in ten days by this organism. Filter paper was used as the source of true cellulose in these experiments.

The investigations cited above should be sufficient to prove that there are substances, more or less well known chemically, which function as essential food substances in promoting growth and accelerating physiological activity on the part of micro-organisms. These substances are usually quite distinct from the food for growth or "building stone" of the organism. According to Eastwood (2), the stimulus causes the cell to

(1) Loc. cit.

(2) Eastwood, A. "Stimulants to Bacterial Variation". Jour. of Hyg. (1924) 23; 317.

function in a particular way, but is not incorporated as a part of the structure of the cell. He states:-

"At the same time it must be remembered that it is often impossible to draw a hard and fast line of demarcation between the two. Some physical or chemical agency may alter the characters of the available food supply, making the cell's task of assimilation either easier or more difficult; such an agency cannot be classed either as a stimulant or as a food, though it indirectly serves both purposes. A bacterium may, to begin with, be unable to break up and utilize a particular substance, e.g., lactose, but it may, by repeated sub-culture in a medium containing lactose, be 'trained' to ferment this substance; the lactose, then, has acted first as a stimulant and afterwards as nutrient material".

One of the chief characteristics of the stimulating substance is its ability in minute amounts to effect a great physiological acceleration on the part of the organism. In the words of Lillie (1):-

"The response is typically greater in extent, both in time and space, than the stimulus".

Numerous instances have been cited above, illustrating this feature of essential food substances. One additional example, from the work of Davidsohn, referred to above, will serve to emphasize this point. Within four hours, at 37°C, 0.044 cc. of orange juice, or 0.0025 gm. of dry substance accelerated the growth of the color organism in broth from 350 million

(1) Lillie, R. S. "Reactivity of the Cell". Sec.4. General Cytology. E. V. Cowdry, (1924) 188, Chicago.

cells, the inoculum, to a figure which doubled the bacterial numbers over the growth in broth without the addition.

The general problem of stimulation seems to be a true physico-chemical phenomenon, in which radiation, electrical conditions at the cell surface, molecular organization of protoplasm, surface tension, and absorption, all play significant rôles. The true nature of stimulation is unknown, and yet through the above physico-chemical media, considerable light is being shed upon such fundamental processes as photosynthesis, nerve physiology, and tissue differentiation. The theories regarding the physico-chemical nature of stimulation, the transmission of the physico-chemical influence, and the response of the irritable system, will not be considered further here, for such a treatise would carry the discussion far afield. Detailed treatment of these matters may be found in the references given below.

The rôle of the essential food substances in the nutrition and metabolism of the microbial cell has come to be a definite and fundamental principle. The elemental requirements of the cell, and the factors of media which foster growth, and increase or maintain virulence and physiological efficiency, are coming to occupy a prominent position in microbial investigation.

In conclusion the following references will serve to interpret more clearly the principle of stimulation. Bovie, whose work is cited above, states:-

"We might describe these results quite correctly if we stated that after killing an ameba by ultra-violet light, the surrounding amebas behaved as if we had released a bios or vitamine principle which stimulated them to greater activity" (1).

Gurwitch (2) has confirmed the existence of a specific "mitogenetic" radiation, giving rise to a mitogenetic factor which induces mitoses in onion tips from other similar root tips, over a distance up to 2 mm. Komuro (3) has obtained excessive cell divisions in root tips by means of X-ray. This immediately suggests the possibility of obtaining radiations from microbial cells, resulting in a stimulation of physiological activity.

In nature, it is probably true that physico-chemical agents are constantly carrying on processes of chemical activation, producing substances which hasten the speed of physiological change.

(1) Loc. cit.

(2) Gurwitch, A. "The Mitogenetic Rays". Bot. Gaz. (1925) 80; 224.

(3) Komuro, - "Preliminary Notes on the Cells of Vicia faba Modified by Roentgen Rays and Their Resemblance to tumor Cells". Bot. Mag. of Tokio, (1922) 36; 41. Jap. Jour. Bot. Abs., Tokio, (1923) 1; 37.

REFERENCES.

(p. 150)

- Lillie, R. S. "Reactivity of the Cell". Sec.4, General Cytology, E. C. Cowdry, (1924), Chicago.
- Bayliss, W. M. "Principles of General Physiology". (1920). London.
- Verworn, M. "General Physiology". (1899). London. Trans. by Lee.
- "Irritability". (1913). Yale Univ. Press.
- Bovie, W. T. "Relation between Physiological Dominance and the Biological Effects of Rays". 25th Ann. Meeting, Amer. Roentgen Ray Soc., (1924).
- Bovie, W. T. "Effect of Sunlight on Growth and Development". The Scientific Monthly, (1925). 21; 70.
- Barr, C. E. "A Stimulating Effect of the Photocytolytic Products of Protoplasm". Jour. Med. Res. (1923) 44; 81.
- Barr, C. E. and Bovie, W. T. "Ultraviolet Cytolysis of Protoplasm". Jour. of Morph. (1923) 38; 295.
- Bovie, W. T. "The Localization of the Physiological Effects of Radiation Within the Cell". Jour. Med. Res. (1918) 39; 265.
- Warburg, O. "Beiträge zur Physiologie der Zelle insbesondere über Oxydationsgeschwindigkeit in Zellen". Ergebn. d. Physiol. (1914) 14; 253.

EXPERIMENTAL PROCEDURE.

In the present investigation the optimum conditions for the growth and fermentative activity of cellulose-destroying micro-organisms are determined. The problem is intimately concerned with the elemental requirements of the cell. A nutrient salt medium containing the essential elements for the cell structure of a large number of soil organisms has been chosen. Besides the chemical elements, certain other factors of the medium are under control. Micro-organisms, moisture and temperature conditions, H-ion concentration, and oxygen supply are, as far as possible, all known factors. As the following experiments will show, this basic nutrient medium, in itself, lacks energy food for the organisms under investigation. This is illustrated, not only by the growth curves, but also by the curves showing the H-ion concentration changes. The following table proves that C. folia is unable to maintain itself in the nutrient medium. Platings were made every four hours using nutrient agar (P_h 7.60).

TABLE I.

The Fate of C. folia in the Basic Nutrient Medium.

Hours	Bacterial Numbers	Hours	Bacterial Numbers
Initial	35,000,000	24	50,000
4	6,000,000	28	20,000
8	2,500,000	32	10,000
12	1,000,000	36	5,000
16	500,000	40	5,000
20	100,000	44	1,000
		48	1,000

The presence or absence of C. folia or Act. colorata does not seem to have any appreciable effect upon the H-ion concentration changes taking place in the medium. It has been shown that these changes are largely chemical. With the introduction of cellulose to the medium, a source of energy for cellulose-destroyers is provided. It is the presence of cellulose which is responsible for any additional increase in the concentration of H-ions during the fermentation of cellulose by C. folia and Act. colorata. As stated above this method furnishes an index to the rate of cellulose decomposition and also a test of the physiological efficiency of these organisms.

One of the foremost factors influencing the cellulose-destroying ability of micro-organisms is the essential food factor, operating conspicuously in nature, and causing stimulation and activation of physiological efficiency. The investigation is set forth in the following experiments, the goal of which is to bring about an artificial fermentation of cellulose of maximum efficiency.

1. Physiological Studies of the Accessory and Stimulating Factors of Certain Media.

2. Physiological Studies of Association.

3. Rôle of Essential Food Substances in Soil.

1. Physiological Studies of the Accessory and Stimulating Factors of Certain Media.

- (a) Loss of Physiological Efficiency on the part of C. folia.

In nature, micro-organisms are physiologically efficient. The maintenance of this effective activity is a function of nature's

medium. Due to certain artificial procedures, as sterilization by heat and the use of drastic chemical reagents, in attempting to control the unknown factors for laboratory investigations, considerable change is usually effected in the general character of the medium. Apparently the important factors so affected are the essential food substances. In order to aid in establishing this point, C. folia was cultivated for nine months, with weekly transfers, upon nutrient (beef-peptone) agar (P_h 7.60). During this time the organism became accustomed to this medium, and soon showed marked ability to split products of protein hydrolysis, with the formation of ammonia. At the end of nine months, C. folia refused to attack cellulose in any form; in other words, the organism had suffered a loss in physiological efficiency, but had acquired other and entirely different physiological proclivities. It is evident that nutrient agar offered C. folia a wholly artificial environment, without any form of true cellulose present, and probably deficient in essential food substances because of the high temperatures and filtration processes used in its preparation.

(b) The Effect of Autoclaving as Revealed by the Activity of C. folia and Act. colorata upon Leaves.

In accord with one of the fundamental principles of pathology, namely, that pathological secretions and animal passage not only preserve virulence, but also develop that property in microorganisms (1), the attempt was made to produce a medium resembling as closely as possible that of nature, in the hopes of eventually

(1) Gurney-Dixon, S. Loc. cit.

re-developing in C. folia the ability to ferment cellulose. In the preparation of this medium, the artificial procedures referred to above were, as far as possible, eliminated. The present experiment was carried on in order to determine the significance of a process such as autoclaving upon the activity of micro-organisms.

Inasmuch as C. folia and Act. colorata were isolated from an active decomposition of leaves in compost, leaves were chosen as the basis for the "leaves medium" described below. Hand-picked maple leaves from the vicinity of the compost, were employed. These were allowed to dry thoroughly at room temperature. Some of the leaves were sterilized in the autoclave for twenty minutes at fifteen pounds pressure in liter flasks containing the basic nutrient solution. Other portions were immersed in sixty per cent. ethyl alcohol for forty-eight hours followed by copious washing with sterile water. The treatment with alcohol proved to be an efficient means of sterilization. The leaves remained sterile in about sixty per cent. of the cases. To ascertain this point, the leaves in two gram quantities were incubated in liter flasks containing 200 c.c. of nutrient solution. Incubation took place at 27° for forty-eight hours. The flasks which remained sterile received inoculations from a mixed culture composed of the two cellulose-decomposing organisms, C. folia and Act. colorata. From time to time during the fermentations microscopic examinations were made of the

medium for contaminating forms. In every case, however, there proved to be no contamination.

Determinations of the hydrogen-ion concentrations (1) produced by the apparent destruction of cellulose, as well as other associated substances in both series, were made at frequent intervals. In the control flasks, without the organisms, the P_h change was very slight. The results are shown below:-

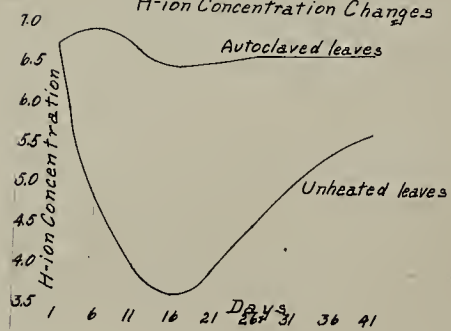
TABLE II.

Effect of Autoclaving as Revealed by the Activity of C. folia and Act. colorata upon Leaves.

Days	Unheated leaves P_h	Autoclaved leaves P_h	Control change of P_h in medium.
Initial	6.80	6.80	6.80
2	6.30	6.85	6.80
4	5.45	6.95	6.80
6	4.87	7.00	6.90
8	4.50	6.95	6.90
10	4.18	6.84	-
12	3.90	6.70	-
14	3.75	6.60	-
16	3.70	6.55	-
18	3.74	6.54	-
20	3.90	6.57	-
22	4.10	6.65	6.94
24	4.35	6.67	-
26	4.55	6.69	-
28	4.75	6.70	-
30	5.00	6.70	-
32	5.20	6.72	-
34	5.35	6.73	-
36	5.48	6.73	-
38	5.60	6.74	-
40	5.70	6.75	7.0

(1) The electrometric method described by Itano was used. Jour. of Bact. (1923) 8; 521.

Graph I
Effect of Autoclaving as revealed
by
H-ion Concentration Changes



From the above experiment it becomes immediately apparent that there is a marked difference in the activity of the organisms upon the leaves in the two cases. This may be shown not only by comparing the changes in H-ion concentration, but also by the differences exhibited in the growth of the organisms, and the comparative ease with which the unheated leaves are attacked.

Of course, heating may so alter the chemical nature of the leaves that they are rendered more resistant to the action of the cellulose destroyers. At the same time it is possible that in the unheated material an essential food factor is operating which may exert an influence upon the physiological efficiency of these organisms. This factor, if thermolabile, would not be present in the autoclaved leaves. The presence of a thermolabile water-soluble essential food substances will be investigated later.

(c) Recovery of the Physiological Efficiency of C. folia.

The value of the unheated, sterile "leaves medium" as a favorable environment for the maintenance and development of cellulose-decomposing ability was investigated using the culture of C. folia which had lost its physiological efficiency. The organism had been cultured upon nutrient agar for nine months as described above. Two cultures of C. folia were prepared. One consisted of this attenuated culture in basic nutrient solution, to be referred to as culture "A". C. folia.

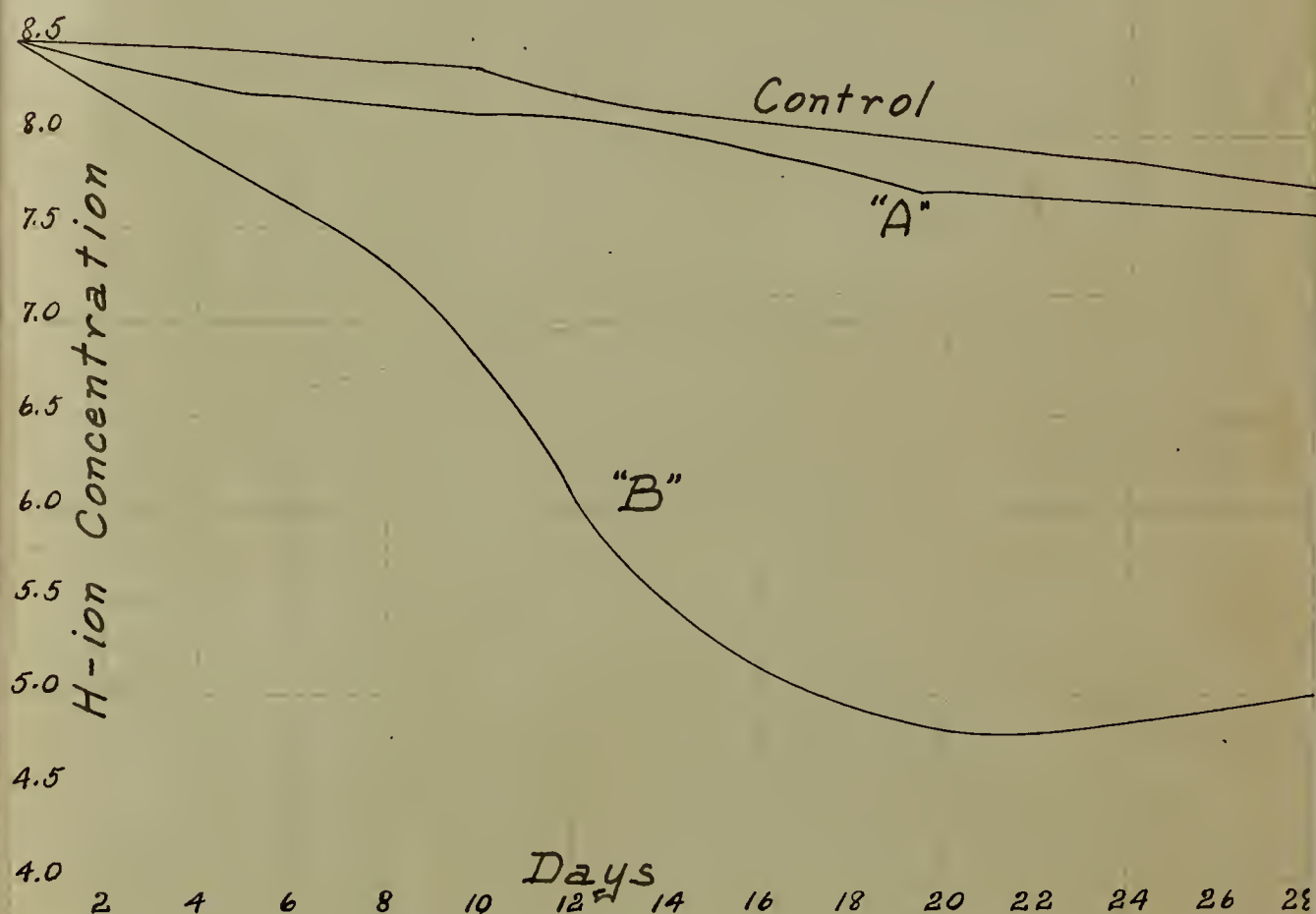
in this physiologically weakened condition, was also cultivated upon the "leaves medium", prepared as described above. As before, care was taken to insure sterility by preliminary incubation and frequent microscopic examinations. The organism was kept upon this medium for four or five weeks, with weekly transfers. The final transfer, which was allowed to incubate from seven to ten days, will be spoken of as culture "B". These cultures were used to inoculate liter Erlenmeyer flasks, each containing 200 c.c. of the basic nutrient solution and three grams of cellulose in the form of raw cotton. The flasks were incubated at 27°. The changes in hydrogen-ion concentration during the decomposition of the cellulose were determined. The results are given in the following table and graph. The control flasks contained the nutrient solution without cellulose.

TABLE III.

Changes in the H-ion Concentration of medium during the decomposition of Cellulose.

Days	A P _h	B P _h	Control without cellulose P _h
Initial	8.50	8.50	8.50
2	8.38	8.20	8.47
4	8.26	7.90	8.45
6	8.20	7.61	8.40
8	8.15	7.28	8.33
10	8.10	6.77	8.34
12	8.08	6.02	8.20
14	8.00	5.50	8.12
16	7.90	5.15	8.06
18	7.80	4.94	8.02
20	7.70	4.82	7.95
22	7.66	4.80	7.90
24	7.63	4.87	7.84
26	7.60	4.94	7.78
28	7.58	5.00	7.73

Graph II
Changes in H-ion Concentration during
"Cellulose Decomposition"



It is evident that cultivation upon the "leaves medium" causes a restoration and development of the physiological efficiency of C. folia to a marked degree. Some significant factor is apparently operating in the untreated leaves which exerts a considerable influence upon the organism.

(d) The Influence of a Leaf Compost Extract upon the
Rate of Cellulose Decomposition by C. folia.

Subsequent investigation of the leaf compost demonstrated the influence of the factor further. The latter, though thermolabile, is readily obtained in the form of an aqueous extract by filtration of an aqueous suspension of the compost through a sterile porcelain filter. The filtrate was found to exert a marked influence upon the efficiency of cellulose-destroying micro-organisms in laboratory experiments. A medium was prepared having the compost as a basis. This material had been autoclaved for fifty minutes at fifteen pounds pressure. A sheet of English filter paper, 11 cm. in diameter and sterile, was pressed against the surface of the compost. The inoculum consisted of a twenty-four^{hour} nutrient solution culture of C. folia. Two cubic centimeter portions of the filtrate were added to a series of flasks containing this medium. Controls were carried at the same time. In the presence of the filtrate complete destruction of the filter paper took place in four weeks, not a trace of the paper remaining. In the control flasks, without

the filtrate, the decomposition was considerably slower and the filter paper was never wholly destroyed during the entire experiment which lasted three months.

In the unheated sterile compost extract some essential food substance is operating, which increases the physiological efficiency of C. folia. This factor is probably present in the untreated plant tissue. During the various microbiological activities which accompany decomposition processes, the accessory factor may be liberated, causing an increase in the rate of cellulose fermentation. This is a fairly accurate and logical conclusion, as later experiments will prove. At the same time, some of the accessory factor present in the compost may come from the microbial cell, liberated as a result of extra- or intra-cellular processes. This possibility, too, will receive consideration later. At present, it is safe to conclude that some factor or factors present in untreated, sterile plant tissue cause a stimulation in the cellulose-decomposing ability of C. folia. These factors, because of their unknown character but definite accessory and activating influence, are denominated essential food substances in this investigation. In the next experiment a commercial preparation of the so-called vitamine B is tested for its stimulating effect.

(e) The Influence of Vitamine B (?) upon the Growth and
Physiological Efficiency of C. folia.

The preparation of vitamine employed (1) was Vitamine-Harris.

(1) Vitamine B (?), Yeast Vitamine, prepared by the Harris Laboratory, Tuckahoe, New York.

the highly-concentrated vitamine, water-soluble-B (fraction 11), described by Osborne and Wakeman (1), and is prepared from fresh fermentations of beer or ale. The yeast is washed to remove excess of beer and soluble products present in the fermentation mass. There may, of course, be small amounts of soluble substances present from the cereals and other food materials used in preparing the fermentation. The preparation consists largely of the intra-cellular content of the yeast cell, and while it is free from native protein, not giving the color reactions for proteins, it does contain nitrogenous derivatives of the yeast proteins, such as amino acids (2). The potency of this Vitamine preparation has been determined by Bailey (3) in a series of biological tests upon young albino rats. The report makes the following statement:-

"In all tests with this preparation the growth of the animals was very conspicuous. The quantities fed have been in accordance with the uniform plan of other tests, but its potency is not properly evaluated in any of these trials since normal growth was secured with the smallest dosage, 25 mg. fed. It is fair to presume that growth would have been secured with substantially less than the minimum quantity which we have used."

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- (1) Osborne, T. B. and Wakeman, A. J. "Extraction and Concentration of the Water-Soluble Vitamine from Brewers' Yeast. Jour. Biol. Chem. (1919) 40; 383.
 - (2) Yeast Vitamine, Harris Laboratory, Bull.14.
 - (3) Bailey, E. M. "The Potency of Some Commercial Vitamine Preparations as Compared with that of Dry Brewers' Yeast". Conn. Agr. Exp. Sta. Bull. 240, (1922).

Itano obtained positive results in his investigation of the influence of vitamine upon azotobacter (1).

TABLE IV.

Growth of azotobacter and Nitrogen Fixed.

Media	Number of organ- isms per c.c.		N per 100 c.c.	
	Initial	after 10 days	Initial	after 10 days
		millions	mgm.	mgm.
Ashby plus Vitamine B (?)	19,000	1,550	0.2	15.0
Plain Ashby	19,000	450	0.2-	5.7

He used the same preparation of vitamine that is employed in this investigation and the one described above.

A one per cent. solution of the yeast vitamine powder, in which vitamine B is highly concentrated, was prepared, and from this a series of vitamine dilutions was set up. One cubic centimeter portions of these dilutions were added to tubes of nutrient solution to give the dilutions indicated below. The inoculum consisted of 0.1 c.c. of a twenty-four hour nutrient solution culture of C. folia.

(1) Itano, A. "Physiological Study of azotobacter chroococcum"
Jour. of Bact. (1923) 8; 483.

Concentration of Vitamine.

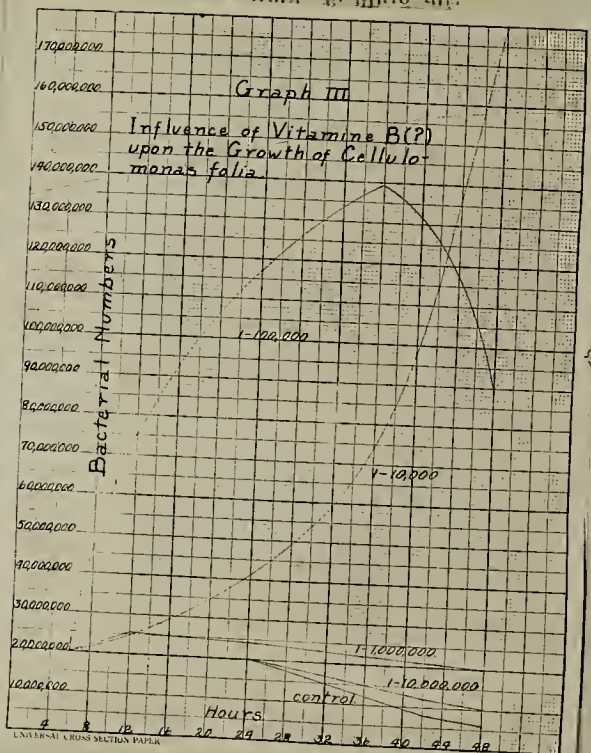
	Control	1-1,000	1-10,000	1-100,000	1-1,000,000
Vitamine	None	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.
Inoculum	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.
Nutrient medium	9.9 c.c.	8.9 c.c.	8.9 c.c.	8.9 c.c.	8.9 c.c.
	10.0 c.c.	10.0 c.c.	10.0 c.c.	10.0 c.c.	10.0 c.c.
Total dilution		1-10,000	1-100,000	1-1,000,000	1-10,000,000

Preliminary tests based upon visual observations of turbidity showed positive results only in dilutions 1-10,000 and 1-100,000 after 24 hours' incubation at 30°.

TABLE V.

Influence of Vitamine B (?) upon the Growth of C. folia.
(Expressed in Millions).

Hours	Control No Vitamine	1-10,000	1-100,000	1-1,000,000	1-10,000,000
6	20	20	20	20	20
12	20	25	75	25	20
18	20	32	100	25	20
24	20	41	117	24	20
30	16	54	130	23	18
36	12	74	139	22	15
42	8	115	126	21	12
48	6	180	90	20	10



The above figures show that vitamine dilutions 1-10,000 and 1-100,000 exert a marked stimulating influence upon the growth of C. folia. Platings were made every six hours, using nutrient agar P_n 7.6. This experiment was repeated several times, and in each case a certain inhibitory influence was manifest in the highest concentration of vitamine during the early part of the experiments. This inhibition was noted also by Itano. The stimulation ^{soon} / became evident, however, and reached greater proportions than the very next lower concentration of vitamine, 1-100,000. In the latter case no inhibitory effect was apparent. These related phenomena of stimulation and inhibition have been well worked out by physiologists (1), and the following quotation from Verworn brings out a principle bearing upon the present problem:-

"It has been seen in a previous section that phenomena of depression can be called out by over-stimulation. This fact is important, for it shows that the same stimuli which with slight intensity or short duration produce excitation, with increased intensity or long duration can produce precisely the opposite effect, namely, depression".

Thus far, hydrogen-ion concentration changes have served as a satisfactory index of the process of cellulose decomposition. Based upon such evidence it is often possible to draw legitimate conclusions regarding the physiological efficiency of C. folia.

(1) Verworn, M. "General Physiology". Trans. by Lee. (1899). 469.

With the addition of growth-accessory substances, however, another factor is introduced which must receive due consideration in the interpretation of results. For example, according to the Harris laboratory, the vitamine preparation contains small amounts of soluble salts, sugars and inorganic substances. The presence of these unknown constituents would have a profound effect upon bacterial action, hence the difficulties attending the use of this preparation in H-ion determinations will be appreciated, particularly when the highly buffered character of such a medium is considered. With the introduction of vitamine into the nutrient solution, the medium becomes very complex and not adapted to the hydrogen-ion technique in its present application. The increase in the rate of cellulose decomposition when vitamine is added is apparent, however, and hydrogen-ion determinations revealed something of the stimulation in the physiological efficiency of the organism. At the same time, due to the complexity of the medium, it is difficult to draw accurate conclusions regarding the increase in the concentration of H-ions resulting from the splitting of cellulose because of the various unknown factors of nutrition operating such as the sugars referred to above. In fact, some other criterion of the process would probably be more satisfactory in this case. The fermentation flasks were set up as in experiment (b), using raw cotton, and suitable concentrations of vitamine in conjunction with the nutrient solution. Microscopical examination of the cotton fibers showed evidence of more active

destruction of cellulose than in the control flasks containing no vitamine. The swelling and dissolution of the cell walls was readily discernible. Stimulation in growth and physiological efficiency was apparent in vitamine dilutions 1-10,000 and 1-100,000. These results are in accord with those obtained by Itano (1) in testing the influence of vitamine upon the growth of Azotobacter.

A stimulative influence is apparent in this intra-cellular product of the yeast cell, and again the true nature of the specific activating agent is unknown. It is called vitamine B, but the more inclusive term, essential food substance, would probably be the more desirable from the standpoint of this investigation.

The purpose of the next experiment is to test extracts from various seeds and seedlings for the presence of essential food substances. This work is similar to the line of investigation of Miller and his associates with regard to "bios" (2).

(f) The Influence of Extracts from Seeds and Seedlings upon the Growth and Physiological Efficiency of C. folia.

Extracts were prepared from a representative each of the Leguminosae, Gramineae, and Polygonaceae. Alfalfa, barley and buckwheat seeds were used, and extracts prepared from the seeds before and after germination; also from the seedlings (3).

(1) Loc. cit.

(2) Loc. cit.

(3) Method used by Thj8tta and Avery, Jour.Exp.Med., (1921) 34; 97.

Ungerminated Seeds.

A weighed quantity of seeds was sterilized for two minutes in mercuric chloride (1-500), and washed thoroughly in sterile water. The seeds were ground in a mortar, and one per cent. aqueous extracts made. The reactions of the latter were adjusted to P_h 4.6, and each extract was boiled for ten minutes (1).

Germinated Seeds.

The seeds were washed and sterilized as described above, then transferred under aseptic conditions to a moistened filter paper in sterile petri dishes. After forty-eight hours' incubation at 27° , during which time most of the seeds germinated, a number of the seeds were transferred under aseptic conditions to sterile weighing bottles, the weights of which had been previously ascertained. The seeds were dried over sulphuric acid until they attained a constant weight, then ground to a powder in a mortar and one per cent. aqueous extracts prepared. The reactions of the latter were adjusted as before followed by boiling for ten minutes.

Seedlings.

The seeds were sown in pots containing sandy loam and grown in the greenhouse under suitable temperature and moisture conditions for a week or ten days.

(1) The extracts in this experiment were subjected to a short autoclaving, 15 pounds for 10 minutes, instead of boiling over free flame as was done in the case of the other extracts prepared. The work of Wildiers and others show "Bios" to be unaffected by boiling in an acid environment, although strong acid destroys it.

	alfalfa	Barley	Buckwheat
Length of time grown	10 days	7 days	10 days
Length of shoot	1-1.5 cm.	2-3.5 cm.	2-3.5 cm.

The entire seedling was removed from the pot and washed free of soil particles. The seedlings were sterilized as in the case of the seeds, washed and dried. One per cent. suspensions were prepared as before. The following table shows the influence of these extracts upon the growth of C. folia.

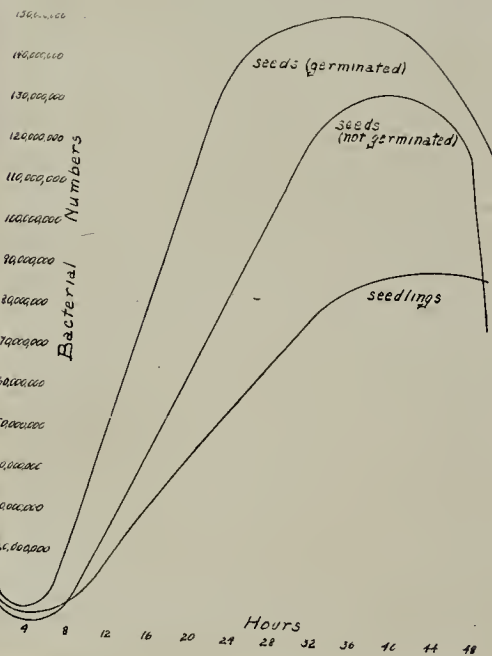
TABLE VI.

Influence of the Extracts upon the Growth of C. folia. (#)

Alfalfa, Barley and Buckwheat Extracts (1-10 dilution) Total dilution (1-1,000) Expressed in Millions.									
Hours	Seeds	alfal- fa Germ-Seed- inated lings seeds	Seeds	Barley Germin- ated seeds	Seed- lings	seeds	Buckwheat Germinat- ed seeds	Seed- lings	
Initial	35	35	35	35	35	35	35	35	35
4	3	6	5	5	9	16	20	26	24
8	8	24	8	12	16	31	30	38	30
12	29	60	20	24	14	53	44	60	42
16	49	92	34	37	17	74	57	84	54
20	70	127	47	50	26	98	71	106	66
24	92	145	59	63	43	120	85	130	77
28	113	150	71	71	86	132	99	146	94
32	129	153	82	79	141	146	113	162	111
36	134	152	88	87	194	159	123	180	128
40	133	147	91	96	250	172	129	196	145
44	127	136	92	105	302	165	132	212	162
48	78	120	90	114	360	200	132	230	180

(#) The "control" for this experiment is given in Table 1.

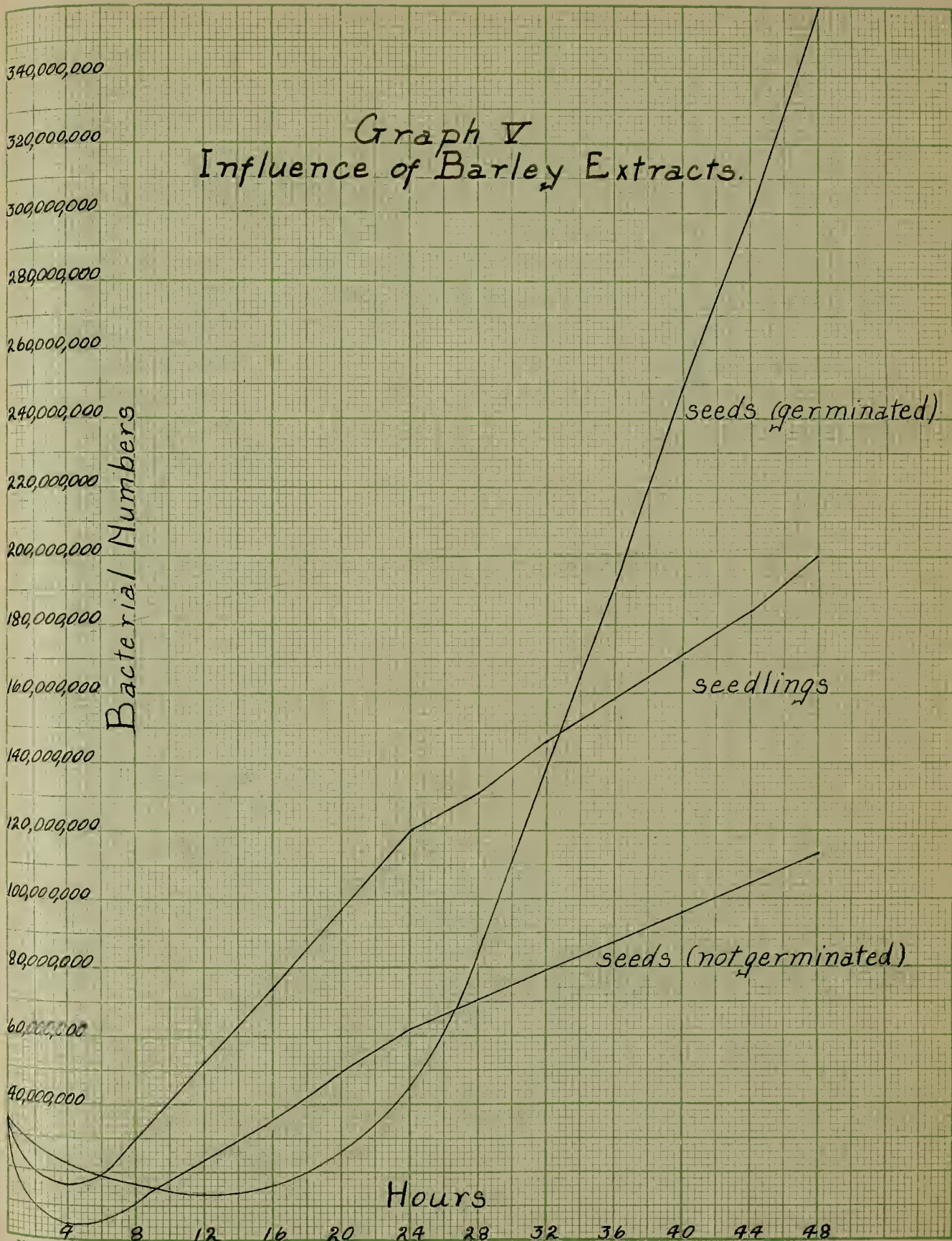
Table IV Influence of Aitai Extract



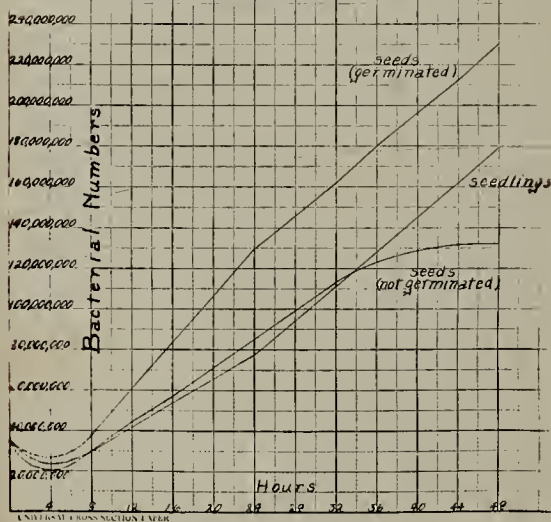
Graph V
Influence of Barley Extracts.

Bacterial Numbers

Hours



Graph VI
Influence of Buckwheat Extracts.



The above table shows the increase in bacterial numbers under the influence of the extracts. The figures recorded apply to an extract dilution of 1 to 10. Other dilutions were employed, namely, 1 to 1,000, 1 to 100,000, and 1 to 1,000,000, but the acceleration in growth became much less apparent.

The stimulating effects of the extracts upon the physiological efficiency of C. folia were readily determined by the H-ion method.

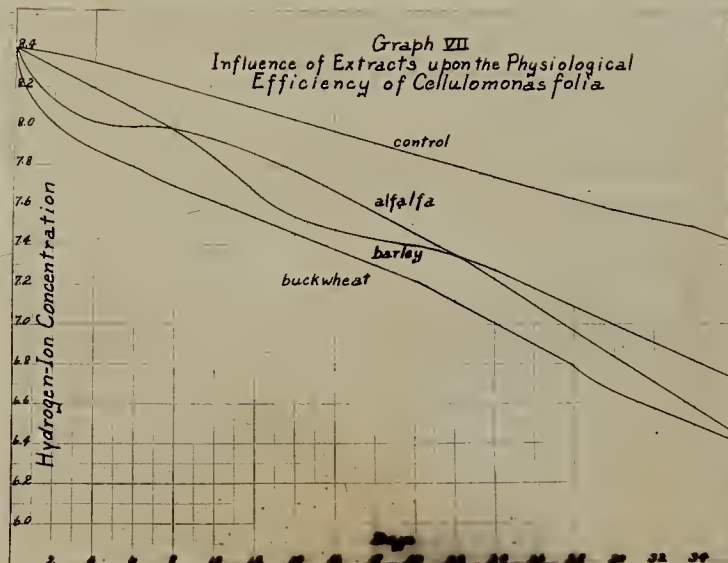
The same general procedure was followed as in previous experiments. Raw cotton was used as a source of true cellulose, and 0.05 per cent. of the active principle of the extracts was added to the nutrient medium. The control represents the action of the organism upon cellulose without the addition of the extracts. The action of C. folia upon nutrient solution containing the same concentration of extract as in the other flasks only without cellulose, was determined. No appreciable change in the H-ion concentration of the medium was observed over the normal changes exhibited by the nutrient solution itself as recorded in the "control" column, Table II.

TABLE VII.

Influence of Alfalfa, Barley and Buckwheat Extracts upon the Cellulose-Decomposing Ability of C. folia.

Days	Control (no extract) P _h	Alfalfa Extracts P _h	Barley Extracts P _h	Buckwheat Extracts P _h
Initial	8.40	8.40	8.40	8.40
2	8.37	8.14	8.30	8.02
4	8.33	8.02	8.20	7.88
6	8.27	8.00	8.10	7.80
8	8.21	8.00	7.98	7.70
10	8.16	7.94	7.84	7.62
12	8.10	7.86	7.67	7.54
14	8.04	7.78	7.54	7.46
16	7.98	7.68	7.48	7.40
18	7.92	7.57	7.44	7.30
20	7.86	7.46	7.40	7.22
22	7.81	7.36	7.36	7.12
24	7.75	7.24	7.28	7.01
26	7.70	7.10	7.18	6.90
28	7.64	6.97	7.09	6.79
30	7.58	6.84	7.00	6.66
32	7.53	6.71	6.91	6.57
34	7.50	6.58	6.82	6.48
36	7.42	6.44	6.72	6.40

Graph VII
Influence of Extracts upon the Physiological
Efficiency of *Cellulomonas folia*



The extracts exerted a marked stimulative influence upon the growth and physiological efficiency of C. folia. In general, the extracts from the germinated seeds exhibited a greater stimulative effect than the extracts from the ungerminated seeds and seedlings as far as growth is concerned.

These differences in the degree of stimulation may be accounted for, at least in part, on the basis of change in the character and amounts of essential food substances present at various stages in the development of the seedling (1). As far as physiological efficiency is concerned, there seem to be no measurable differences in the stimulations obtained by the various extracts.

C. folia may find sufficient food for growth in the extract alone, but the significant fact is that these extracts probably contain essential elements which stimulate the physiological activity and growth of the organism, whether they be of the nature of vitamins, bios, or essential food nutrients. Nevertheless, the physiological activity of C. folia in a medium composed of nutrient solution and extract, without cellulose, is negligible, as far as H-ion concentration change is concerned. In the presence of cellulose, however, the stimulative effect of the extracts is marked. Consistent results were obtained in several control experiments substantiating the above figures. Stimulation was obtained in growth and in cellulose-decomposing ability in extracts containing 0.1 per cent. up to 0.001 per

(1) Miller, W. L. Loc. cit.

cent. of the active principle of the extracts.

(g) Discussion.

The above experiments show clearly that there are factors in nature which cause appreciable stimulation in the growth and physiological efficiency of cellulose-destroyers. These factors, present in plant tissue which has not been exposed to the drastic procedures of the laboratory, may be called essential food substances. This is a generous and inclusive term, though of necessity rather indefinite. These substances undoubtedly exert considerable influence upon the decomposition of cellulose in nature. This influence will be dealt with more in detail later under the heading, "Rôle of Essential Food Substances in Soil".

It is not the purpose of these experiments to convey the idea that the essential food substances or the accessory factors operating in plant tissues are identical with vitamine B. They may or may not be similar substances, though both have in common the accessory influence. On the other hand, until the nature of the vitamine or bios factor is more definitely known, who can judge whether accessory action or stimulation is due to food substances, making the problem one of food requirement; or to the presence of a minute quantity of some complex organic compound; or even to some force not yet sufficiently recognized? As in the case of Gray and Chalmers' findings (1), the stimulation may be due to the presence of some definite chemical compound, such as xylose or lignin.

(1) Loc. cit.

These accessory factors, referred to in this investigation as essential food substances, play an important rôle as constituents of media in which maximum physiological activity is the goal.

2. Physiological Studies of Association.

Besides the artificial features mentioned above, characteristic of laboratory investigations of natural phenomena, the influence of another factor of great importance in nature is often neglected. This factor has to do with the mutual relations of micro-organisms, and the general term "association" is applied to this type of influence.

Nature works largely with mixed cultures and in investigating these same processes of nature, the general tendency is to ignore the association factor, largely because of its complexity, and to attempt to secure results with pure cultures. In certain cases pure cultures do yield results, and the physiological efficiency of the species concerned is beyond question. In the recovery or maintenance of physiological efficiency it has been shown that essential food substances play a significant rôle. It is probable, too, that in microbial associations this same factor may be intimately concerned.

It is extremely difficult to classify types of association and no such attempt will be made in this discussion. Pfeffer's classification (1), including "Conjunctive symbiosis" and

(1) Pfeffer, W. Handbuch der Pflanzenphysiologie, (1897).
Bd. I, Leipzig.

"Disjunctive Symbiosis", which permits of much careful analysis and subdivision, would probably not fulfil the need effectively. There is a certain indefiniteness and vagueness in the interpretations which may be placed upon it, and with the increasing complexity of the problem of association, there is great need for decisive and exact construction. The case of the ultramicrobe, for example, parasitic on bacteria, but exercising a protective action within the body; how is one to interpret this symbiotic relationship suggested by d'Herelle (1) ?

For the present, it may be well to revert to the chapter on media and review the external conditions of life. There one finds that the general conditions for the growth of an organism, food supply, oxygen supply, temperature, moisture, hydrogen-ion concentration, essential food substances, are all elements or factors of the medium. Changes and alterations in these conditions may constitute a stimulating or inhibiting factor in the growth and physiological efficiency of a micro-organism. Now the influences wrought through association may be explained and determined to a large extent from this point of view. Marshall states:-

"If associations of micro-organisms dependent or otherwise are subjected to analysis, there may be traced through them all some functional factor or principle as temperature, oxygen supply, food supply or condition of food (whether acid or alkaline, whether dry or moist, whether composed of

(1) d'Herelle, F. "Immunity in Natural Infectious Disease". (1924). Balt. 79.

one class of elements or another) or the production of metabolic products" (1).

It would seem possible to approach the subject of classification of microbial associations from the standpoint of the medium.

There is evidence that association among micro-organisms of the same strain as well as of different species often resolves itself upon analysis into a problem of essential food substances (2). The food supply of the medium undergoes some change resulting in the formation of substances which stimulate physiological activity. These may be products of metabolism, extracellular or intracellular, or substances formed in the medium due to alteration of its chemical constituency. Investigation of the influence of association upon the cellulose-destroying micro-organism reveals the fact that there is a definite stimulation in the rate of fermentation, and that this is due to the essential food factor. The results of the present work indicate that the effect of association is very similar to the activation obtained in the previous experiments where a small amount of essential food substance was added.

One of the most prominent organisms which has been employed in association experiments is Azotobacter, significant because of its ability to synthesize the growth-accessory or

(1) Marshall, C. E. "Microbial Associations". Science, (1915), 41; 306.

(2) Gurney-Dixon, S. "The Transmutation of Bacteria". (1919), Cambridge, 22.

Eastwood, A. "Stimulants to Bacterial Variation". Jour. of Hyg. (1924) 23; 323.

essential food factor. Hunter (1) has shown that Azotobacter is capable of synthesizing an accessory factor similar to vitamin B. He also showed that this organism exhibits growth-promoting properties and curative effects in experiments with rats and pigeons. Mockeridge (2) noted the growth-promoting effect of Azotobacter and Rhizobium upon green plants.

The work of Hutchinson and Clayton (3) indicates that breakdown products of cellulose-decomposition stimulate the process of nitrogen assimilation by Azotobacter, and therefore function as essential food substances. They calculated that the fixation of nitrogen per gram of mannite supplied was equal to 3.18 mgms., while that per gram of cellulose actually decomposed was no less than 19.3 mgms.

The results obtained by Fringsheim have already been referred to (4). He also showed that cleavage products resulting from the methane and hydrogen fermentations of cellulose may function as essential food substances

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- (1) Hunter, O. W. "Production of Growth-Promoting Substance by Azotobacter". Jour. Agr. Res., (1923) 23; 825.
 - (2) Mockeridge, F. A. "The Formation of Plant Growth-Promoting Substances by Micro-organisms". Ann. Bot. (1924) 38; 723.
 - (3) Hutchinson, H. B. and Clayton, J. "On the Decomposition of Cellulose by an Aerobic Organism (Spirochaeta cytophaga, n. sp.)". Jour. Agr. Sci., (1919) 9; 143.
 - (4) Fringsheim, H. "Ueber die Verwendung von Cellulose als Energiequelle zur Assimilation des Luftstickstoffs". Cent. f. Bakt., etc. (1909) 23; 300.
"Weiteres über die Verwendung von Cellulose als Energiequelle zur Assimilation des Luftstickstoffs". Ibid., (1910) 26; 222.

for nitrogen-fixing organisms. Small concentrations of certain carbohydrates caused stimulation in nitrogen assimilation, but the most marked accessory influence was exerted by the products of cellulose fermentation.

These activating substances caused greater stimulation in the lower concentrations, which allies them definitely with the essential food substances. The essential elements may be present in suitable media, but if conditions are not favorable in this regard, they may be supplied through association. As in Pringsheim's work, such substances may be products resulting from the splitting of certain constituents of the medium by the associate, or they may be metabolic products from the microbial cell, as in the case of Azotobacter.

Because of the intimate relationship between Azotobacter and the essential food factor, and because of the intimate connection between nitrogen assimilation by Azotobacter and cellulose fermentation in the active breakdown processes of the soil, this organism was chosen for these experiments. Preliminary experiments showed that Act. colorata, an associate of C. folia in nature, exerted a stimulating action upon the latter organism. The Actinomyces, too, was selected for further study of association. Three additional species, not cellulose-destroyers, were isolated from soil in which active decomposition was taking place. These also

revealed a stimulating action upon the cellulose-decomposing ability of C. folia, and have been identified as B. mycoides, B. subtilis and B. cereus.

(a) The Influence of Association with Act. colorata upon the Growth and Physiological Efficiency of C. folia.

The same general procedure was followed in this experiment as in the previous ones, using the basic nutrient solution and raw cotton. In fact, in all of these experiments, the details of technique are uniform, and only the changes in the routine procedure need be repeated. Nutrient agar was used for the plating of C. folia and N-free agar (1) for Act. colorata. For the H-ion series, liter Erlenmeyer flasks, containing 200 c.c. of the nutrient solution, and 3 grams of raw cotton, received inoculations from a 24-hour nutrient solution culture of C. folia (approximately 100,000,000 cells per flask), and from a four-day old culture of the Actinomyces in nutrient solution containing raw cotton. Incubation took place at 27°. The following results show the influence of Act. colorata upon the growth and physiological efficiency of C. folia. Because of the filamentous character of the Actinomyces growth and its tendency to break up into segments and form conidia, an accurate plate count, particularly with the Actinomyces above, proved impossible.

(1) O.A.C. Medium.

TABLE VIII.

The Influence of Act. colorata upon the Growth of C. folia.

Days	Control		Association of <u>C. folia</u> and <u>Act. colorata</u> in basic nutrient solution with cellulose.	
	<u>Act. colorata</u> alone	<u>C. folia</u> alone	<u>C. folia</u>	<u>Act. colorata</u>
1	100 †	30 x	55 x	100 †
2	Rapid increase in growth	42	98	66
3	Filaments form	54	137	46
4	Segments	66	148	30
5	Conidia appear	78	146	22
6	1000	100	130	20
7	1000 †	150	110	18

x Expressed in Millions.

† Expressed in Thousands.

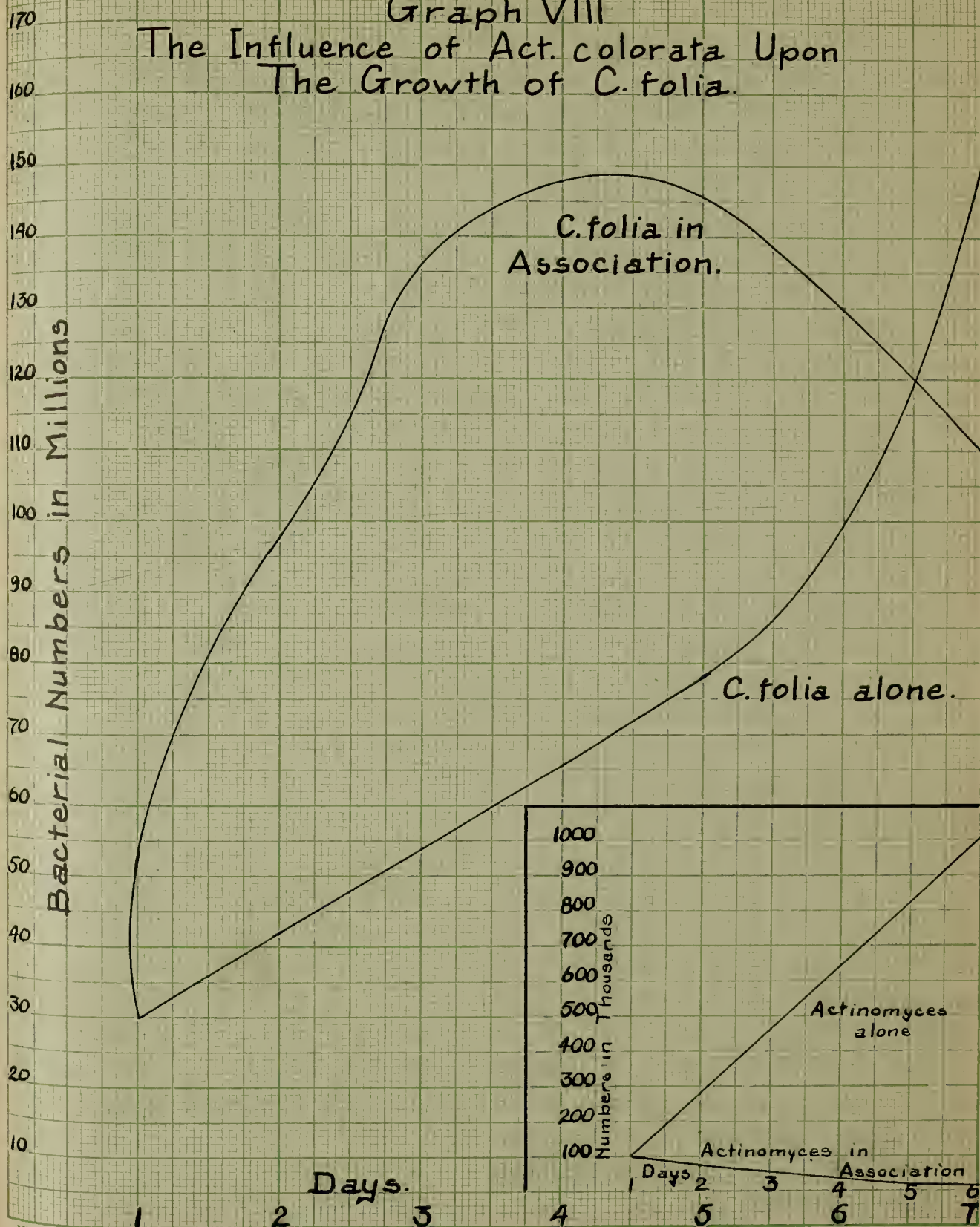
TABLE IX.

The Influence of the Association of Cellulose-Destroyers upon the Rate of Cellulose Decomposition.

Days	<u>C. folia</u> alone	<u>Act. colorata</u> alone	Association of <u>C. folia</u> and <u>Act. colorata</u>
	P_h	P_h	P_h
Initial	8.40	8.40	8.40
4	7.84	7.56	7.30
8	7.30	7.18	6.20
12	6.96	7.00	5.34
16	6.76	6.90	5.00
20	6.68	6.84	4.84
24	6.66	6.78	4.80
28	6.64	6.70	4.80
42	6.46	6.30	-
56	6.16	6.00	-
63	6.00	5.82	-
70	5.80	5.60	-

Graph VIII

The Influence of *Act. colorata* Upon The Growth of *C. folia*.



The above tables indicate that act. colorata exerts a stimulative action upon the growth and physiological efficiency of C. folia at the expense of the former organism which gradually dies out. In the fermentation flasks, in five weeks' time no trace of the Actinomyces could be found. In order to determine more definitely the nature of the stimulation, an extract was made from the Actinomyces cells. Cells from a four-day old culture growing upon N-free agar were washed in saline solution and then suspended in nutrient solution. The suspension was acidified to P_h 4.6 and boiled for 10 minutes on each of two consecutive days. The reason for this technique has been referred to above. The extract contains the water-soluble substances present in the living Actinomyces cells, and inasmuch as the failure of the Actinomyces results in the activation of C. folia, such an extract should contain the same essential food substances and produce stimulation. The results are as follows:-

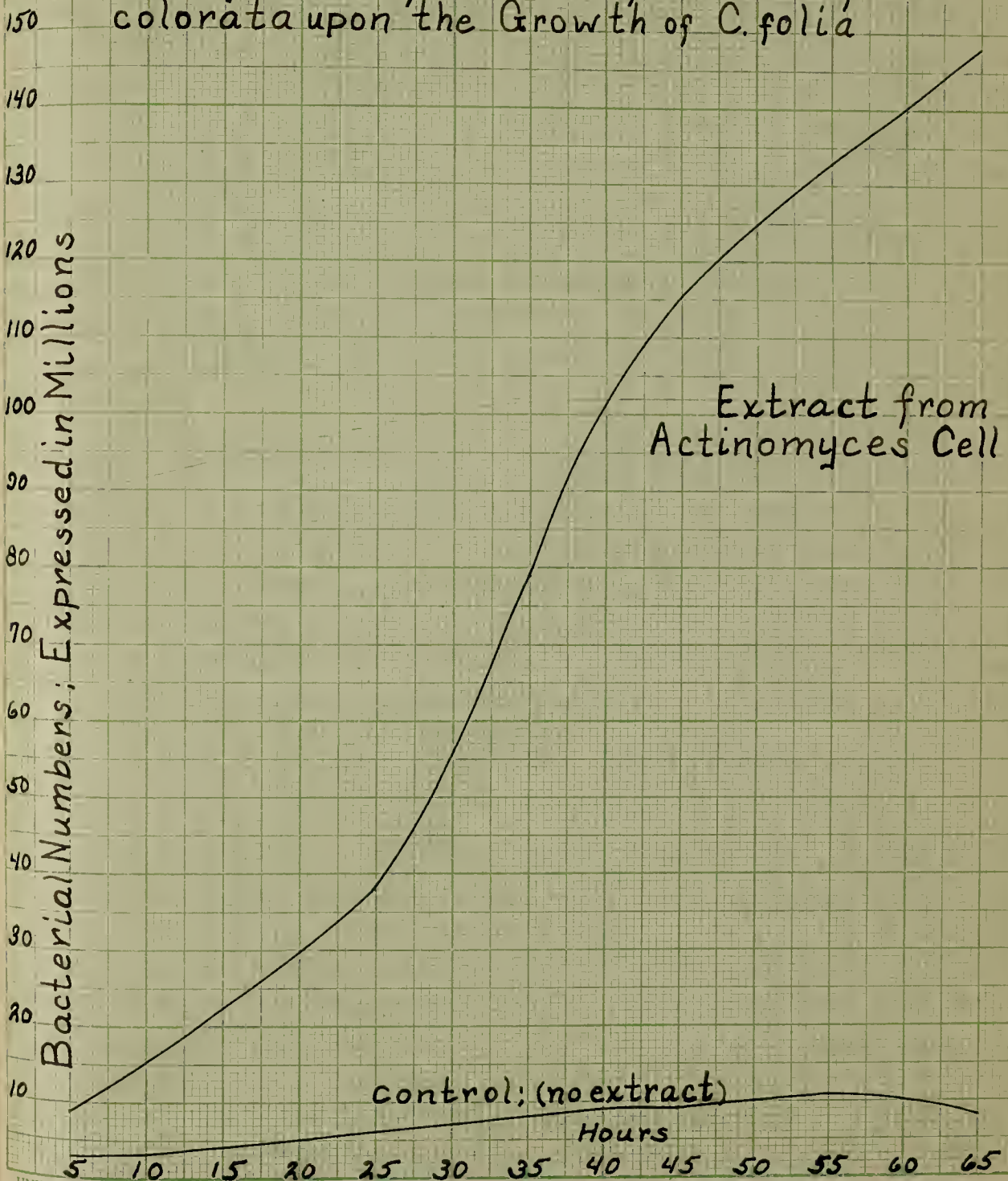
TABLE A.

The Influence of Extracts from cells of act. colorata upon the Growth of C. folia in Basic Nutrient Solution.
Expressed in Millions.

Hours	Extract from <u>actinomyces</u> cell	Control (no extract)
Initial	2	2
5	9	3
10	16	3
15	23	4
20	30	5
25	38	6
30	56	7
35	79	8
40	101	9
45	116	9
50	125	10
55	133	11
60	140	10
65	148	8

Graph X

The Influence of Extracts from Cells of *Act. colorata* upon the Growth of *C. folia*



The results of the above experiments show that association with Actinomyces causes a stimulation in the growth and cellulose-decomposing ability of C. folia. The stimulation is very similar to that previously reported in the presence of the growth-accessory or essential food substances. The association in this case may be considered equivalent to the addition of such substances, for Actinomyces, though gradually dying out, apparently furnishes C. folia with some essential food factor.

(b) The Influence of Azotobacter upon the Growth and Physiological Efficiency of C. folia.

The object of this experiment is to determine the influence of association with Azotobacter upon the growth and cellulose-decomposing ability of C. folia. One loopful from a five-day old culture of Azotobacter (A4 Jones) on Ashby agar was introduced into 100 c.c. of the basic nutrient solution. A uniform suspension was made. A suspension of C. folia from a 24-hour nutrient agar culture was also made in 100 c.c. of the nutrient solution. One c.c. portions from this suspension, containing approximately 3,000,000 cells, were used to inoculated test tubes, each containing 8 c.c. of nutrient solution.

From the Azotobacter suspension dilutions were

prepared in nutrient solution. One c.c. portions from these dilutions were also added to the tubes of nutrient solution. 200 Azotobacter cells were added in one case; 40,000 in a second; and in the last set, 2,800,000 cells. The living Azotobacter cells added were determined by the plate method using Ashby agar. The results are given below:-

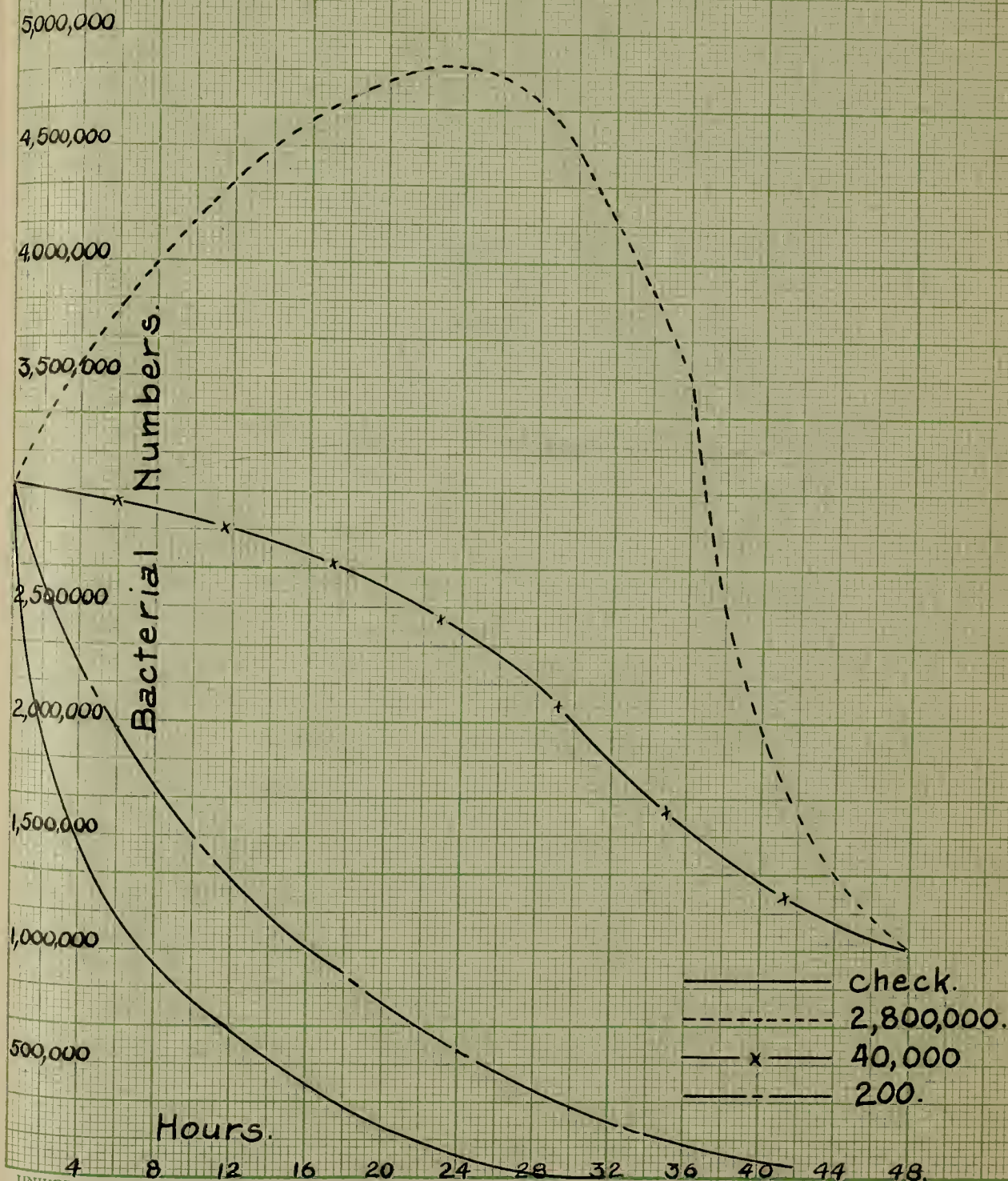
TABLE XI.

The Influence of Azotobacter upon the Growth of C. folia in Nutrient Solution.

Hours	<u>Azotobacter:</u> 200 cells + <u>C. folia</u>	40,000 cells + <u>C. folia</u>	2,800,000 cells + <u>C. folia</u>	Control <u>C. folia</u> alone
Initial	3,000,000	3,000,000	3,000,000	3,000,000
6	1,950,000	2,950,000	3,800,000	1,150,000
12	1,300,000	2,850,000	4,350,000	650,000
18	900,000	2,650,000	4,700,000	300,000
24	550,000	2,400,000	4,850,000	100,000
30	300,000	2,000,000	4,450,000	10,000
36	150,000	1,550,000	3,500,000	10,000
42	60,000	1,200,000	1,600,000	10,000-
48	10,000	1,000,000	1,000,000	10,000-

Graph XI

The Influence of Azotobacter upon the Growth of C. folia



These figures show that association with Azotobacter results in a marked stimulating effect upon the growth of C. folia. In order to ascertain more definitely the seat of the factor of stimulation, the influence of Azotobacter was investigated more in detail. The following preparations were made from a five-day old culture of Azotobacter cultivated in ashby solution without CaCO_3 .

1. Suspension of washed Azotobacter cells (living).
2. Extract from Azotobacter cell.
3. Extract from culture medium.

The culture of Azotobacter was centrifuged, throwing down the cells and leaving the supernatant liquid clear. The latter was retained for the preparation of the extract from the culture medium. The cells were washed several times with sterile physiological salt solution, after which a cell suspension in 10 c.c. of saline solution was prepared.

A similar suspension was used in preparing the extract from the Azotobacter cells. In this case the saline suspension of washed cells was acidified to P_h 4.6 as in the previous experiment, and boiled for ten minutes. The suspension was boiled only once.

From the supernatant liquid obtained after centrifuging the original culture, the extract from the culture medium was made. This liquid was filtered through a sterile porcelain filter and the resulting extract remained sterile. The influence of these preparations upon the growth of C. folia was determined as before.

TABLE XII.

The Influence of Washed Azotobacter Cells; of Extract Prepared from Azotobacter Cells; and of Extract from the Culture Medium in which Azotobacter has been Growing, upon the Growth of C. folia.

Hours	Washed <u>Azotobacter</u> Cells.	Extract from <u>Azotobacter</u> Cells	Extract from Culture Medium
6	750,000	100,000	20,000
12	1,020,000	20,000	10,000
18	1,310,000	10,000	5,000
24	1,560,000	10,000	5,000
30	1,670,000	10,000	5,000-
36	1,700,000	10,000	5,000-
42	1,650,000	10,000	5,000-
48	1,460,000	5,000	5,000-

The above table indicates that the stimulative effect of Azotobacter upon the growth of C. folia is caused by association with the living Azotobacter cell, and not with extracts from the cell or culture medium.

To determine the influence of Azotobacter upon the physiological efficiency of C. folia, a twenty-four hour nutrient solution culture of C. folia was made, also a suspension of Azotobacter in nutrient solution. Approximately equal numbers of each species were used as inocula, (500,000,000 cells). The fermentations were carried on as in the previous experiment using 3 grams of raw cotton in 200 c.c. of nutrient solution, and incubating at 27°.

Graph XII

The Influence of *Azotobacter* upon the Growth of *C. folia*

1,700,000

1,600,000

1,500,000

1,400,000

1,300,000

1,200,000

1,100,000

1,000,000

900,000

800,000

700,000

600,000

500,000

Bacterial Numbers

A

A - *Azotobacter* (washed cells)
B - Extract from *Azotobacter* cell
C - Extract from culture medium

B

C

Hours

4

8

12

16

20

24

28

32

36

40

44

48

TABLE XIII.

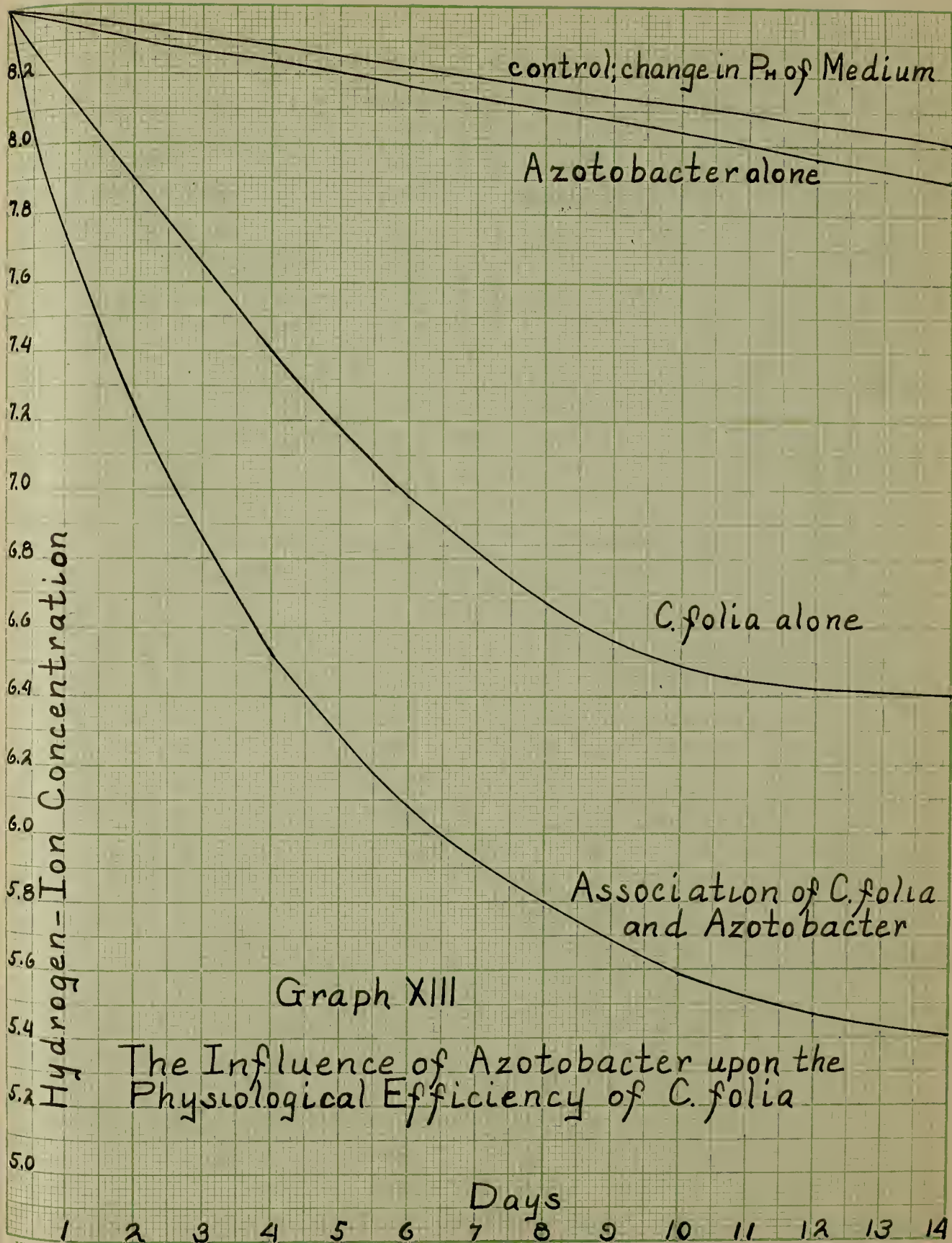
The Influence of Azotobacter upon the Physiological Efficiency of C. folia.

Days	<u>C. folia</u> alone P_h	<u>Azotobacter</u> alone P_h	Control: change in P_h of medium without organ- isms.	Association of <u>C. folia</u> and <u>Azoto-</u> <u>bacter</u> P_h
Initial	8.40	8.40	8.40	8.40
2	7.90	8.30	8.34	7.24
4	7.40	8.24	8.28	6.52
6	6.98	8.16	8.22	6.08
8	6.68	8.10	8.16	5.80
10	6.48	8.04	8.12	5.58
12	6.42	7.96	8.06	5.46
14	6.40	7.90	8.00	5.40

This experiment shows that Azotobacter, itself not a cellulose-destroyer, exerts a stimulating action upon C. folia both in growth and in cellulose-decomposing ability. This influence is manifest when C. folia is in association with living Azotobacter cells. This work was repeated and a similar result obtained using Azotobacter chroococcum which was isolated from soil in which active cellulose decomposition was taking place.

(c) The Influence of B. mycoides, B. subtilis, and B. cereus, upon the Growth and Physiological Efficiency of C. folia.

Following the same general technique, B. mycoides, B. subtilis, and B. cereus were investigated for their stimulating action. Preliminary experiments based upon visual tests indicated that these organisms, associated with active cellulose



Graph XIII

The Influence of Azotobacter upon the Physiological Efficiency of *C. folia*

fermentation in soil, exerted an accelerating influence upon the rate of cellulose decomposition. Because of the inability of these organisms to multiply in the basic nutrient medium or to attack cellulose, their numbers rapidly decreased. This decrease could not be traced quantitatively by the plate method because of the similar nutritive proclivities of these organisms and C. folia. Microscopic examinations, however, revealed the gradual dying out of the associated bacteria, resulting at the same time in a marked stimulation of C. folia in growth and physiological activity. These organisms were cultivated upon nutrient agar (P_h 7.60). Aqueous suspensions of the organisms were made, and after washing and centrifuging the cells extracts were prepared as before by acidifying the washed cell suspension to P_h 4.60 and boiling for 10 minutes. In this particular experiment the boiling process was repeated upon three consecutive days. All of the extracts were sterile. The influence of these extracts upon the growth of C. folia was tested in tubes of nutrient solution, treated uniformly as in the previous experiments. C. folia was plated out at intervals upon nutrient agar.

TABLE XIV.

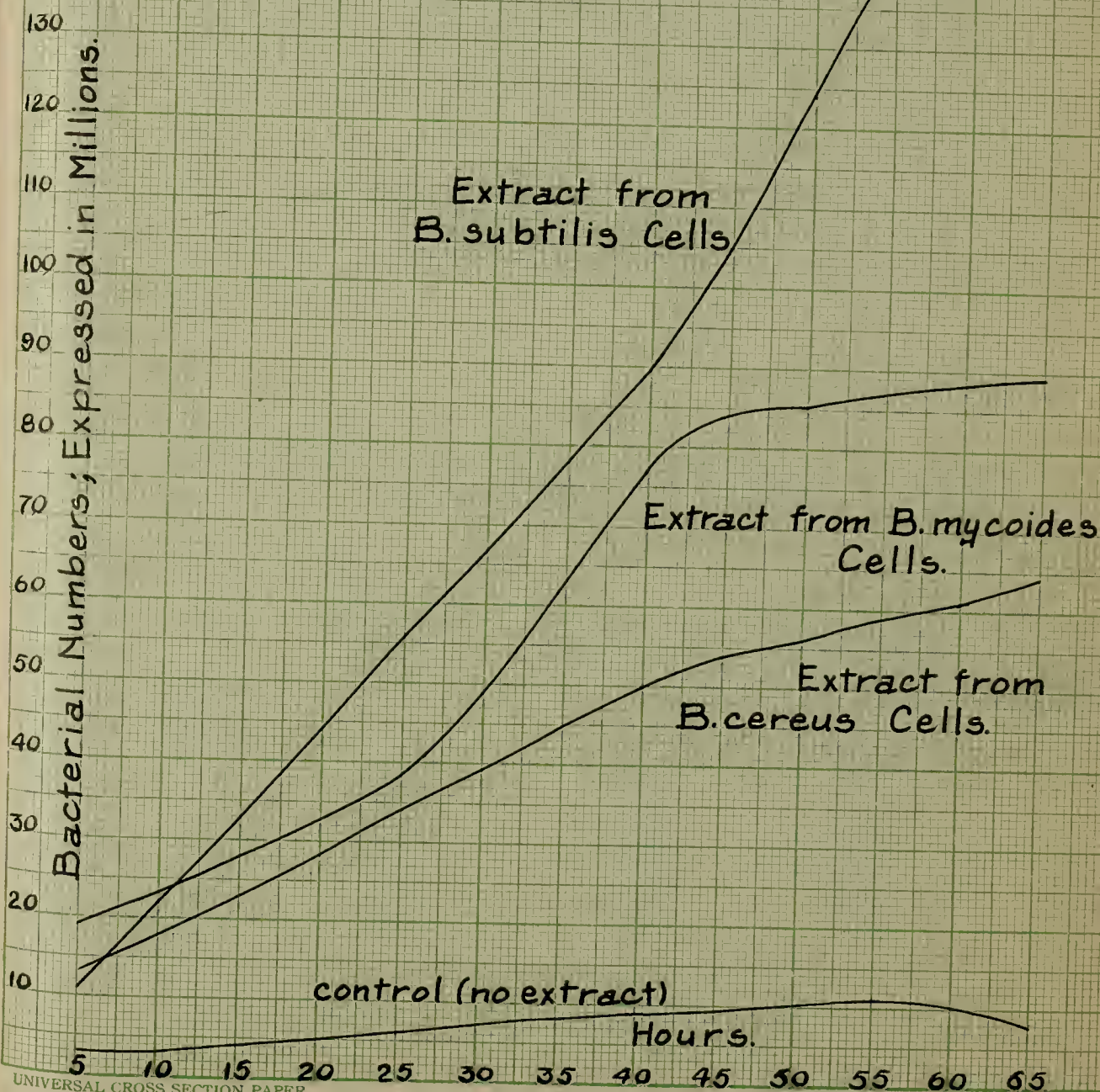
The Influence of Extracts Prepared from the Cells of B. mycoides,
B. cereus, and B. subtilis, upon the Growth of C. folia
in Nutrient Solution.

Expressed in Millions.

Hours	Control No extract	Extracts from <u>B. mycoides</u> cells	Extracts from <u>B. cereus</u> cells	Extracts from <u>B. subtilis</u> cells
Initial	2	2	2	2
5	3	19	13	11
10	3	23	18	22
15	4	28	23	33
20	5	33	28	44
25	6	38	34	56
30	7	49	39	67
35	8	63	45	78
40	9	77	50	89
45	9	84	54	105
50	10	85	56	124
55	11	87	59	142
60	10	88	61	161
65	8	89	64	175

Graph XIV

The Influence of Extracts prepared from the Cells of *B. mycoides*, *B. cereus*, and *B. subtilis* upon the Growth of *C. folia*.



These results show that some essential food factor may be extracted from the cells of B. subtilis, B. mycoides, and B. cereus, which accelerates the growth of C. folia in nutrient solution. In the association of these organisms with C. folia in nature, this factor probably influences the physiological efficiency of the cellulose-destroyer. To determine this influence the same method was employed as before. Each fermentation flask contained nutrient solution and raw cotton, and received an inoculation of 300,000,000 cells of C. folia and 50,000,000 cells of the associated organism. The following table and graph give the result of the association.

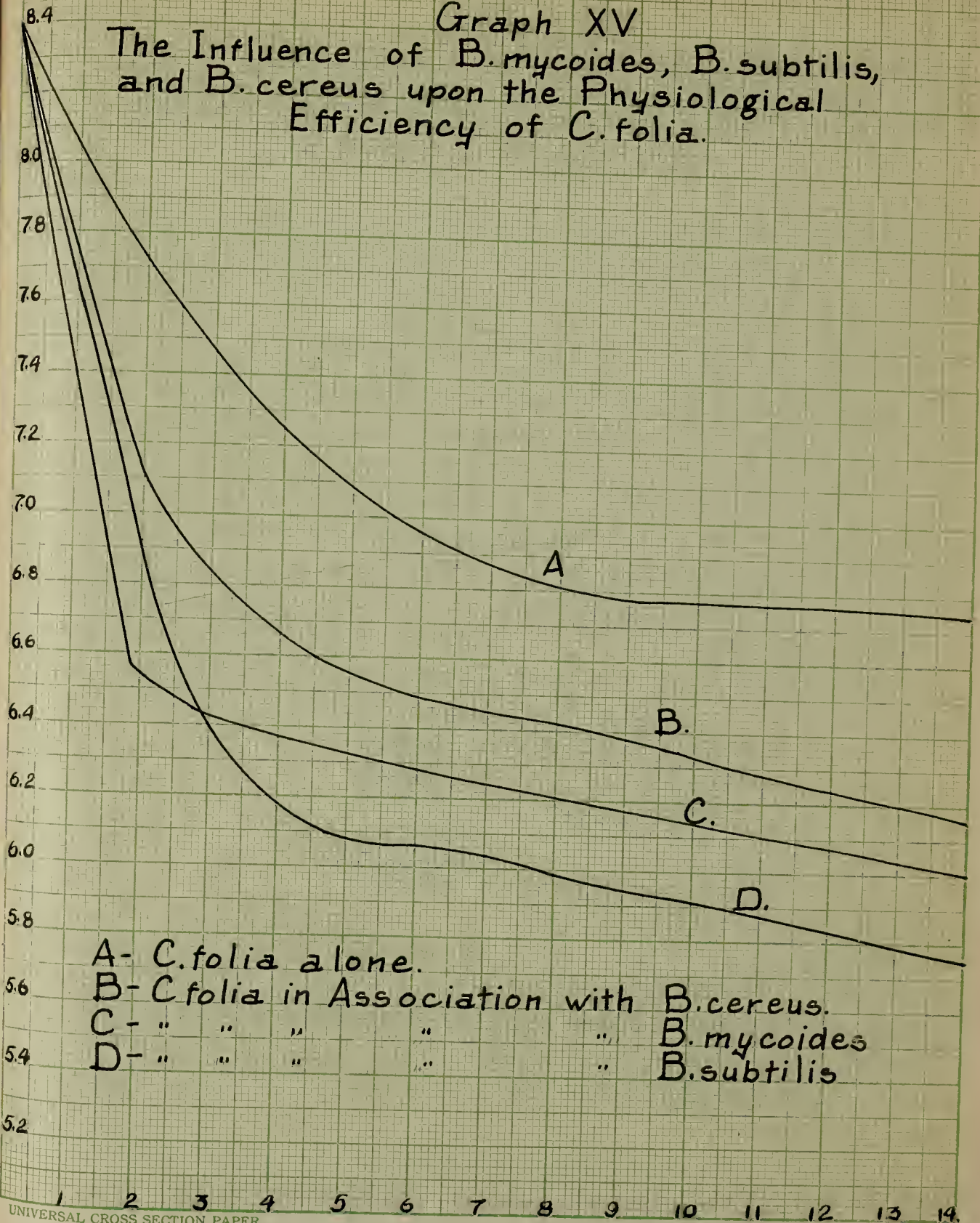
TABLE XV.

The Influence of B. mycoides, B. subtilis, and B. cereus upon the Physiological Efficiency of C. folia.

Days	Control <u>C. folia</u> alone	Association of:		
		<u>B. mycoides</u> with <u>C. folia</u>	<u>B. subtilis</u> with <u>C. folia</u>	<u>B. cereus</u> with <u>C. folia</u>
Initial	8.40	8.40	8.40	8.40
2	7.73	6.54	6.92	7.10
4	7.24	6.37	6.16	6.66
6	6.96	6.28	6.07	6.48
8	6.80	6.20	5.98	6.42
10	6.77	6.14	5.91	6.32
12	6.76	6.07	5.83	6.24
14	6.74	6.00	5.75	6.15

Graph XV

The Influence of *B. mycoides*, *B. subtilis*,
and *B. cereus* upon the Physiological
Efficiency of *C. folia*.



A- *C. folia* alone.

B- *C. folia* in Association with *B. cereus*.

C- " " " " " *B. mycoides*

D- " " " " " *B. subtilis*

B. mycoides, B. subtilis and B. cereus exert a stimulating action upon the growth and physiological effect of C. folia.

(d) The study of Microbial Associations by means of the China blue-Rosolic Acid-Cellulose Medium.

In association studies such as those carried on in this investigation, there is often need of a medium which will reveal in detail the results of association. In this experiment such a medium is described. Through its use the influence of association in cellulose fermentation may be observed conveniently and with a fair amount of accuracy. The method is based upon the China blue-rosolic acid reaction described by Bronfenbrenner (1). The preparation of the "CR-cellulose" medium is as follows:-

Basin nutrient solution

K_2HPO_4	1 gm.
$MgSO_4$	1 "
Na_2CO_3	1 "
$(NH_4)_2SO_4$	2 "
H_2O (dist.)	1000 c.c.
Raw cotton	30 gms.
(chemically untreated)	

The salts are dissolved in 500 c.c. of the water, and of the remaining portion a 0.5% agar is prepared. Before sterilization the two portions are mixed and the cotton added, cut into small fragments. With constant stirring, 1.0% "CR"

(1) Bronfenbrenner, J. "A new Indicator for Direct Reading of Hydrogen Ion Concentration in Growing Bacterial Cultures". Jour. Med. Res. (1918) 39; 25.

preparation (1) is introduced. In transferring the medium to petri plates, care is taken to insure a uniform distribution of cotton over the plate. The dishes are then autoclaved. The percentage of agar present proved very satisfactory in offering a smooth homogenous surface, yet allowing the microbial suspensions added to reach the entire mass of cotton. The P_H of the medium prepared in this way is 8.40 and is colored a brilliant red.

After cooling, the surface of the medium is inoculated uniformly with the cellulose-destroyer; one-half of the plate only receives treatment with the associated organism. The indicator has its turning point very close to neutrality, and with the production of acid, the medium changes to a deep blue.

All of the organisms investigated above were tested out upon this medium in association with C. folia. The following table shows the influence of association upon the rate of cellulose decomposition as revealed by H-ion concentration changes, using the "CR-cellulose" medium.

(1) CR indicator is obtained by mixing equal parts of one-half per cent. aqueous solution of China blue with one per cent. solution of rosolic acid in ninety-five per cent. alcohol. The indicator has its turning point very close to neutrality. On the alkaline side China blue is water clear. Rosolic acid gives on the acid side different shades of pale yellow, which is masked by the deep blue of the China blue and gives sharp color values in media. In alkaline environment, China blue being colorless, the rosolic acid gives a pure red.

TABLE XVI.

The Influence of Association upon the Rate of Cellulose Fermentation as Determined by the "CR-cellulose" Reaction.

Days	<u>C.folia</u> alone x	<u>B.mycoides</u>	<u>C. folia</u> in association with:					<u>Azotobacter</u>
			<u>B.cereus</u>	<u>B.subtilis</u>	<u>Act.col-</u> <u>orata.</u>			
Initial	Medium red (8.40)	Red	Red	Red	Red		Red	
1	"	"	"	"	"		"	
2	"	"	"	"	"		"	
		(slight reduction of indicator observed)						
3	Medium red	Blue ++	Blue +	Blue ++	Blue ++		Blue +++	
4	" "	" +++	" +	" ++	" ++		" +++	
5	" "	" +++	" ++	" +++	" +++		" +++	
6	" "	" +++	" +++	" +++	" +++		" +++	

- (1) + = medium slightly bluish (P_h 6.8)
 ++ = blue color definite (P_h 6.4 - 6.6)
 +++ = intense blue (P_h 6.0 - 6.2)

x C. folia alone caused the acid reaction in 8 to 10 days.

The results observed with this medium are quite in accord with the quantitative results recorded above. The greatest stimulation seems to occur when C. folia is associated with Azotobacter, B. subtilis, Act. colorata, or B. mycoides. In the case of B. cereus the stimulation was not so marked. With the other organisms tested, Rhizobium radiocolum, Radiobacter, and soil Actinomyces, the stimulating effect was negligible.

The value of the China blue-rosolic acid-cellulose medium lies in the ease of preparation, its adaptability to the H-ion technique, and the accuracy with which the indicator responds to stimulation in the physiological activity of cellulose-destroyers. Other advantages of the medium are as follows: The buffer index is negligible, the color change occurs with definiteness at very nearly P_h 7.0, the indicator is not affected by heat, shows no

evidence of bactericidal action, is comparatively stable, and its constituents are definitely known chemical substances (1).

(e) Discussion.

Association with other organisms appears to be a factor of considerable significance in the problem of maintenance or recovery of the physiological efficiency of cellulose-destroyers. C. folia is stimulated both in growth and cellulose-destroying ability through association with Act. colorata, Azotobacter (A4 Jones), also Azotobacter chroococcum, B. subtilis, B. mycoides, and B. cereus. In the case of the Actinomyces there is marked stimulation. Influenced by this association, C. folia splits cellulose with great rapidity, and in twenty-four days the P_h increases from 8.40 to 4.80. Deprived of this stimulating influence, C. folia is not such an active destroyer of cellulose, and in twenty-four days the concentration of H-ions increases to a less extent, from P_h 8.40 to 6.66. This work actually demonstrates the influence of association upon the physiological efficiency of C. folia, for the stimulation of the organism occurs at the expense of Act. colorata, which gradually dies out. Some essential food factor is contributed to the medium by the Actinomyces, however, which is the agent of activation.

Association with Azotobacter, B. subtilis, B. mycoides,

(1) Bronfenbrenner, J. Loc. cit.

and B. cereus, also results in acceleration in the growth and physiological efficiency of C. folia. Azotobacter does not appear to attack cellulose, but influences the cellulose-destroyer directly through the elaboration of an extracellular factor which functions as an essential food substance.

This essential substance for C. folia, produced by Azotobacter, does not accumulate in the culture medium as the above experiment shows. The living cell is a necessary feature. During the life of the Azotobacter cell a "stimulation environment", probably of the nature of the "radiation environment" of Bovie⁽¹⁾ may be set up, influencing the cells of C. folia. When this "stimulation environment" is disturbed due to the death of the Azotobacter cell, the stimulative effect is no longer felt by the cellulose-destroyer.

The other organisms studied also secrete essential food substance or substances, but here the stimulation seems to be an intracellular process.

These studies show that, as far as cellulose fermentation is concerned, stimulation through association resolves itself into a problem of essential food substances. The associated organism in each case adds to the medium (in these experiments a deficient medium), essential food elements. This factor seems to be the most significant one involved in the maintenance and recovery of the physiological efficiency of C. folia.

3. Essential Food Substances in Soil.

The investigation thus far has proved that there is, operating in nature, a factor, which is called here the essential food factor. It has been shown that many substances, some well-known and some relatively obscure chemically, may function as essential food substances, and that these play an important role in nature's medium as manifested by close observation of nature at work.

(1) Loc. cit.

The addition of these substances to the more or less artificial media of the laboratory also gives definite results. These results have been observed and determined quantitatively in the case of cellulose fermentation. The rate of this fermentation process is profoundly affected by the essential food factor. The cellulose-destroying micro-organism responds to its presence by a stimulation in growth and cellulose-decomposing ability.

Association with living cells seems to be one very significant source of the essential food factor. Plant cells and microbial cells synthesize substances which supply essential elements to the medium in which cellulose-fermentation is taking place. The result is increased growth and physiological efficiency on the part of the cellulose-decomposing organism. According to Mockeridge (1), soil bacteria liberate from the soil organic matter the essential food factors, which thus stimulate cellular activity. Some of these factors may be a product of microbial metabolism as demonstrated in a previous experiment in the case of B. subtilis, B. mycoides, B. cereus, Azotobacter, and Act. colorata. On the other hand it has been shown that plant cells, e.g., leaves, seeds and seedlings, contain essential food substances which also stimulate the growth and physiological efficiency of cellulose-destroyers. The seeds and seedlings investigated were those of alfalfa, barley and buckwheat.

(1) Mockeridge, F. A. "The Occurrence and Nature of the Plant Growth-Promoting Substances in Various Manurial Composts". Biochem. Jour. (1920) 14; 432.

The purpose of the present investigation is to determine, in the light of Mockeridge's contention, if, during the decomposition of green manure, the essential food factor is elaborated and influences the cellulose-fermenting organisms in the ways indicated.

(a) The Influence Exerted by the Essential Food Substances Elaborated during the Decomposition of Plant Material in Soil, upon the Growth and Physiological Efficiency of C. folia.

A poor soil (sandy loam) was prepared by mixing sand and loam, using equal parts to each. This soil was subjected to thorough mixing in order to insure a homogeneous product. The crops to be investigated were grown in pots upon the sandy loam. These were incubated at the proper greenhouse temperature with control pots. After the period of growth indicated below, the crops were "plowed under", and allowed to decompose.

Examinations of the decompositions from time to time revealed, in a general way, the progress of the fermentation. When the processes appeared to be well on the way to completion, only a modicum of the skeletal remains being visible, 40 per cent. aqueous extracts were prepared of all the soils, including the controls.

Crop	Age in days	Number of days allowed for Decomposition.
Alfalfa	25	8
Barley	15	10
Buckwheat	17	8
Red Clover	28	10

The aqueous suspensions of soil were filtered through sterile porcelain filters (Chamberland), and the filtrates tested for sterility by incubation and culturing upon beef-peptone agar. The sterile extracts were employed to test the influence of essential food substances elaborated during the decomposition of plant material in the soil, upon the growth and physiological efficiency of C. folia.

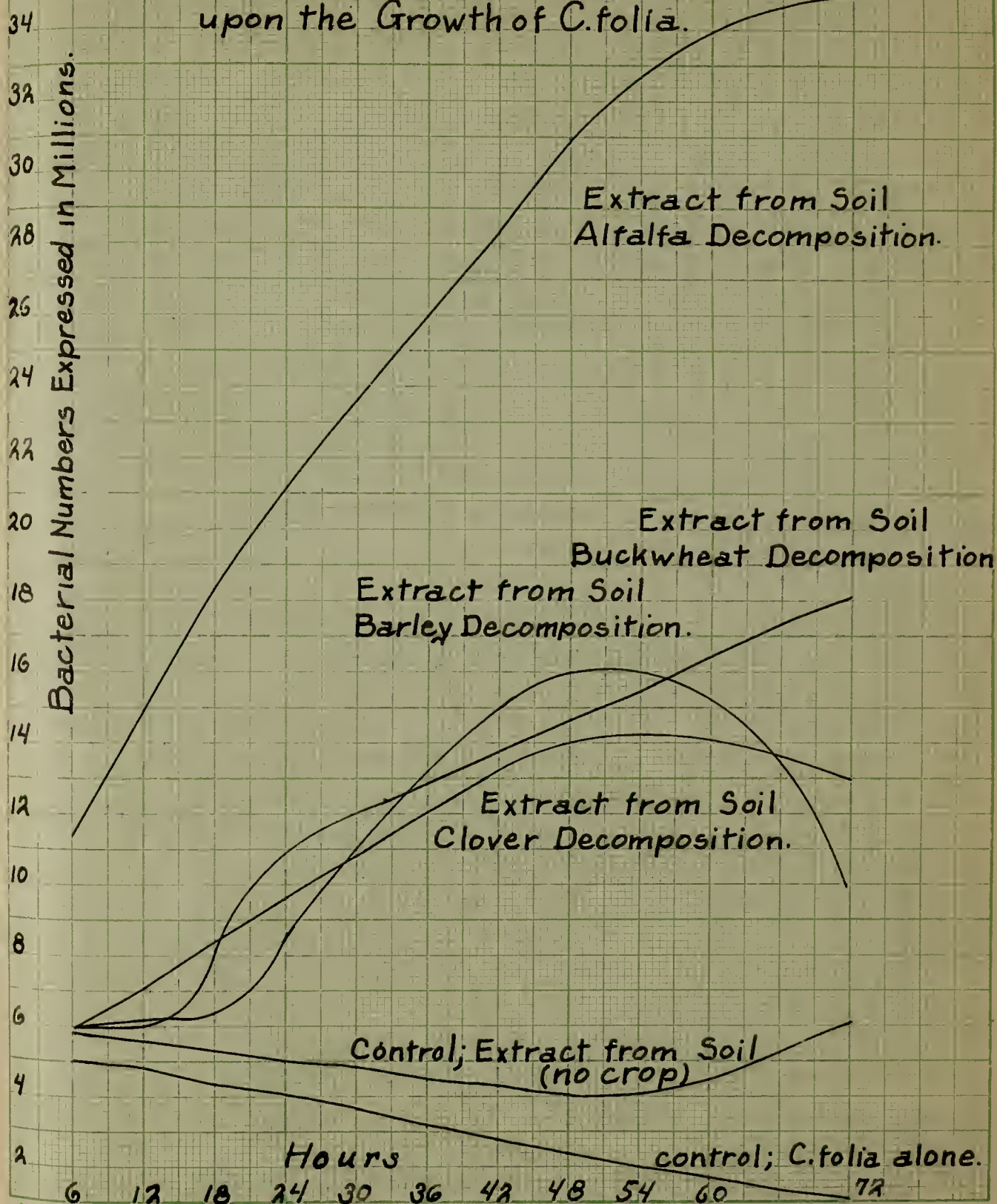
The influence of these extracts upon the growth of C. folia was first tested. The extracts were added to the basic nutrient medium making a total extract concentration equivalent to 0.4 gm. of soil. A uniform inoculation with C. folia (approximately 6,000,000 cells) was given each flask, and the cultures were incubated at 27°. The results are recorded below:-

TABLE XVII.

The Influence of Soil Extracts. Prepared from Soils in which Alfalfa, Barley, Buckwheat, and Clover have undergone Decomposition, upon the Growth of C. folia in Basic Nutrient Solution.

Hours	Control <u>C. folia</u> alone	Control Extract from soil alone.	Extract from Extract from Extract from		
			Soil, Alfalfa Decomposi- tion.	Soil, Barley Decomposi- tion.	Soil, Clover Decomposi- tion.
	No extract	No crop added			
6	5,000,000	5,800,000	11,400,000	6,000,000	6,000,000
12	4,900,000	5,600,000	15,000,000	6,200,000	7,000,000
18	4,300,000	5,300,000	18,400,000	6,400,000	8,400,000
24	4,000,000	5,000,000	21,000,000	8,700,000	9,600,000
30	3,600,000	4,800,000	23,600,000	11,000,000	10,800,000
36	3,200,000	4,500,000	26,100,000	13,400,000	12,000,000
42	2,800,000	4,300,000	28,300,000	15,000,000	13,200,000
48	2,400,000	4,000,000	31,000,000	16,000,000	14,000,000
54	2,000,000	4,000,000	32,700,000	16,000,000	14,200,000
60	1,700,000	4,400,000	34,000,000	15,200,000	14,100,000
66	1,300,000	5,200,000	34,700,000	13,400,000	13,600,000
72	1,000,000	6,000,000	35,000,000	10,000,000	13,000,000

Graph XVI The Influence of Soil Extracts upon the Growth of C.folia.



The above figures show that the extracts prepared from the soils in which the crops had been plowed under and allowed to decompose, exert a marked stimulating influence upon the growth of C. folia. The control soil, which received no crop treatment, reveals negligible stimulation; in the nutrient medium alone, there^{is}/a steady decline in bacterial numbers. It is evident that some essential food substance or substances are operating in the extracts from the soils in which the crops decomposed.

The influence of these substances upon the rate of cellulose decomposition by C. folia was tested next. Two gram quantities of raw cotton were added to the flasks of nutrient solution, and an extract concentration equivalent to 8 grams of soil was present in each 100 c.c. of medium. Uniform inoculations^{were made} from a young culture of C. folia in nutrient solution, approximately 300,000,000 cells. The flasks were incubated at 27°. The electrometric method was supplemented by the colorimetric in this experiment. The influence of the extracts upon the rate of cellulose fermentation by C. folia is indicated below.

The rates of the decomposition processes were readily estimated in a preliminary experiment, by visual observation of the macroscopic and microscopic changes; +++ rapid, ++ moderate, + slow.

Crop	After 7 days' fermentation
Alfalfa	+++
Buckwheat	+++
Barley	++
Clover	++
Control	+

The quantitative results based upon H-ion concentration changes are as follows:-

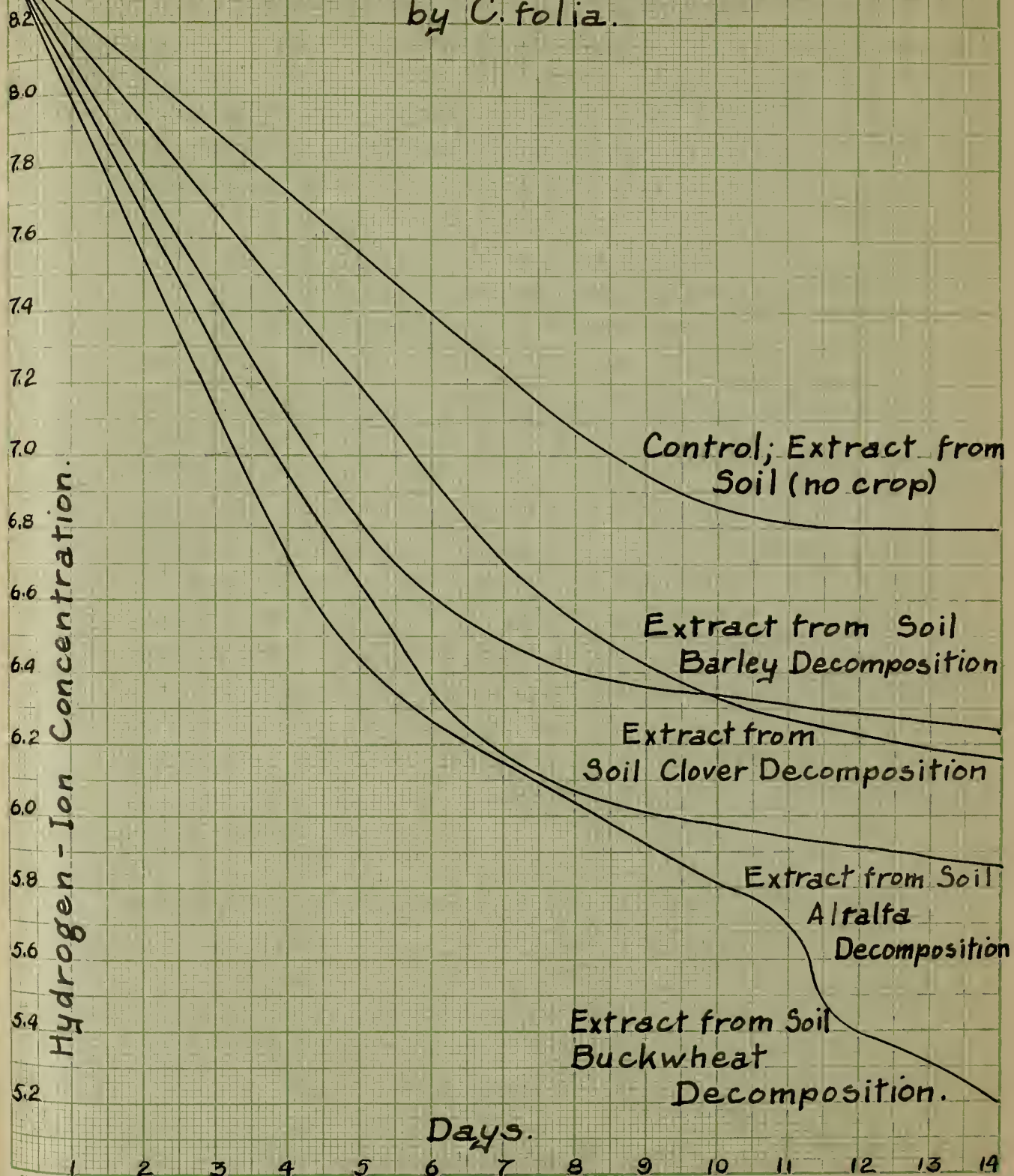
TABLE XVIII.

The Influence of Extracts Prepared from Soils in which Alfalfa, Barley, Buckwheat, and Clover have Undergone Decomposition upon the Rate of Cellulose Fermentation by C. folia in a Medium Composed of Nutrient Solution and Raw Cotton.

Days	Control Extract from Soil alone No Crop added	Extract from Soil Alfalfa Decomposition In terms of P_h	Extract from Soil Buckwheat Decomposition	Extract from Soil Barley Decomposition	Extract from Soil Clover Decomposition
Initial	8.40	8.40	8.40	8.40	8.40
1	8.23	8.04	8.00	8.10	8.16
2	8.07	7.68	7.54	7.76	7.92
3	7.90	7.30	7.12	7.44	7.68
4	7.74	6.94	6.72	7.12	7.44
5	7.57	6.65	6.44	6.82	7.20
6	7.40	6.34	6.26	6.62	6.94
7	7.24	6.18	6.15	6.49	6.70
8	7.06	6.06	6.04	6.40	6.54
9	6.95	6.00	5.92	6.46	6.42
10	6.86	5.98	5.80	6.34	6.32
11	6.82	5.94	5.70	6.31	6.27
12	6.80	5.92	5.40	6.29	6.22
13	-	5.88	5.31	6.26	6.18
14	-	5.86	5.20	6.24	6.16

Graph XVII

The Influence of Soil Extracts upon the Rate of Cellulose Fermentation by *C. folia*.



The above figures indicate that the stimulation is manifest early in the decomposition process, i.e., during the first two weeks. After that the accessory influence becomes less marked. The stimulation in the presence of the extracts is evident.

(b) [^]Rôle of the Essential Food Factor in the Decomposition of Green Manure.

This experiment is designed to reveal a practical aspect to the essential food problem. The results recorded above indicate that acceleration in the rate of cellulose decomposition and stimulation in the physiological efficiency of cellulose-destroyers takes place early in the process of destruction. Although in the decomposition of green manure the cellulose-destroyers eventually obtain essential food substances, and hence receive due stimulation, a preliminary dissolution of the cell wall of the plant must take place before the essential food substances are liberated. Of course, under normal conditions there is probably a supply of the essential food factor in the soil, so that the entire process is accelerated more or less without a definite lag period. Under abnormal conditions, some other factor such as moisture conditions or oxygen supply may dominate the decomposition process and the essential food factor becomes relatively less important. In this investigation, however, normal conditions prevailing in good soil are considered, and here the stimulation

in cellulose fermentation would seem to form a cycle in which cellulose-destroyers receive stimulation from present and past processes to attack fresh plant material, later liberating from this material the essential food factor, which is thus rendered available for other organisms. If this be true, it should be possible, by controlling the conditions of the experiment, to demonstrate this lag period, during which the medium is deficient in essential food elements. The cellulose-destroyers are therefore without the accessory influence until they succeed in splitting the plant material sufficiently to liberate the essential food substances for themselves. The addition of the extracts containing this factor, to the medium, should result in aggressive decomposition from the first with no lag period.

Crops of approximately the same ages as before were grown upon the sandy loam. The entire plant was then removed, washed thoroughly in water, and sterilized in HgCl_2 (1-1000) for 3 to 4 minutes. After copious washing in sterile water the plants, in small bundles of 3 grams each, were transferred under aseptic conditions to sterile quartz sand which had been previously ignited and washed with sulphuric acid. In transferring, the process of "plowing under" was simulated. The plants were inoculated with pure cultures of C. folia and Act. colorata. Each pot received a uniform inoculation. The plant tissue was then treated with the extract, delivered from a sterile pipette. Extracts from the four crops and the control were tested upon the green crops recently "plowed under" and treated with pure cultures of micro-organisms.

In observing the influence of the extracts it was found that the greatest change in the plant material occurred during the first three or four days; after that the control pots also showed marked stimulation, which according to theory, was to be expected. In other words, in four days the cellulose-destroyers had liberated enough of the essential food factor from the plant cells to cause a very considerable increase in the rate of cellulose decomposition. In the presence of the extracts, however, the green manure was largely destroyed during this four-day interval.

From day to day detailed macroscopical and microscopical examinations of the plant tissues were made. The changes in the control pots were negligible until after the fourth day. In the majority of the pots which had received the extracts from the decomposed crops, the plant tissue was largely destroyed at the end of the third day of the fermentation. Of course, in the control pots the associative action of C. folia and Act. colorata, in the light of the above experiments, might cause stimulation in the cellulose-decomposing ability of the former organism. As a matter of fact, however, the Actinomyces was growing steadily during these first few days and existed largely in the vegetative stage. The stimulation exerted by Act. colorata would be intracellular as previous experiments have shown, therefore, activation from this source would probably not occur until later in the process when the older filaments would have undergone dissolution. The effect of the extracts was very apparent, however, and only

the skeletal remains of the green manures were visible on the third and fourth days.

This experiment sheds considerable light upon nature's method of working. It is probable that by a concentration of the forces utilized by nature, more rapid and efficient processes may be obtained in the laboratory than are commonly found in nature. This has actually been accomplished in this experiment.

GENERAL DISCUSSION.

At the outset of this investigation the author was faced by certain problems which presented real difficulties. The first was the problem of isolating cellulose-destroyers in pure culture while still capable of exercising their cellulose-fermenting powers. Cellulose is an extremely resistant substance and a particular set of conditions are necessary for the efficient functioning of these organisms. Such conditions, though supplied effectively by nature, are lacking in the usual laboratory methods attendant upon isolation of pure cultures. When the proper conditions exist and each factor participating is functioning favorably, cellulose-destroyers are actively engaged in fermenting cellulose to a greater or lesser extent, depending upon the individual characteristics and proclivities of the species. The point is, each species is decomposing cellulose at a maximum rate, in other words, is physiologically efficient. Instances of maximum efficiency are not frequently observed in the laboratory. Even in nature where conditions are usually favorable, and where favorable conditions exert such an effective influence upon micro-organisms, a decrease in physiological efficiency, or in the realm of pathology, virulence, is sometimes noted. The underlying reasons for these phenomena are not always apparent. They revert, without doubt, to the problem of favorable or unfavorable conditions. Yet the factors dominating a set of

conditions may be so complex in character that considerable research may be required to bring them to light. For example, the possible pathogenic rôle of the ultramicrobe in diseases ascribed to well-known bacteria.

The operations of nature are superior to man-devised methods, but at the same time it is possible to secure even more rapid or more efficient results than are usually observed in nature. The present investigation was designed to bring about an artificial cellulose fermentation of maximum efficiency.

Many suggestions were obtained from nature, and a study was made of the individual physiological proclivities of the organisms under examination.

In the isolation, nutrient (beef-peptone) agar was used. During this relatively short exposure to the artificial conditions accompanying this procedure, the organism suffered a decided loss in cellulose-decomposing ability. The only means available for again bringing about efficient functioning consisted apparently either in returning the organism to its natural habitat or cultivating it in mixed culture with associated organisms. In other words the secret of physiological efficiency, and, in reality, the essence of the whole work was hidden among a series of unknown and complex factors. Thus nature works: efficiently utilizing the factors available in constructing a complex series of conditions. Some of the factors involved, though contributory, may play relatively unimportant parts. There are, however,

usually the dominating factors in nature's processes, to which may be ascribed leading rôles in the maintenance of physiological efficiency.

Close observation of nature and careful analysis of situations found there usually reveal the features of nature's medium and the relative importance of the factors operating. In the case of cellulose fermentation, analysis and experimentation showed the secret of physiological efficiency to be intimately concerned with essential food substances. This factor is readily altered or inhibited by physico-chemical treatment and unnatural laboratory manipulation. It is sometimes furnished by nature in the form of definite chemical compounds such as sugar, occurring as constituents of the substratum. The factor may be produced through the biological or physico-chemical alteration of certain components of the medium. Products of extracellular or intracellular metabolism may function as essential food substances; and finally there is evidence that some force not yet sufficiently recognized may operate in a manner resembling closely, if not definitely, the essential food factor.

The essential food factor offers essential elements - elements which are required for the maximum efficiency, or at least the normal functioning, of the cell. Such is the factor under investigation; and in its present application it has been found that maximum efficiency in cellulose fermentation is dependant directly upon the presence of these substances in the medium.

Emphasis is laid in this work upon the role of association in furnishing the essential food factor. Organisms may build up within their cells substances akin to vitamins or growth-accessory substances which supply this essential element. They may be of the nature of nucleic acid or some allied compound which, if not akin to vitamins, at least have in common with them the accessory or stimulating action. Thus azotobacter influences C. folia, and the latter is stimulated to increased growth and physiological efficiency. The associated organism may change the character of the medium, producing from the constituents present, substances which exert a similar stimulating action.

The nature of association is not yet well understood, and its complexity tends to lessen the number of investigations having as their goals the study of the mutual relationships of micro-organisms. This factor is a significant one, however, without any doubt, and recognition of its importance should be suitably and duly made. The present investigation shows that one phase of association, that affecting the rate of cellulose fermentation, is intimately concerned with the provision of essential food substances. This is, however, only one phase of the general problem of media. Association also contributes in other ways, affecting the conditions which go to make up the medium; such, for example, as oxygen supply, H-ion concentration, moisture, and food supply. In the last case, however, close contact is made with the essential food factor, and at times the line of demarcation seems to be largely imaginary. Food supply is taken

to mean the so-called "building stones" of the cell; substances which the organism can use for its maintenance.

In active fermentations and other aggressive microbiological phenomena, some essential food substance must of necessity be present. Great variations in fermenting ability and pathogenicity are often observed among ordinarily physiologically active species. At times remarkably rapid and efficient results are obtained; while occasionally the organism exhibits a disinclination to grow or function in an efficient manner upon the same medium - the same, that is, with respect to food supply. The difference lies in the presence of the essential food factor, the effects of which this investigation attempts to demonstrate.

This investigation has been confined to aerobic cellulose decomposition. Although in certain processes of nature the anaerobic fermentation would seem to progress with particular aggressiveness, yet, generally speaking, the aerobic process appears to be more wide-spread and of greater significance in its relation to soil fertility.

The present treatment of the subject of cellulose fermentation from the standpoint of the essential food factor of media refers typically to aerobic processes. In the absence of oxygen other factors are introduced which probably overweigh the essential food factor.

The stated purpose of this investigation was to bring about an artificial fermentation of cellulose of maximum efficiency.

What is maximum efficiency? When has an organism attained maximum efficiency? It will be evident that this is a goal constantly before the investigator, but probably never attained; or, if actually obtained, there is no criterion by which it can be recognized. In this work, however, by controlling the factors governing the process, an unknown factor has been revealed which causes the cellulose destroyer to attain great efficiency in cellulose fermentation under artificial laboratory conditions. A maximum state of efficiency has been obtained, one which, from close observation and experimentation, is obviously greater than is found in most of the cellulose fermentations of nature. The tremendous importance of the essential food factor is therefore apparent.

SUMMARY AND CONCLUSIONS.

A specific summary of the results of the present investigation follows:-

Preliminary observations of natural processes showed that cellulose-destroyers were most efficient under the conditions provided by nature, and that in pure cultures they were capable of carrying on active cellulose fermentation. Under cultivation in the laboratory, however, upon beef-peptone agar, these organisms eventually lost the ability to decompose cellulose, in other words, had suffered a loss in physiological efficiency.

The goal of the investigation, therefore, was to ascertain what factors operated for increased efficiency on the part of these organisms and by incorporating these factors in the environmental medium to obtain an artificial fermentation of maximum efficiency.

The external conditions for growth, temperature, oxygen supply, moisture, reaction, inorganic nutrients, purified filter paper, and pure cultures of cellulose destroyers were all, as far as possible, known and controlled factors of the medium. Under these conditions the cellulose destroyers proved inefficient, and practically none of the cellulose was attacked. To obtain a fermentation of maximum efficiency, therefore, the organisms must be rendered physiologically active.

First, the medium was supplied with a more nearly natural cellulose; not the commercial product, rendered resistant to microbial attack through drastic chemical treatment, but a cellulose of nature, raw cotton. This feature of the medium contributed toward a more active fermentation.

There proved to be a factor operating in leaves and other plant tissue which, when present in the medium, resulted in an increase in the growth and cellulose-decomposing ability of micro-organisms. The leaves, unheated and sterile, were added to a sterile nutrient salt solution which, by itself, would not support the growth of the cellulose destroyers under investigation. Autoclaving the leaves so altered the composition of the medium that the activities of the organisms were considerably changed and the stimulation, so apparent in the case of the unheated leaves, was no longer manifest.

This unheated leaves medium, therefore, proved to be a decided factor in enhancing the physiological efficiency of cellulose destroyers. After several weeks' cultivation upon this medium in pure culture, C. folia acquired the ability to destroy raw cotton vigorously, while upon beef-peptone agar the organism became accustomed to a different type of medium and refused to attack cellulose. Accompanying the active fermentation there was a definite increase in the concentration of hydrogen-ions, probably the main physiological feature of C. folia in its rôle of cellulose destroyer. The increase in twenty-eight days was from

p_H 8.50 to p_H 5.00. In the case of the culture habituated to beef-peptone agar there appeared to be little action upon cellulose, and the increase in hydrogen-ion was very slight, viz., from p_H 8.50 to p_H 7.58. The factor responsible for the stimulation was readily obtained in the form of an aqueous extract, by filtration. The influence exerted by this extract was effective in increasing and re-establishing the physiological efficiency of C. folia.

The presence of a minute amount of a commercial preparation of vitamine B also caused marked stimulation in the growth and cellulose-destroying activity of C. folia. The stimulation was most apparent in vitamine dilution 1-10,000 and 1-100,000.

The seeds, germinated and ungerminated, and seedlings of alfalfa, barley, and buckwheat secreted substances which functioned as a stimulating factor for C. folia. This organism was influenced both in growth and cellulose-destroying ability when small amounts of such substances in aqueous extract were added to the basic nutrient medium.

Another factor making for the increased physiological efficiency of cellulose-dissolving organisms was revealed in association studies. Association with Act. colorata, Azotobacter, B. subtilis, B. mycoides, and B. cereus, caused a stimulation in the growth and physiological activity of C. folia. In the association with Act. colorata, also a cellulose destroyer,

the stimulation occurred at the expense of the Actinomyces, which gradually died out. In the case of Azotobacter the stimulation came from association with the living cell, and not, as the experiment revealed, with extracts from the cell or culture medium. The nature of the factor operating seemed to be such that the accelerating effect was manifest only when C. folia was in association with the living Azotobacter cell. This points to a possible physico-chemical property of the factor which may prove to be a means of differentiation and identification. The association of C. folia with B. subtilis, B. mycoides, and B. cereus resulted in a marked stimulation of the cellulose destroyer due to the secretion of an accessory factor by the three associated bacteria. This factor was readily extracted from the cell.

In addition to the relatively minor elements of the medium, therefore, such as temperature, reaction, inorganic nutrients, moisture, and oxygen supply, which are capable of great variation, the form of cellulose employed; the presence of a minute amount of substance from plant tissue, furnishing an essential factor of stimulation; and the stimulative effects of association with other species, also apparently providing an essential food factor, are all of first importance as features of the medium for maximum efficiency in cellulose fermentation.

In the decomposition of green manures (alfalfa,

barley, buckwheat, and clover) by soil bacteria, substances were elaborated which exerted a stimulating influence upon C. folia when added in small amounts to the basic nutrient salt solution. This factor was added in the form of aqueous soil extracts. The stimulation was readily observed during the first two weeks' incubation. The decomposition of green manure by C. folia and Act. colorata was tested in a medium composed of sterile quartz sand and with environmental conditions under control as far as possible. The medium was deficient in the stimulating factor which was locked up in the plant tissue. In four days' time the organisms had liberated enough of the accessory factor to cause a considerable increase in the rate of cellulose decomposition. In the presence of the soil extracts containing the accessory factor from the decompositions of green manure by the soil bacteria, the lag period of four days was eliminated. With the factor of stimulation present in the medium, C. folia and Act. colorata started a vigorous decomposition of the green manure at once, and the plant tissue was largely destroyed at the end of the third day. The activation was due to the accessory factor present in the extract, and not to the association of these two cellulose destroyers, for stimulation from this source, as previous experiments showed, occurs only at the expense of the Actinomyces which gradually dies out. In this instance, the organism was present largely in the vegetative stage. From

the results obtained in this investigation it is possible to draw certain conclusions.

1. The physiological efficiency of a cellulose decomposing organism is influenced markedly by the composition of the medium in which it has been cultivated.
2. Unheated plant tissue furnishes the medium with a factor, referred to in this investigation as an essential food factor, which stimulates cellulose destroyers in growth and physiological activity. Maple leaves, and the seeds and seedlings of alfalfa, barley and buckwheat, provide the essential food factor for C. folia. That the yeast cell too may also supply this factor is suggested by the accelerating influence exerted upon C. folia by Yeast Vitamine (Harris).
3. The influence of association of micro-organisms upon cellulose fermentation is one of stimulation, and in the light of the experimental evidence and present understanding of the subject, it is legitimate to consider the stimulation obtained in this investigation as equivalent to the presence of the essential food factor. Act. colorata, Azotobacter, B. subtilis, By. mycoides, and B. cereus in association with C. folia resulted in increased growth and cellulose-decomposing ability on the part of this organism.
4. The essential food factor present in plant tissue is elaborated into the surrounding medium during the

decomposition of the plant material. In the decomposition of green manure by soil micro-organisms, the factor is elaborated into the soil.

5. These essential food substances stimulate cellulose fermentation. C. folia receives stimulation both in growth and physiological efficiency in the presence of this factor. If soil is deficient in these substances an activation of cellulose destroyers with the resultant stimulation in the decomposition process does not take place until the factor is liberated from the plant material which is allowed to decompose. Normally, however, through such agencies as decomposition of organic matter and microbial associations, soil contains essential food substances for the rapid and efficient decomposition of cellulose.
6. A maximum state of efficiency in cellulose fermentation may be obtained by providing a natural, chemically untreated form of cellulose, and through the agency of the essential food factor, under the influence of which cellulose destroyers maintain a high degree of physiological activity.

TRANSLATIONS.

The membrane consists of cellulose, as the bluish-violet color with chlor-zinc-iodide and the blue color with iodine and sulphuric acid indicate. Schütt. (8).

It was again the mixture or the interposition of foreign materials, giving to the resistant framework of certain organisms new properties and a variable composition which determined the adoption of the names of "fungine", "lignine", "lichenine", "medullene", which it is now necessary to eliminate from scientific nomenclatures. Payen. (9).

In the early stages of development of the plant the cell membrane consists throughout of pure cellulose; with progressive development, however, other materials, the incrusting substances, are deposited in the cell membrane or formed from it. To these depositions or incrustations in the unligified membranes may be added the bitter principle, pigments, tannin, and especially the pectin compounds - even the gummy - resiniferous - and slime-forming materials are classed with the incrustants. Entering the ligified membrane in addition are the never failing aromatic substances of wood, hadromal, conifer, and vanilla; later, cork or corky material. Lignin is one of the most important incrustants of the cell membrane, by which is generally understood chemically, those constituents which have a higher carbon content than cellulose

and with which cellulose is intimately associated, that is to say, either penetrated or enveloped. König. (26).

The hemicelluloses, hexosans as well as pentosans, are hydrolyzed and dissolved by dilute mineral acids (also in part by organic acids).

The incrusting substances consist of:-

- (a) The bitter principle, tannin, pigments, pectin compounds, the gummy and mucilaginous materials, the aromatic aldehyde (hadromal, conifer, and vanilla) likewise soluble in acids or in dilute alkalies.
- (b) The ester-like compounds cutin and suberin, soluble in acid and alkali, chiefly oxidizable, however, by weak oxidizing agents and in this way separable from true cellulose.

True cellulose or celluloses insoluble in dilute acids and alkalies, unoxidizable by weak oxidizing agents, but soluble in concentrated mineral acids and copper-ammonium (also in a solution of zinc chloride in two parts by weight of acetic anhydride). König (27).

- das Besenried - broom reed.
das Bohnenstroh - bean-straw.
das Brot - bread.
die Dattel - date.
die Eichel - acorn.
die Erbse - pea.
das Gemüse - vegetables.
die Gerste - barley.
die Gurke - cucumber.
der Hafer - oats.
das Kakaopulver - cocoa powder.
die Kapuzinerkresse - Indian cress; nasturtium.
die Kartoffel - potato.
das Kleeheu - clover hay.
die Kleie - bran.
der Kohl - cabbage.
der Kohlrabi - cabbage-turnip.
die Kokosnuss - coconut.
der Kopfsalat - head lettuce (Staudensalat).
der Kuchen - cake.
die Linsen - lentil.
die Möhre - carrot.
die Nusschale - nut-shell.
das Reismehl - rice flour.
die Rohfaser - crude fiber.

- der Roggen - rye.
die Rübe - turnip; rape.
der Sägespäne - sawdust.
die Wurzel - root.
das Schrot - groats.
der Selleriesalat - celeriac-salad.
der Sesam - sesame.
die Sojabohne - soy bean.
der Spinat - spinach.
die Steckrübe - turnip.
das Strohheksel - chopped straw.
(der Weisskohl -)
() white cabbage.
(das Weisskraut -)
die Weizenkleie - wheat bran.
die Wicke - vetch.
das Wiesenheu - meadow hay.

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B I B L I O G R A P H Y.

THE CELL MEMBRANE.

- Abrams, A. "The Effect of Chemical Reagents on the Micro-structure of Wood". Jour. Ind. Eng. Chem. (1921) 13; 786.
- Huhn, Fr. "Die Chemie der Pflanzlichen Zellmembran". Zeitschr. der Nahrungs- und Genussmittel. (1914). 27; 21.
- Klebs, G. "Beiträge zur Physiologie der Pflanzenzelle". Untersuch. aus. d. botan. Institut zu Tübingen (1888) 2; 489.
- König, J. "Die Zellmembran und Ihre Bestandteile in Chemischer und Physiologischer Hinsicht". Die landw. Versuchs- Stationen (1907) 65; 55.
- _____ "Chemie der Menschlichen Nahrungs- und Genussmittel". (1910) 3; 451.
- Krabbe, G. "Ein Beitrag zur Kenntnis der Structur und des Wachstums Vegetabilischer Zellhäute". Jahrb. f. wiss. Bot. (1887) 18; 347.
- Krauch, C. and W. v. d. Becke. "Über die Holzfaser bestimmung und ihre Mängel". Die landw. Versuchs- Stationen (1882) 27; 387.
- Mangin, L. "Sur la callose, nouvelle substances fondamentale existant dans la membrane". Comptes Rendus, Paris, (1890) 110; 644; Jour. Myc. (1894) 7; 140, U.S.D.A.
- Mohl, H. von. "The Vegetable Cell", (1851), Bot. Zeit., (1853) 11; 753, 769, (1858) 16; 373.
- Molisch, H. "Microchemie der Pflanze", (1921).

- Müller, K. "Die Chemische Zusammensetzung der Zellmembran bei verschiedenen Kryptogamen", Zeitschr. physiol. Chem. (1905) 45; 264.
- Nägeli, C. "Die Stärkekörner", Zurich, (1858), Bot. Mittheilungen, (1864), München, Bd. 2.
- Payen, M. "Memoires sur les Developpements des Vegetaux", (1844), 211.
- Potter, M. C. "On the Occurrence of Cellulose in the Xylem of Woody Stems". Ann. Bot. (1904) 18; 121.
- Ritter, G. J. "Distribution of Lignin in wood". Jour. Ind. Eng. Chem. (1925) 17; 1194.
- Schleiden, M. J. "Beitr. z. Phytogenesis", Müller's Arch. (1838).
- Schulze, F. "Beitrag zur Kenntniss des Lignins", Chem. Zentralblatt, (1857), 321.
- Schulze, E. Steiger and Maxwell. "Zur Chemie der Pflanzenzellmembranen. Hoppe Seyler's Zeitschr. physiol. Chem. (1890) Bd. XIV, XVI, XIX.
- Schulze, E. "Über die zur Gruppe der stickstofffreien Extraktstoffe Gehörenden Pflanzenbestandteile", Jour. f. Landwirts. (1904) 52; 1.
- Schütt, F. "Centrifugales Dickenwachsthum der Membran und Extramembranöses Plasma", Jahrb. f. wiss. Bot., (1899) 53; 598.
- Sorauer, P. Handbuch der Pflanzen-krankheiten (1921) 1; 939.
- Strasburgen, E. "Die Pflanzlichen Zellhäute", Jahrb. f. wiss. Bot., (1898) 31; 511.
- Timberlake, H. G. "The Development and Function of the Cell Plate in Higher Plants", Bot. Gaz. (1900) 30; 73, 154.

"Ontogenie der Pflanzenzelle", Handwörterbuch der Naturwissenschaften, (1915) 10; 777.

CELLULOSE CHEMISTRY.

Bain, J. W. and Kay, G. F. "The Effect of Heat upon Cellulose".
Roy. Soc. Can. (1924) 18; 269. Jour. Soc. Chem. Ind. (1923)
42; 140 A.

Bersch, J. "Cellulose, Cellulose Products, etc.", Trans. by Brannat
(1904) Phila.

Birtwell, C., Clibbens, D. A. and Ridge, B. P. "Chemical Analysis
of Cotton. Oxycellulose I." J. Text. Inst. (1925) 16; 13-
52 1.

Bray, M. W. and Andrews, T. M. "An Improved Method for the
Determination of Alpha, Beta and Gamma Cellulose". Jour. Ind.
Eng. Chem. (1923) 15; 377.

Castoro, N. "Beiträge zur Kenntnis der Hemizellulosen".
Zeitschr. physiol. Chem. 49; 100.

Cross and Bevan series:-

Cross, C. F. and Bevan, E. J. "The Ketonic Constitution of
Cellulose". Jour. Chem. Soc. (1901) 79; 366.

"Cellulose" (1916) London.

"Researches on Cellulose" (1895-
1900) London.

"Researches on Cellulose" (1900-
1905).

Cross, C. F., Bevan, E. J., and Sindall, E. W. "Wood Pulp and
its Uses". (1911) London.

- Cross, C. F. and Doree, C. "Researches on Cellulose". (1910-1921).
- Deming, H. G. "Some New Solvents for Cellulose and Their Action on This Substance". Jour. Amer. Chem. Soc. (1911) 33; 1515.
- Denham, W. G. and Woodhouse, H. "The Methylation of Cellulose". Jour. Chem. Soc. (1913) 103, 1735; (1914) 105, 2357.
- Dore, W. H. "The Proximate Analysis of wood". Jour. Ind. Eng. Chem. (1919) 11; 556.
- _____ "The Determination of Cellulose in Woods". Ibid. (1920) 12; 264.
- Duncan, R. K. "The Chemistry of Commerce". (1907) N.Y.
- Esselen, G. J. "Cellulose and its Derivatives", Chap. 27, "Colloidal Behavior", by R. H. Bogue (1924) 2; 627.
- _____ "A Few High Spots in the Chemistry of Cellulose". Jour. Ind. Eng. Chem. (1923) 15; 306.
- Gallagher, F. E. and Pearl, I. L. "Hydrolysis of Cellulose and Ligno-Cellulose". Congress of App. Chem. (1912) 13; 147.
- Green, A. G. and Perkins, A. G. "Note on the Constitution of Cellulose". Jour. Chem. Soc. (1906) 89; 811.
- Haas, P. and Hill, T. C. "An Introduction to the Chemistry of Plant Products" (1921) London.
- Haworth, W. N. and Hirst, E. L. "The Constitution of Disaccharides. Part V: Cellobiose (Cellose)". Jour. Chem. Soc., (1921) 119; 193.
- Hess, K. "Cellulose". Z. Angew. Chem. (1924) 37; 993.
- Heuser, E. "Textbook of Cellulose Chemistry". Trans. by West, C. J. and Esselen, G. J. (1924) New York.

- Hibbert, H. "Studies on the Chemistry of Cellulose. I. The Constitution of Cellulose". Jour. Ind. Eng. Chem. (1921) 13; 256. 13; 334.
- Hibbert, H. and Parsons, J. L. "Studies on the Reactions Relating to Carbohydrates and Polysaccharides". Jour. Soc. Chem. Ind. (1925) 44; 473 T.
- Hoffmeister, W. "Die Rohfaser und Einige Formen der Cellulose". Landw. Jahrb. (1888) 17; 239.
-
- "Die Cellulose und Ihre Formen Das Cellulosegummi". Die landw. Versuchs- Stationen: (1891) 39; 461.
-
- "The Quantitative Separation of cellulose-like Carbohydrates in Vegetable Substances". Die landw. Versuchs- Stationen (1897) 48; 401.
- Irvine, J. C. and Soutar, C. W. "The Constitution of Polysaccharides". Jour. Chem. Soc. (1920) 117; 489.
- Irvine, J. C. and Hirst, E. L. "The Constitution of Polysaccharides". Jour. Chem. Soc., (1923) 123; 518.
- Irvine, J. C. "Some Constitutional Problems of Carbohydrate Chemistry". Jour. Chem. Soc. (1923) 123 and 124; 908.
- Johnsen, B. and Hovey, R. W. "The Determination of Cellulose in Wood". Jour. Soc. Chem. Ind. (1918) 37; 132 T. Paper, (1918) Vol. 21.
- Johnsen, B. "The Estimation of Cellulose in wood". Jour. Ind. Eng. Chem. (1921) 13; 358.
- Kleiber, A. "Versuche zur Bestimmung des Gehalts einiger Pflanzen und Pflanzenteile an Zellwandbestandteilen an Hemicellulosen und an Cellulose". Die landw. Versuchs- Stationen. (1900) 54; 161.

- Kolthoff, I. M. "Significance of Adsorption in Analytical Chemistry". Pharm. Weekblad. (1921) 58; 233.
- König, J. "Die Bestimmung der Cellulose und ihre Mängel". Die landw. Versuchs-Stationen (1873) 16; 415.
- _____ "Chemie der Menschlichen Nahrungs-und Genussmittel". (1910) 3; 457.
- Lange, G. "Zur Quantitative Bestimmung der Cellulose". Zeitschr. Physiol. Chem. (1890) 14; 283.
- Lindsey, J. B. and Tollens, B. "Ueber Dextrose aus Sulfit-cellulose and aus Tannenholz". Ann. d. Chemie. (1892) 267; 370.
- Mahood, S. A. "Some Observations on the Determination of Cellulose in Woods". Jour. Ind. Eng. Chem. (1920) 12; 873.
- Mahood, S. A. and Jable, D. E. "Comparison of Wood Cellulose and Cotton Cellulose". Jour. Ind. Eng. Chem. (1922) 14; 727.
- _____ "The Chemistry of Wood". Jour. Ind. Eng. Chem. No.10 (1922) 14; 933.
- Miller, R. N. and Swanson, W. H. "Chemistry of the Sulphite Process". Jour. Ind. Eng. Chem. (1925) 17; 843.
- Monier-Williams, G. W. "The Hydrolysis of Cotton Cellulose". Jour. Chem. Soc., (1921) 119; 803.
- Ost, H. and Wilkening, L. "Die Verzuckerung des Zellstoffs". Chem.-Ztg. (1910) 34; 461.
- Ozawa, T. "Wood Cellulose". Jour. Chem. Ind., Japan, (1924) 27; 884.
- _____ Soc.
Renker, M. Jour./Chem. Ind. 1910, 28. 1269. "Bestimmungsmethoden der Cellulose". Berlin, (1910).

- Ritter, G. J. and Fleck, L. C. "Chemistry of wood". Jour. Ind. Eng. Chem. (1922) 14; 1050.
- Ritter, G. J. "Distribution of Lignin in wood". Ibid., (1925) 17; 1194.
- Rogers, L. "Industrial Chemistry", (1921) N.Y.
- Sachsse, R. "Die Chemie und Physiologie der Farbstoffe, Kohlenhydrate, und Proteinsubstanzen". Leipzig, L. Voss, (1877).
- Schorger, A. W. "The Chemistry of wood. Methods and Results of Analysis of Some American Species". Jour. Ind. Eng. Chem. (1917) 9; 556.
-
- "Seed Hairs of Milkweed". Ibid., (1925) 17; 642.
- Schulze, E. "Über die Cellulose". Chem.-Ztg. (1895) 19; 1465.
- Schulze and Castoro. "Beiträge zur Kenntnis der Hemizellulosen". Zeitschr. physiol. Chem. (1902) Vol.37; (1903) Vol.39.
- Sherrard, E. C. "Manufacture of Ethyl Alcohol from Wood Waste". Chem. Age, (1921) 29; 76, New York.
- Sherrard, E. C. and Blanco, G. W. "The Preparation and Analysis of a Cattle Food Consisting of Hydrolyzed Sawdust". Jour. Ind. Eng. Chem. (1921) 13; 61.
- Simon, O. and Lohrlich, H. "Eine Neue Methode der Quantitativen Cellulose bestimmung in Nahrungsmitteln und Faeces". Hoppe-Seyler's Zeitschr. physiol. Chem. (1904), 42; 55.
- Small, J. O. "Notes on the Viscosity of Cotton Cellulose". Jour. Ind. Eng. Chem. (1925) 17; 515.
- Stephenson, J. H. "The Manufacture of Pulp and Paper". (1922), Vol.3, Executive Comm. Voc. Ed. Prep. Paper Ind. of U.S. and Canada.

Tollens, B. "Les Hydrates de Carbone". (1896), Paris, 224.

_____ "On Plant Materials, Especially the Pentosans in
Feedstuffs, Their Determination and Properties". Jour. f.
Landw. (1896), 44; 171.

Uyeda, Y. "The Proximate Compositions of Korean Hemp and Ramie".
Jour. Ind. Eng. Chem. (1920) 12; 573.

_____ "Studies on Bast Fibers". Jour. Ind. Eng. Chem. (1921),
13; 141.

Willstätter, I. R. and Zechmeister, L. "Hydrolysis of Cellulose".
Ber., (1913) 46; 2401.

Wise, L. E. and Russell, W. C. "Contributions to the Chemistry
of Wood Cellulose". Jour. Ind. Eng. Chem. (1922) 14; 285.

Worden, E. C. "Nitrocellulose Industry", (1911), N.Y.

"The Preparation of Standard Cotton Cellulose and the Proposed
Methods of Analysis". Rpt. of Comm. Div. of Cellulose Chem.,
Amer. Chem. Soc., Jour. Ind. Eng. Chem., (1923) 15; 748.

CYTOHYDROLYTIC ENZYMES.

Bary, A. de. "Ueber Einige Sclerotinien und Sclerotienkrankheiten".
Bot. Zeit. (1886) 44; 377.

Brown, A. J. "An Acetic Ferment Which Forms Cellulose". Jour.
Chem. Soc. (1886), 432.

Brown, H. J. "On the Search for a Cellulose-Dissolving (Cyto-
hydrolytic) Enzyme in the Digestive Tract of Certain Grain-
Feeding Animals". Jour. Chem. Soc. (1892) 61; 352.

- Brown, H. J. and Morris, G. H. "On the Existence of a Cellulose-Dissolving Enzyme (Cyto-hydrolyst) in the Germinating Seed of the Grasses". Jour. Chem. Soc. (1890) 57; 497.
- Brown, H. "On the Distribution of Cytase in Cultures of Botrytis cinerea." Ann. Bot. (1917) 31; 489.
- Buller, A. H. R. "Enzymes of Polyporus squamosus". Ann. Bot. (1906) 20; 49.
- Euler, H. "General Chemistry of the Enzymes", (1912) trans. by Pope.
- Green, J. R. "On Vegetable Ferments". Ann. Bot. (1893) 7; 93.
- Kellerman, K. F. "Formation of Cytase by Penicillium pinophilum". U.S.D.A. Bur. Plant Ind., Circ. 113, (1912) 29.
- Newcomb, Fred C. "Cellulose Enzymes". Ann. Bot. (1899), 13; 49.
- Reinitzer, F. "Ue. d. Zellwandlösende Enzym d. Gerste". Zeitschr. physiol. Chem. (1897) 23; 175.
- Schellenberg, H. C. "Untersuchungen über das Verhalten Einiger Pilze gegen Hemizellulosen". Flora, (1908) 98; 257.
- Schmitz, H. and Zeller, S. M. "Studies in the Physiology of the Fungi". Ann. Missouri Bot. Gard. (1919) 6; 193.
- Ward, H. M. "A Lily-Disease". Ann. Bot. (1888-89) 2; 319.

CELLULOSE DECOMPOSITION BY MICRO-ORGANISMS.

- Behrens, J. Beiträge zur Kenntnis der Obstfaulnis". Cent. f. Bakt. etc., 1898.
- Buchanan, R. E. "General Systematic Bacteriology", (1925) 1; 247, Balt.

- Daszewska, W. Bul. soc. Bot. Geneve 2 ser., 4 No.7, pp.255-316.
(1912).
- Fred, E. B., Peterson, W. H. and Viljoen, J. W. "The Fermentation
of Cellulose by Thermophilic Bacteria". Abs. Bact. (1924),
8; 11.
- Gescher, M. V. "Über Zellulosezersetzende Bakterien". Faserforsch.
(1922) 2; 28. Abs. in Chem. Centralbl. (1922) 11; 276.
- Groenewege, J. "Untersuchungen über die Zersetzung der Zellulose
durch aërobe Bakterien". Bull. du Jardin, Botan. de Buitenzorg.
ser.IV, T. 2, Fasc.3, (1920) 261; Cent. f. Bakt. etc. (1921)
53; 414.
- Hoppe-Seyler, F. "Gärung der Cellulose". Ber. der Deutsch. chem.
Gesell. (1883) 16; 122.
- _____ "Über Gärung der Cellulose mit Bildung von Methan
und Kohlensäure". Zeitschr. physiol. Chem. (1886) 10; 201, 401.
- _____ "D. Methangärung d. Essigsäure". Ibid. (1887)
11; 561.
- Hutchinson, H. B. and Clayton, J. "On the Decomposition of Cellu-
lose by an Aerobic Organism (Spirochaeta cytophaga, n. sp.).
Jour. Agr. Sci. (1919) 9; 143.
- Jones, D. H. "Bacterial Action in the Septic Tank system of Sewage
Disposal". O.A.C. Bul. 267, (1918).
- Kellerman, K. F. and McBeth, I. G. "Soil Organisms which Destroy
Cellulose". Cent. f. Bakt. etc., (1912) 34; 63.
- _____ "The Fermentation of Cellulose".
Cent. f. Bakt., etc., (1912) 34; 485.

Kellerman, K. F., McBeth, I. G., Scales, F. M., and Smith, N. R.

"Identification and Classification of Cellulose Destroying Bacteria". Cent. f. Bakt., etc., (1913-14) 39; 502.

Krainsky, A. "Zur Frage der Zellulosezersetzung durch Mikro-organisms". Zhur. Opytn. Agron. Russ. Expt. Landw. (1913) 14; 255.

"Die Aktinomyceten und Ihre Bedeutung in der Natur".

Cent. f. Bakt., etc., (1914) 41; 649.

Kroulik, A. "Über Thermophile Zellulosevegärer". Cent. f. Bakt. (1912) 36; 339.

Lafar, F. "Handbuch der Technischen Mykologie" (1904-06, 3; 248.

Lieske, K. "Morphologie und Biologie der Strahlenpilze (Actinomyceten)". (1921), Leipzig.

Löhnis, F. and Lochhead, A. G. "Über Zellulose-Zersetzung". Cent. f. Bakt., etc. (1913) 37; 490.

"Experiments on the Decomposition

of Cellulose by Aerobic Bacteria". Cent. f. Bakt., (1922) 58; 430.

Macfayden, A. and Blaxall, F. R. "Thermophilic Bacteria". Trans. Jenner Inst. of Prev. Med., ser. 2, (1899) 162.

McBeth, I. G., Scales, F. M., and Smith, N. R. "Characteristics of Cellulose Destroying Bacteria". Science (1913) 38; 415.

McBeth, I. G., and Scales, F. M. "The Destruction of Cellulose by Bacteria and Filamentous Fungi". U.S.D.A. Bur. of Plant Ind. Bul. 266, (1913).

- Mitscherlich, E. "Zusammensetzung der wand der Pflanzenzelle"
Bericht über die zur Bekanntmachung geeigneten Verhandlungen d.
Kgl. preuss. Akad. d. wissensch. zu Berlin, (1850) 102.
- Mütterlein, C. "Studien über die Zersetzung der Zellulose im
Dünger und Boden". Cent. f. Bakt. (1913) 39; 167.
- Neuberg, C. "Vom Abbau der Cellulose in der Natur". Naturwissen-
schaften (1923) 30; 657.
- Omelianski, W. "Sur la fermentation de la cellulose". Compt.
Rend. (Paris) (1895) 121; 653.
- _____ "Sur la fermentation cellulosique". Ibid.,
(1897), 125; 970.
- _____ "Ueber die Gärung der Cellulose". Cent. f. Bakt.
etc., (1902) 8; 193, 225, 257, 289, 321, 353, 385.
- _____ "Die Histologischen und Chemischen Veränderungen
der Leinstengel unter Einwirkung der Mikroben der Pektin- und
Cellulosegärung". Cent. f. Bakt., (1904) 12; 33-43.
- _____ "Ueber die Trennung der wasserstoff- und Methan-
gärung der Cellulose". Cent. f. Bakt., etc., (1904) 11; 369.
- _____ "Über Methanbildung in der Natur bei Biologischen
Prozessen". Cent. f. Bakt., (1906) 15; 673.
- _____ "Zur Frage der Cellulosegärung". Ibid. (1913)
36; 472.
- Orla-Jensen, S. "Die Hauptlinien des natürlichen Bakteriensystems".
Cent. f. Bakt., etc. (1909) 22; 305.
- _____ "Vorschlag zur Einer Neuen Bakteriologischen
Nomenklatur". Ibid., 24; 477.

- Otto, H. "Untersuchungen über die Auflösung von Zellulosen und Zellwänden durch Pilze". Beiträge zur allgemeinen Botanik, G. Haberlandt. Bd. 1, (1918) Berlin.
- Popoff, L. "Über Sumpfgasfäulnis". Pflüger's Archv. f. d. ges. Physiol. (1875) 10; 113.
- Prazmowski, A. "Untersuchungen über die Entwicklungsgeschichte und Fermentwirkung Einiger Bakterien-Arten, Leipzig, (1880) 58.
- Primm, R. R. "Bacterial Digestion of Fiber or Cellulose". wis. Agr. Exp. Sta., Bull. 240, (1914).
- Pringsheim, H. "Über den Fermentativen Abbau der Zellulose". Cent. f. Bakt. (1912) 35; 302. Zeitschr. physiol. Chem. (1912) 78; 266.
-
- "Über Vergärung der Zellulose durch Thermophile Bakterien". Cent. f. Bakt. etc., (1913) 38; 513.
-
- "Die Beziehungen der Zellulosezersetzung zum Stickstoffhaushalt in der Natur". Mitt. Deut. Landw. Gesell. (1913) 28; 26, 43, 295.
- Pringsheim, H. and Lichtenstein, S. "Zur Vermeintlichen Reinkultur der Zellulosebakterien". Cent. f. Bakt., etc. (1923) 60; 309.
- Pringsheim, H. and Genin, A. "Hemicelluloses, VI. Fermentative Fission of Salep manna". Zeitschr. physiol. Chem. (1924) 140; 299.
- Sack, J. "Zellulose Angreifende Bakterien". Cent. f. Bakt., etc. (1924) 62; 77.

Scales, F. M. "Some Filamentous Fungi Tested for Cellulose Destroying Power". Bot. Gaz. (1915) 60; 149.

"A New Method of Precipitating Cellulose for Cellulose Agar". Cent. f. Bakt., etc., (1915) 44; 661.

Schellenberg, N. C. "Untersuchungen über das Verhalten Einiger Pilze gegen Hemizellulosen". Flora, (1908) 98; 257.

Tanner, Fred W. "Microbiology of Flax Retting". Bot. Gaz. (1922) 74; 174.

Thaysen, A. C. and Bunker, H. J. "The Destruction of Cellulose Fibers by Micro-organisms, and the Importance of the Microscope in the Study of this Destruction". Jour. Roy. Micros. Soc., (1923), 303.

Treoul, A. "Production de plantules amyliferes dans les cellules vegetales pendant la putrefaction. Chlorophyll cristallisee". Compt. Rend. de l'Acad. des Sci., Paris, (1865) 61; 432.

"Reponse a trois notes de M. Nylander concernant la nature des amylobacter". Ibid., (1867) 65; 513.

Van Iterson, C., Jr. "Die Zersetzung von Cellulose durch Aerobe Mikroorganismen". Cent. f. Bakt., etc., (1904) 11; 689.

Van Senus, A. H. C. "Bijdrage tot de kennis der cellulose gisting". Proefschrift, Leiden, (1891) 136.

Van Tieghem, P. E. L. "Sur le Bacillus amylobacter et son role dans la putrefaction des tissus vegetaux". Bul. de la Soc. Bot. de France (1877) 24; 128.

"Sur la fermentation de la cellulose". Compt. Rend. de l'Acad. des Sci., Paris, (1879) 88; 205.

Van Tieghem, P. E. L. "Identite du B. amylobacter et du Vibrion butyrique de Pasteur". Ibid., 89; 5.

"Sur le ferment butyrique (Bacillus amylobacter) a l'epoque de la houille". Ibid., 89; 1102.

"Remarques sur l'etat ou se trouvent les graines silicifiees de Saint-Etienne". Bull. de la Soc. Bot. de France, (1881) 3; 243.

Waksman, S. A. "Cultural studies of Species of Actinomyces". Soil Sci., (1919) 8; 87.

ROLE OF CELLULOSE-DESTROYERS ON GREEN AND BARNYARD MANURES.

Barthel, Chr. and Bengtsson, N. "Action of Stable Manure on the Decomposition of Cellulose in Tilled Soil". Soil Sci., (1924), 18; 185.

Deherain, P. "Recherches sur les fermentations du fumier de ferme". Ann. Agron. (1884) 10; 385.

"Sur la fabrication du fumier de ferme". Compt. Rend. (1884) 98; 377. 99; 45.

"Über die Buttersäuregärung in den Diffusionsgefassen". Zeitschr. d. Vereins- f. d. Rübenzucker- Ind. (1884) 21; 269.

Gayon, U. "Recherches sur la fermentation du fumier". Compt. Rend. (1884) 98; 528.

"Studies of Methane Fermentation". Mem. Soc. Sci. Physiq. et Naturel. de Bordeaux, (1884) 1; 51-52, 57.

Hebert, A. "Etudes sur la preparation du fumier". Ann. Agron. (1892) 18; 536.

- Hutchinson, E. B., and Richards, E. H. "Artificial Farmyard Manure". Jour. Min. Agr. (1921) 28; 398.
- Martin, T. L. "Decomposition of Green Manures at Different Stages of Growth". Agr. Exp. Sta., Cornell Univ., (1921) Bull. 406.
- Potter, R. S. and Snyder, R. S. "Decomposition of Green and Stable Manures in Soil". Jour. Agr. Res. (1917) 11; 677.
- Reiset, J. "Experiences sur la putrefaction et sur la formation des fumiers". Compt. Rend. (1856) 42; 53.
- Richards, E. H. "Dissolved Oxygen in Rainwater". Jour. Agr. Sci., (1917) 8; 331.
- Stoklasa, J. "Ueber die Wirkung des Stallmistes". Cent. f. Bakt., etc., (1908) 20, 301; Fuhling's Land. Ztg. (1907) 56, 409; Oesterr., (1907) 10; 440.
- Schloessing, T. "Sur la fermentation formenique du fumier". Compt. Rend. (1889) 109; 835.
-
- "Contribution a l'etude des fermentations du fumier". Ann. Agron., (1892) 18; 5.
- "Barnyard Manure", Farmers' Bull., 192, (1915).
- Whiting, A. L. and Schoonover, W. R. "The Comparative Rate of Decomposition of Green and Cured Cloved Tops in Soil". Soil Sci., (1920) 9; 137.

DECAY OF WOOD.

- Atkinson, G. F. "Studies of Some Shade Tree and Timber-Destroying Fungi". Cornell Univ. Agr. Expt. Sta., Bull. 193 (1901).
- Bray, H. W. and Staidl, J. A. "The Chemical Changes Involved

- during Infection and Decay of Wood and Wood Pulp". Jour. Ind. Eng. Chem. (1922) 14; 35.
- Buller, A. H. H. "The Destruction of Wooden Paving Blocks by the Fungus Lentinus lepideus Fr." Part 1. Jour. Econ. Biol. (1905), 1; 1.
-
- "The Biology of Polyporus squamosus Huds." Part 3. Ibid., (1906) 1; 101.
- Fritz, C. W. "Cultural Criteria for the Destruction of Wood-Destroying Fungi". Roy. Soc. Can. (1923) 17; 191.
- Hartig, R. "Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Nische". (1878).
- Long, W. H. and Harsch, R. M. "Pure cultures of Wood-Rotting Fungi on Artificial Media". Jour. Agr. Res. (1918) 12; 33.
- Rumbold, C. "Beiträge zur Kenntnis der Biologie Holzzerstörender Pilze". Naturw. Ztschr. Forst. u. Landw. Jahrg. 6. (1908) Heft 2; 81.
- Schacht, H. "Ueber die Veränderungen durch Pilze in Abgestorbenen Pflanzenzellen". Pringsheim's Jahrb. f. wiss. Bot. (1863) 3; 442.
- Schmitz, H. "Studies on the Physiology of the Fungi". Ann. Missouri Bot. Gard. (1919) 6; 93.
- Snell, W. H. "Studies of Certain Fungi of Economic Importance in the Decay of Building Timbers". U.S.D.A. Bur. of Plant Ind., Bull. 1053. (1922).
- Viljoen, J. A. and Fred, E. B. "The Effect of Different Kinds of Wood and Wood Pulp Cellulose on Plant Growth". Soil Sci., (1924) 17; 199.

Ward, H. M. "Penicillium as a Wood-Destroying Fungus". Ann. Bot. (1898) 12; 565.

Zeller, S. M. "Studies on the Physiology of the Fungi". Ann. Missouri Bot. Gard. (1916) 3, 439; (1917) 4, 93.

CELLULOSE DECOMPOSITION IN THE SOIL.

Ames, J. W. "The Function of Organic Matter in the Maintenance of Soil Fertility". Proc. Soc. Prom. Agr. Sci. (1917) 38; 28.

Anderson, J. A. "The Influence of Available Nitrogen on the Fermentation of Cellulose in the Soil". Soil Sci., (1926) 21; 115.

Barthel, Chr. and Bengtsson, H. "Action of Stable Manure on the Decomposition of Cellulose in Tilled Soil". Soil Sci. (1924) 18; 185.

Christensen, H. R. "Ein Verfahren zur Bestimmung der Zellulosezer-
setzenden F  hrgkeit des Erdbodens". Cent. f. Bakt. etc. (1910) 27; 449.

Lochhead, A. G. "Some Trends in Soil Microbiology". Sci. Agr., (1924) 4; 308.

L  hnis, F. "Handbuch der Landwirtschaftlichen Bakteriologie", Berlin, (1910).

McBeth, I. G. "Studies on the Decomposition of Cellulose in Soils". Soil Sci. (1916) 1; 437.

Potter, R. S. and Snyder, R. S. "Decomposition of Green and Stable Manures in Soil". Jour. Agr. Res. (1917) 11; 677.

Fringsheim, H. "Ueber die Verwendung von Cellulose als Energiequelle zur Assimilation des Luftstickstoffs". Cent. f. Bakt. etc., (1909) 23; 300.

"Weiteres über die Verwendung von Cellulose als Energiequelle zur Assimilation des Luftstickstoffs". Ibid., (1910) 26; 222.

Rahn, O. "Soil and Soil Problems", Thirteenth Rept. Mich. Acad. Sci., (1911).

Richards, E. H. "Dissolved Oxygen in Rainwater". Jour. Agr. Sci. (1917) 8; 331.

Russell, E. J. and Appleyard, A. "The Influence of Soil Conditions on the Decomposition of Organic Matter in the Soil". Jour. Agr. Sci., (1917) 8; 385.

Whiting, A. L. and Schoonover, W. R. "The Comparative Rate of Decomposition of Green and Cured Clover Tons in Soil". Soil Sci., (1920) 9; 137.

Waksman, S. A. "The Importance of Molds in the Soil". Soil Sci., (1918) 6; 137.

Waksman, S. A. and Heukelekian, O. "Microbiological Analysis of Soil as an Index of Soil Fertility. VIII Decomposition of Cellulose". Soil Sci., (1924) 17; 275.

Waksman, S. A. "Influence of Micro-organisms upon the Carbon-Nitrogen Ratio in the Soil". Jour. Agr. Sci., (1924) 14; 555.
"Chemical Nature of Soil Organic Matter". U.S.D.A., Bur. of Soils, Bull. 74.

FATE OF CELLULOSE IN THE ANIMAL BODY.

- Brown, H. J. "On the Search for a Cellulose-Dissolving (Cytolytic) Enzyme in the Digestive Tract of Certain Grain-feeding Animals". Jour. Chem. Soc. (1892) 61; 352.
- Choukevitch, J. "Etude de la flore bacterienne du gros intestin du cheval". Ann. de l'Inst. Pasteur, (1911) 25; 247.
- Distaso, A. "Sur un microbe qui desagregue la cellulose (Bacillus cellulosa desagregans n. sp.) Compt. Rend. (1911) 70; 995.
- Ekin, H. "Cellulose as a Feeding Stuff". Meddel. Centralanst. Föreskäv. Jordbruks. (1918) Vol.15. K. Lanthr. Akad. Handl. och Tidskr. (1918) 57; 278.
- Fries, J. A. "Intestinal Gases of Man". Amer. Jour. Physiol. (1906) 16; 468.
- Hall, A. D. "Rothamsted Experiments". (1919).
- Haubner, K. "Amts- und Anzeigeblatt für die Landwirtsch. Vereine des Königreiche Sachsen", (1854).
- Haubner, K. and Sussdorf. "Fütterungsversuche über die Verdaulichkeit der Pflanzenfaser bei Schafen Berichte über das Veterinärwesen im Königreich Sachsen, (1859) 104.
- Henneberg, J. W. J. and Stohmann, F. "Ueber die Bedeutung der Cellulose-Gärung für die Ernährung der Thiere". Zeitschr. f. Bio. (1885) 3; 613.
-
- "Beiträge zur Begründung einer Rationellen Fütterung der Wiederkäuer, Braunschweig". (1860-1864) - 2 pts.

- Holderfleiss. "D. Bed. d. verd. Anteils d. Rohfaser f. d. tier. Ernähr." C. f. Agr. Ch. (1896): 372.
- Hopffe, A. "Bakteriologische Untersuchungen über die Celluloseverdauung". C. f. B. I Abt., Orig., (1919) 83; 374, 531.
- Kellner, O. "Die Ernährung die Landwirtschaftlichen Nutztiere" (1909).
- Khouvine, Y. "Digestion de la cellulose par la flore intestinale de l'homme". Ann. de l'Inst. Pasteur, (1923) 37; 711.
- Krogh, A. and Schmit-Jensen, H. O. "The Fermentation of Cellulose in the Paunch of the Ox and its Significance in Metabolism Experiments". Biochem. Jour. (1920) 14; 686.
- Lohrlich, H. "Über die Bedeutung der Cellulose im Haushalte des Menschen". Hoppe-Seyler's Zeitschr. physiol. Chem. (1906) 47; 200.
-
- "Bemerkungen zur Frage der Celluloseverdauung beim Rinde und über die Methoden der quantitative Cellulosebestimmung". Ibid., (1910) 69; 143.
- Markoff, J. "Continuous Investigations on the Fermentation Processes in the Digestion of the Ruminant and Pig". Biochem. Zeit. (1913) 57; 1.
- Neuberg, C. "Der Harn etc." (1911) 1 Teil.
- Nuttall and Thierfelder. "Tier. Leben ohne Bakt. im Darmkanal". Zeitschr. physiol. Chem. (1895) 21; 109, (1897) 23, 231.
- Scheunert, A. "Verdauung Vorgänge im Enddarm (Dickdarm)". Handb. d. Biochem. (1909) 3; 134.

- Scheunert, A. and Lötsch, E. "Über die quantitative Cellulosebestimmung mit Hilfe der Methoden von Lange und Simon und Lohrlich". Hoppe-Seyler's Zeitschr. physiol. Chem. (1910) 65; 219.
- Scheunert, A., Grimmer and Hopffe. "Zur Frage der Celluloseverdauung". Zeitschr. physiol. Chem. (1915) 96; H.3.
- Schmidt and Strasburger. "Die Faeces des Menschen", Berlin (1905).
- Schmidt and Lohrlich. "Ue. d. Bed. d. Cellulose. f. d. Kraftwechsel d. Diabetiker". D. med. woch. (1907) H.47.
- Schotten, C. "Ueber die Fluchtigen Säuren des Pferdeharns und das Verhalten der Fluchtigen Fettsäuren im Organismus". Zeitschr. physiol. Chem. (1882) 7; 375.
- Tappeiner, H. "Die Darmgase der Pflanzenfresser". Ber. der Deutsch. Chem. Gesell. (1881) 14; 2375.
- _____ "Ueber Celluloseverdauung". Ber. der Deutsch. Chem. Gesell. (1882) 15; 999.
- _____ "Die Gase des Verdauungsschlauches der Pflanzenfresser". Zeitschr. f. Bio. (1893) 19; 228.
- _____ "Untersuchungen über die Gärung der Cellulose, Insbesondere über deren Lösung im Darmkanale". Zeitschr. f. Bio. (1884) 20; 52.
- _____ "Nachträge zu den Untersuchungen über die Gärung der Cellulose". Ibid. (1888) 24; 105.
- Thomson, W. H. "A Treatise on Clinical Medicine". (1918).
- Weiske. "Ist Cellulose ein Nahrungsmittel?" Chem. Centralblatt (1884) 385.

Wille, F. "Beiträge zur Kenntnis der Hemizellulosenverdauung bei Höheren Tieren und über das Vorkommen einer Hemizellulase in Tierischen Drüsen, nebst Einigen Ergänzungen zur Anatomie der Weizenkleie". Landw. Jahrb. (1918) 52; 411.

Zuntz, N. Gesichtspunkte z. krit. Stud. d. neueren Arb. auf d. Gebiete d. Ernährung". Landw. Jahrb. (1879) 8; 65.

Bronfenbrenner, J. "A New Indicator for Direct Reading of Hydrogen Ion Concentration in Growing Bacterial Cultures". Jour. Med. Res. (1918) 39; 25.

Bronfenbrenner, J. and Schlesinger, M. J. "Carbohydrate Fermentation by Bacteria as Influenced by the Composition of the Medium". Proc. Soc. Exp. Bio. and Med. (1918) 16; 44.

Brown, J. H. "Hydrogen Ions, Titration, and the Buffer Index of Bacteriological Media". Jour. Bact. (1921) 6; 555.

Charpentier, C. A. G. "Studien über den Einfluss des Rindvieh und Pferdestallmistes auf die Zersetzung der Zellulose in der Ackererde". Inaug. Diss. Tavaskhus (Finland) Thesis, Helsingfors. (1921).

Gurney-Dixon, S. "The Transmutation of Bacteria". (1919) Cambridge, 54.

d'Herelle, F. "Immunity in Natural Infectious Disease", Trans. by Smith, (1924) Balt.

ESSENTIAL FOOD SUBSTANCES.

- Ayers, S. H. and Mudge, C. S. "The Relation of Vitamines to the Growth of Streptococcus". Jour. Bact. (1922) 7; 449.
- Bachman, F. W. "The Vitamine Requirements of Certain Yeasts". Jour. Bio. Chem. (1919) 39; 235.
- Bailey, E. M. "The Potency of Some Commercial Vitamine Preparations as Compared with that of Dry Brewers' Yeast. Conn. Agr. Exp. Sta. Bul. 240, (1922).
- Bailey, S. F. "Hormone Mediums". Jour. Infect. Dis. (1925) 36; 340.
- Barr, C. E. and Bowie, W. T. "Ultraviolet Cytolysis of Protoplasm". Jour. Morph. (1923) 38; 295.
- Barr, C. E. "A Stimulating Effect of the Photocytolytic Products of Protoplasm". Jour. Med. Res. (1923) 44; 79.
- Bayless, W. M. "Principles of General Physiology". (1920) London.
- Bottomley, W. B. "Some Accessory Factors in Plant Growth and Nutrition". Proc. Roy. Soc., B, (1914) 88; 237.
- Bottomley, W. B. "The Significance of Certain Food Substances for Plant Growth". Ann. Bot. (1914) 28; 531.
-
- "Some Effects of Organic Growth-Promoting Substances (Auximones) on the Growth of Lemna minor in Mineral Culture Solutions". Proc. Roy. Soc. Lon., Ser. B, (1915-1917) 89; 102, 481. Beneficial effect of water

extract and fractionated "Bacterised peat" upon the growth of *Lemna minor* (water plant).

Bottomley, W. B. "The Isolation from Peat of Certain Nucleic Acid Derivatives". Proc. Roy. Soc. Lond., B., (1917) 90; 39.

"The Effect of Nitrogen-fixing Organisms and Nucleic Acid Derivatives on Plant Growth". Proc. Roy. Soc., B. (1919) 91; 83.

"The Effect of Organic Matter on the Growth of Various Water Plants in Culture Solution". Ann. Bot., (1920) 34; 353.

"The Growth of *Lemna* Plants in Mineral Solutions and in Their Natural Medium". Ann. Bot. (1920) 34; 345.

Bovie, W. T. and Hughes, D. M. "The Effects of Quartz Ultra-Violet Light on the Rate of Division of Paramecium caudatum". Jour. Med. Res. (1918) 39; 223.

Bovie, W. T. "An Approximation of the Value of the Absorption Index of Fluorite Rays in Protoplasm". Jour. Med. Res. (1918) 39; 239.

Bovie, W. T. "The Localization of the Physiological Effects of Radiation within the Cell". Jour. Med. Res. (1918) 39; 251.

Bovie, W. T. and Deland, G. A. "New Experiments on the Sensitization of Protoplasm to Heat by Exposure to Light

- of Short Wave-Length". Amer. Jour. Physiol. (1923) 66; 55.
- Bovie, W. T. "Relation Between Physiological Dominance and the Biological Effects of Rays". 25th Ann. Meeting. Amer. Roentgen Ray Soc., (1924).
- _____ "The Effect of Sunlight on Growth and Development." The Scientific Monthly, (1925) 21; 70.
- Bronfenbrenner, J. and Schlesinger, M. J. "Carbohydrate Fermentation by Bacteria as Influenced by the Composition of the Medium". Proc. Soc. Exp. Bio. and Med. (1918) 16; 44.
- Clark, H. A. "The Effect of Certain Chemicals on the Rate of Reproduction of Yeast". Roy. Soc. Can. (1921) 15; 47.
- _____ "The Rate of Formation and Yield of Yeast in Wort". Jour. Phys. Chem. (1922) 26; 42.
- Cole, S. W. and Lloyd, D. J. "The Preparation of Solid and Liquid Media for the Cultivation of the Gonococcus". Jour. Path. & Bact. (1917) 21; 267.
- Coward, K. H. and Drummond, J. C. "The Formation of Vitamine A in Living Plant Tissues". Biochem. Jour. (1921) 15; 530.
- Cowdry, E. V. "General Cytology". (1924) Chicago.
- Davidsohn, H. "Vitaminstudien". Biochem. Zeitschr. (1924) 150; 304.
- Davis, D. J. "Food Accessory Factors (Vitamines) in Bacterial

- Culture with Especial Reference to Hemophilic Bacilli".
Jour. Infect. Dis. (1917) 21; 392, (1918) 23; 248.
- Eastcott, E. V. "The Natural Distribution of Bios I and Bios II". Roy. Soc. Can. (1923) 17; 157.
-
- "The Formation of Bios in Infusions". Roy. Soc. Can. (1924) 18; 117.
- Eastwood, A. "Stimulants to Bacterial Variation". Jour. Hyg. (1924) 23; 317.
- Eijkman, C., van Hoogenhuyze, C. J. C., and Derks, T. J. "The Vitamine Content of Micro-Organisms in Relation to the Composition of the Culture Medium". Jour. Bio. Chem. (1922) 50; 311.
- Ellis, C. and Macleod, A. L. "Vital Factors of Foods". (1922) New York.
- von Euler, H. et al. "Growth Factors". Zeitschr. physiol. Chem. (1925) 144; 123, 132.
- Folin, O. and Macallum, A. B. "On the Blue Color Reaction of Phosphotungstic Acid (?) with Uric Acid and other Substances." Jour. Bio. Chem. (1912) 11; 265.
- Fulmer, E. I., Nelson, V. E. and Sherwood, F. F. "The Nutritional Requirements of Yeast". Jour. Amer. Chem. Soc. (1921) 43; 186.
-
- and White, A. "The Growth of Yeast on a Medium of Wholly Synthetic Origin". Jour. Bio. Chem. (1923) 57; 397.

- Fulmer, E. I. and Nelson, V. E. "Studies on Yeast". Jour. Infect. Dis., (1923) 33; 130.
- Fulmer, E. I. "The Effect of Ammonium Salts upon the Swelling of Colloids and upon the Growth of Yeast at Various Temperatures". Colloid Symposium Mon. H. N. Holmes, (1925) New York.
- Funk, C. and Dubin, H. E. "The Vitamine Requirements of Certain Yeasts and Bacteria". Jour. Bio. Chem., (1921) 48; 437.
- Funk, C. "The Vitamine". (1922) Balt.
- Gray, P. H. H. and Chalmers, C. H. "On the Stimulating Action of certain Organic Compounds on Cellulose Decomposition by Means of a New Aerobic Micro-organism that Attacks both Cellulose and Agar". The Ann. of App. Bio., (1924), Nos. 3 and 4, 11; 324.
- Guilliermond, A. "The Yeasts", Trans. by Tanner, (1921), New York.
- Gurwitch, A. "The Mitogenetic Rays". Bot. Gaz. (1925) 80; 224.
- Heller, V. G. "Vitamine B in Yeast". Jour. Bio. Chem. (1923) 55; 385.
- d'Herelle, F. "Immunity in Natural Infectious Disease". (1924) Balt.
- Hunter, O. W. "Production of Growth-Promoting Substance by Azotobacter". Jour. Agr. Res., (1923) 23; 825.

- Huntoon, F. M. "Hormone Medium". Jour. Infect. Dis., (1918) 23; 169.
- Itano, A. "Physiological Study of Azotobacter". Jour. of Bact., (1923) 8; 483.
- Komuro, "Preliminary Notes on the Cells of Vicia faba Modified by Roentgen Rays and Their Resemblance to Tumor Cells". Bot. Mag., Tokio, (1922) 36; 41. Jap. Jour. Bot. Abs., Tokio, (1923) 1; 37.
- Leichtentritt, B. and Zielaskowski, M. "Untersuchungen über den Wachstumsfördernden Faktor des Zitronensaftes". Biochem. Zeitschr. (1922) 131; 513.
- Linossier, G. "Les Vitamines et les Champignons". Compt. Rend. Soc. Biol., (1920) 83; 346.
- Livingston, B. E. "Further Studies on the Properties of Some Unproductive Soils". U.S.D.A. Bur. of Soils, Bull. 36, (1907).
- Lloyd, D. J. "On Vitamines, Amino Acids and other Chemical Factors Involved in the Growth of the Meningococcus". Jour. Path. Bact. (1916) 21; 113.
- Lucas, G. H. W. "The Effect on the Growth of Yeast of an Unknown Constituent of Malt". Roy. Soc. Can., (1921) 15; 53.
- _____ "The Quantitative Determination of Bios". Roy. Soc. Can. (1922) 16; 269.
- _____ "Chemical Study of Bios". Roy. Soc. Can.

(1923) 17; 157.

MacDonald, M. B. "Synthesis of 'Bios' by Yeast Grown in a Solution of Purified Nutrients". Jour. Bio. Chem. (1923) 56; 489.

_____ and McCollum, E. V. "The Cultivation of Yeast in Solutions of Purified Nutrients". Ibid., (1921) 45; 307.

McCollum, E. V. and Davis, M. J. "The Cause of the Loss of Nutritive Efficiency of Heated Milk". Jour. Bio. Chem. (1911) 23; 247.

Meyerhof, O. "Chemical Dynamics of Life Phenomena". (1924) Phila.

Miller, E. W. "The Effect of Cooking on the Water-soluble Vitamins in Carrots and Navy Beans". Jour. Bio. Chem. (1920) 44; 159.

Miller, W. Lash. "Wildiers' Bios". Science, (1924) LIX; 197.

_____ "Further Developments in the Study of Wildiers' Bios". Can. Chem. Metal. (1924) 8; 57.

Mockeridge, F. A. "Some Effects of Organic Growth-Promoting Substances (Auximones) on the Soil Organisms Concerned in the Nitrogen Cycle". Proc. Roy. Soc. Lond., B. (1917) 99; 508.

_____ "The Occurrence and Nature of Plant Growth-Promoting Substances in Various Organic Manurial Composts". Biochem. Jour. (1920) 14; 432.

Mockeridge, F. A. "The Occurrence of Nucleic Acid Derivatives in Nitrogen-fixing Bacteria". Ibid., (1924) 18; 550.

"The Formation of Plant Growth-Promoting Substances by Micro-organisms". Ann. Bot., (1924) 38; 723.

"The Occurrence of Nucleic Acid Derivatives in Nitrogen-fixing Bacteria". Biochem. Jour. (1924) 18; 550.

Nelson, V. E., Fulmer, E. I., and Cessna, R. "Nutritional Requirements of Yeast". Jour. Bio. Chem., (1921) 46; 77.

Orton, C. R., McCollum, E. V., and Simmonds, N. "Observations on the Presence of the Antineuritic Substance, Water-soluble B, in Chlorophyll-free Plants". Jour. Bio. Chem., (1922) 53; 1.

Pacini, A. J. P. and Russell, D. W. "The Presence of a Growth-Producing Substance in Cultures of Typhoid Bacilli". Jour. Bio. Chem. (1918) 34; 43.

Pasteur, L. "Mémoire sur la fermentation alcoolique". Ann. de Chemie et de Physique. (1860) 58; 323.

Pfeffer, W. Handbuch der Pflanzenphysiologie, (1897) 1; Leipzig.

Puri, Amar Nath. "Effect of Methyl and Ethyl Alcohol on the Growth of Barley Plants". Ann. Bot. (1924) 38; 745.

Robbins, W. J. "The Effect of Autolysed Yeast and Peptone on the Growth of Excised Corn Root-tips in the Dark". Bot. Gaz., (1922) 74; 59.

- Robertson, R. C. "Food Accessory Factors (Vitamins) in Bacterial Growth". VIII. Relation of Substances Formed by B. coli to the Growth of Yeast. IX. Growth of several Common Bacteria in a Synthetic Medium and Relation of Substances Formed by Them to Growth of Yeast. Jour. Infect. Dis., (1924) 34, 395; 35, 311.
- Robertson, T. B. "Experimental Studies on Cellular Multiplication". Biochem. Jour. (1921) 15; 595, 612.
- Schreiner, O., Reed, H. S. and Skinner, J. J. "Certain Organic Constituents of Soils in Relation to Soil Fertility". U.S.D.A. Bur. of Soils, Bull. 47, (1907).
- _____ and Skinner, J. J. "Nitrogenous Soil Constituents and Their Bearing on Soil Fertility". Ibid., Bull. 87 (1912)
- Shorey, E. C. "Some Organic Soil Constituents". U.S.D.A., Bull. 88, (1913) Bur. of Soils.
- Sims, H. des B. "The Preparation and Purification of Bios I". Roy. Soc. Can., (1924) 18; 116.
- Spinka, J. "Effect of Ultra-Violet Radiations on Accessory Food Factors". Biochem. Zeitschr. (1924) 153; 197.
- Thatcher, R. W. "The Chemistry of Plant Life". (1921). New York.
- Thjötta, P. and Avery, O. T. "Studies on Bacterial Nutrition". III. Plant Tissue, as a Source of Growth Accessory Substances, in the Cultivation of B. influenzae. Jour. Exp. Med., (1921) 39; 355.

- Tinti, M. "Einfluss von Vitaminen auf das Wachstum einiger Bakterienarten". Cent. f. Bakt., etc., (1923) Abt. 1, Orig. 90; 401.
- Verworn, M. "General Physiology" - an outline of the Science of Life; Trans. by F. S. Lee, (1899).
- Warburg, O. "Beiträge zur Physiologie der Zelle, insbesondere über Oxydationsgeschwindigkeit in Zellen". Ergebn. d. Physiol. (1914) 14; 253.
- Warrington, K. "The Effect of Boric Acid and Borax on the Broad Bean and Certain other Plants". Ann. Bot. (1923) 37; 629.
- Werkman, C. H. "The Rôle of Accessory Food Factors in the Physiology of Micro-organisms". Jour. Bact. (1926) 11; 86
- Whipple, B. K. "Water-Sol. B in Cabbage and Onion". Jour. Bio. Chem. (1920) 44; 175.
- Wildiers, E. "Nouvelle substance indispensable au développement de la levûre". La Cellule. (1901) 18; 313.
- _____ "La Cellule". (1901) 18; 313., *ibid.*, (1904) 21; 327. Devloo, (1906) 23; 361.
- Williams, R. J. and Seidell, A. "The Chemical Nature of Vitamines". Jour. Bio. Chem., (1916) 26; 431.
- Williams, R. J. "The Vitamine Requirement of Yeast". Jour. Bio. Chem., (1919) 38; 465.
- "Yeast Vitamine". Bull.No.14, Vitamine-Harris, The Harris Laboratories, Tuckahoe, New York.

