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THE FUNCTION OF VITAMINES IN THE METABOLISM OF SCLEROTINIA CINEREA.[1,2]

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I. Introduction.

In the spring of 1918 the writer began an investigation of some of the biochemical phenomena concerned in the parasitism of certain phytopathogenic fungi. *Sclerotinia cinerea* (Bon.) Schröter was selected as a good subject to begin with, since it is both a virulent parasite and a ready saprophyte, and undergoes its principal life history phases under cultural conditions in the laboratory. It was proposed to begin at the very bottom, and to ascertain first the bare fundamentals of its nutrition, since most questions of parasitism must in the last analysis be referred to the nutrition of the fungus. The mechanism of penetration of the host, the mechanical and chemical equipment of the latter for warding off the attacking fungus, are in many cases of parasitism more or less secondary to the primal question, does the host furnish the necessary nutrients for the particular fungus in question? It was, therefore, thought desirable to make a preliminary study of the fundamental nutritional requirements of *Sclerotinia*, the brown-rot organism of drupe and pome fruits.

Currie[3] has come the nearest to making this sort of a study. Using *Aspergillus niger*, he found that the only ions required are potassium, magnesium, sulfate and phosphate (PO_4^{+++}); calcium and iron, at least in so far as it is possible to exclude them from a medium by the most careful methods of purification, are not essential. Nitrogen from almost any mineral or organic form will serve. By varying the proportions of the mineral elements, nitrogen, and sugar, Currie could induce profound changes in the metabolism of this fugus, as measured by the by-products. The organism grows luxuriantly on a medium containing only the most highly purified salts, sucrose, and nitrate; it also thrives on practically all dead plant tissues and on many animal tissues; and it will readily attack and live on many living tissues, especially fruits, roots, and tubers. In other words, *Aspergillus* can run the gamut from parasitism to saprophytism.

When Currie's solutions were tried with the brown-rot fungus, failure was met with at every combination of pure salts and sugar. Pure organic

[1] Published with the approval of the Director as Paper No. 181, Journal Series, Minnesota Agricultural Experiment Station.

[2] This paper was submitted to the Ogden Graduate School of the University of Chicago as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[3] J. N. Currie, *J. Biol. Chem.*, 31, 15 (1917).

nitrogen compounds, such as asparagine and amino acids, did not improve the media. When, however, a small amount of prune, peach, or apple juice was added to them, the fungus grew at a normal rate. Decoctions from other plant materials were tried, with similar results. It was apparent that *Sclerotinia* had very different nutritional requirements from those of *Aspergillus*, and that some substance, or substances, was furnished by natural materials that were essential to the growth of the fungus. A long list of nitrogen compounds, sugars, pectin, inorganic salts, and salts of organic acids, under various conditions of acidity, temperature, and light, were tried, but in all cases where the substances involved were pure, and were *not contaminated by mother liquor from a plant juice*, growth either did not take place or was very weak. The writer presented these data before the Cleveland meeting of the American Chemical Society in September, 1918,[1] and at that time postulated that, for the proper growth of this fungus there is required, besides suitable mineral, nitrogen, and carbohydrate food, some accessory nutrients, which are furnished by plant materials, and especially by the natural hosts of the organism. Work had not progressed far enough at this time, however, to furnish any idea as to the nature of these accessory substances. When the writer took this problem to the University of Chicago in April, 1919, Dr. William Crocker immediately suggested a comparison with the work of Williams[2] on the vitamine requirement of baker's yeast. The latter had found that the yeast was dependent for normal growth on an adequate supply of a water-soluble vitamine in the medium, and that this vitamine was probably identical with the antineuritic vitamine B of animal nutrition.

When *Sclerotinia* was transferred on to media containing vitamine preparations from yeast and from wheat embryo[3] practically normal growth took place. The present paper deals with the further investigations of the vitamine requirements of *Sclerotinia cinerea*.

II. The Vitamines.

A brief review of the literature on vitamines insofar as it pertains to the work in hand follows.

There have been many arguments for and against the continued use of the term vitamine, principally on the basis of its implied amine structure, the proof of which has not as yet been satisfactorily established. However, the term "vitamine" is so convenient, and it is so widely used in both the scientific and the popular vernacular, that its continued use has much to recommend it, provided its meaning is generally understood and

[1] J. J. Willaman, *J. Ind. Eng. Chem.*, 10, 863 (1918).

[2] R. J. Williams, *J. Biol. Chem.*, 38, 465 (1919).

[3] The yeast vitamine was very kindly furnished by Dr. R. J. Williams, at that time student in the University of Chicago, and the wheat embryo vitamine by Prof. R. A. Dutcher of the University of Minnesota.

accepted. In the present paper the writer uses the term "vitamine" in the sense of the following definition: *Vitamines constitute a class of substances the individuals of which are necessary for the normal metabolism of certain living organisms, but which do not contribute to the mineral, nitrogen, or energy factors of the nutrition of those organisms.* This definition (1) does not assign any specific function to the vitamines, hence it will include the substances which may prevent disease, promote growth and reproduction, cause dormancy by their temporary absence, or affect any other phase of metabolism; (2) does not limit vitamines to either plant or animal organisms; (3) does not claim that a given metabolic activity, such as growth, will not take place in the absence of the vitamine, but that it will not take place normally; (4) does not imply anything as to the nature, properties, composition, or number of the vitamines; (5) does not exclude, but actually invites the use of the terms "vitamine A," "vitamine B," "vitamine C," and so on *ad libitum*, provided this specific use of the word is specifically defined in each case. "Vitamine" then is a generic term, like "carbohydrate" and "salt." The writer has taken pains to establish this meaning of the word as it is used in the following pages, so that his free and unqualified use of it will not be misunderstood. He also submits it to the critical consideration of others.

It has been established beyond all doubt that the higher animals are dependent on plant sources for their vitamines. This is at least the case for the 3 such vitamines so far described, the growth-promoting, fat-soluble A, the antineuritic B, and the antiscorbutic C. What the function of these substances in plants is has not been touched upon at all by investigators. Other vitamines for animals may be discovered by further research; and whether these will also be phytogenic can only be conjectured.

Certain of the plants themselves may have exogenous vitamines. Bottomley, in his well-known work on bacterized peat, isolated substances which aided materially in the normal development of a number of seed-plants. In some cases very small quantities of his preparations had marked stimulative properties when used as a fertilizer. The vitamines, which he called "auximones," were produced by the action of certain soil organisms on peat and are apparently connected with nitrogen fixation. The auximones, like vitamine B, were precipitated by phosphotungstic acid and by silver nitrate. Other investigators have corroborated Bottomley's results in some cases, but have failed to do so in others. A few papers by Bottomley and others are cited for further reference.[1]

[1] W. B. Bottomley, *Proc. Roy. Soc. (London)*, **88B**, 237 (1914); *Ann. Botany*, 28, 531 (1914); *Proc. Roy. Soc. (London)*, **89B**, 481 (1917); D. H. Jones, *Abstracts Bact.*, 1, 43 (1917); G. D. Knox, "The Spirit of the Soil," **1916**, London; O. Rosenheim, *Biochem. J.*, 11, 7 (1917).

Appleman[1] believes that potato tubers contain growth-promoting substances which are essential for the proper growth of sprouts. These substances are present in limited amounts; hence, his recommendation for a minimum size of seed piece in cutting tubers for seed purposes. He ascribes the "spindling sprout" disease of potato tubers to a low content of these growth-promoting substances.

Among the fungi other examples of vitamine requirements have been brought to light. Williams[2] found that baker's yeast is absolutely dependent on a supply of vitamine in the medium in which it is to grow. He could use the rate of multiplication of a single yeast cell in hanging drop culture as an index of the vitamine content of the medium. A single cell in a purely synthetic medium would fail to grow. If many cells were present, they became less sensitive to a lack of vitamine until, if a mass of yeast "as big as a pin head," as recommended by Pasteur, were used in a flask of purely synthetic medium, growth would proceed normally for a time without the addition of vitamine, ultimately, however, coming to a standstill. This indicates some accommodation on the part of the yeast to small amounts of vitamine. Williams isolated the vitamine from wheat embryo, milk, pancreatin, beer wort, and autolyzed yeast, and since these are typical sources of vitamine B in animal feeding experiments, he considered the yeast vitamine to be identical with the water-soluble B. It is interesting to point out in this connection that Harden and Zilva[3] found no vitamine B in beer, as measured by feeding tests with pigeons. Apparently the growth of the yeast in fermenting the beer had removed the bulk of the vitamine.

There are many examples in the literature of the ability of fungi and bacteria to grow on synthetic media with apparently no source of vitamine whatsoever. The most extreme of these media are those of Doryland[4] and of Pieper, *et al.*[5]

But in none of these cases has the growth of a single spore or bacterium been tried; and a loopful of the previous medium, or several hundred spores, may furnish an initial supply of vitamine sufficient to maintain the organism. Thus we do not know the real relation of any of these organisms to a vitamine supply.

Several instances of vitamines for bacterial growth are on record. Davis[6] reports that the influenza bacillus requires, besides the protein and mineral factors of its nutrition, two substances in very small amounts.

[1] C. O. Appleman, Agr. Exp. Sta., Md., *Bull.*, 212 (1918); *Science, N. S.*, 48, 319 (1918).

[2] R. J. Williams, *J. Biol. Chem.*, 38, 465 (1919).

[3] A. Harden and S. S. Zilva, *J. Inst. Brewing*, 24, 197 (1918).

[4] C. J. T. Doryland, *J. Bact.*, 1, 135 (1916).

[5] E. J. Pieper, C. J. Humphrey and S. F. Acree, *Phytopath.*, 7, 214 (1917)

[6] D. J. Davis, *J. Infec. Dis.*, 21, 392 (1917); 23, 248 (1918).

One of them is hemoglobin and the other is a vitamine-like substance, as yet unidentified, which is elaborated by a number of other bacteria when grown in the same culture with the influenza bacillus, and which can also be isolated from many animal tissues and from potatoes, carrots, and sprouted rice and wheat. There is evidence that the second factor is required to make the iron of the hemoglobin available. In fact, Davis suggests a possible role for other vitamines in making available iron, calcium, phosphorus, iodine, and amino acids. Miss Lloyd[1] maintains that such is the function of the vitamines for the meningococcus. This organism must have amino acids in its nutrition, but it is unable to obtain them from proteins unless the vitamines, which it obtains from body fluids, are present.

Shearer[2] found "an accessory food factor" in the nasal secretion, which greatly stimulates the growth of meningococcus, pneumococcus, *B. typhosus B. coli communis*, some fecal streptococci, and some throat bacteria. He did not demonstrate that the substance was absolutely essential to the normal growth of the organisms, but simply that it greatly stimulated their growth; hence his substance may or may not fall under our definition of a vitamine.

Pacini and Russell[3] report that the typhoid bacillus elaborates a vitamine in cultures; that the vitamine, when extracted from the cultures with alcohol and then with water, will promote the growth of experimental animals and that it is a clinical observation that patients recovered from an attack of typhoid often experience a marked acceleration in growth. Their feeding experiments cover too short periods, however, to be thoroughly convincing.

In summary of the present status of the studies of vitamines, it may be said that they are no doubt very widely distributed in both animals and plants; that in some cases plants obtain their vitamine from other plants, in some cases animals obtain theirs from plants, and in other cases plants from animals; that the best known vitamines are the A, B and C vitamines in the nutrition of higher animals; and that these vitamines have their origin in plants, but that their function there is unknown.

III. Methods Employed.

Description of *Sclerotinia* in Cultures.

Only the Monilia stage of the fungus appears in culture; that is, the mycelial felt, bearing the asexual spores, or conidia. On certain media and under certain conditions microconidia appear,[4] but the characteristic life cycle on cultural media is the growth of the mycelium and then the

[1] D. Lloyd, *J. Path. Bact.*, 21, 113 (1916).

[2] C. Shearer, *Lancet*, 1917, p. 59.

[3] A. J. P. Pacini and D. W. Russell, *J. Biol. Chem.*, 34, 43 (1918).

[4] W. D. Valleau, *J. Agr. Res.*, 5, 365 (1915).

production of the conidia. In this paper these two phases will be spoken of as vegetative growth and reproduction, without qualification.

The juice of peach, plum, prune, or apple is probably one of the best and most normal medium for the laboratory propagation of this fungus. There is a certain range of dilution of such juices for optimum growth; too concentrated or too dilute juices, especially the former, will check the growth entirely. Within 24 hours after inoculation with spores on the surface of the medium, a white, cottony growth can be seen. This rapidly spreads along the surface, at the same time sending hyphae into the solution. The central or older portion of the mat becomes very dense and felty, while the rim is thinner. A normal rate of growth is almost a centimeter a day in diameter for 5 or 6 days, and then considerably slower as the limits of the containing flask and the exhaustion of the medium are approached.

The greater bulk of the mycelium is contained in the now leathery mat, very few hyphae, especially from the older portion, extending down into the medium. The surface layers are white, while beneath this the mycelium is black. The medium always contains a gel of calcium pectate coagulated by the pectase secreted by the fungus. Depending on the concentration of the pectins, this gel varies from a little flocculent precipitate to a completely solid medium; and a portion of this gel is emmeshed by the hyphae and made a part of the mycelium, as has been reported on briefly by the writer.[1]

The spores may appear any time from the second to the sixth day. They may cover an area a centimeter in diameter, they may appear in scattered areas or circles, or they may cover practically the entire surface of the mat with a dense buff-colored mass. On a healthy mycelium these spores persist and remain virile for some time, at least for 2 or 3 weeks; while on unhealthy mycelium they may shrivel up within 4 or 5 days.

Both vegetation and reproduction apparently depend almost entirely on the character of the medium; they will take place in either light or darkness, and at temperatures at least from 15° to 35°, although the writer has not made any special attempt to determine the temperature limits or the formative effect of light, except insofar as to prove that they did not need to be taken into account in the present work.

Culture Methods.

100 cc. Erlenmeyer flasks, containing 25 cc. of culture solution, and incubated at 30° ± 1°, were used throughout these experiments. The surface of these solutions had a diameter of 6 cm. The amount of growth at any particular time was judged by inspection, by estimating the diameter of the mat in centimeters. In recording these observations, the figure indicating the size of the growth was often qualified by such terms as "thin," "heavy," "wrinkled," and in comparing various cultures, such

[1] J. J. Willaman, *Botan. Gaz.*, 1920 (in press).

modifications were taken into consideration. Admittedly this method is not exact; but practice has given the writer what he considers is all the accuracy warranted by the eccentricities and irregularities of such culture studies. The determination of the dry weight of mycelium was impracticable in these studies for two reasons: first, because of the difficulty of freeing the mats of fungus from mother liquor without losing parts of the mycelium; and second, because not only the total growth but the rate of growth was wanted. Duplicate, and sometimes triplicate, sets were employed, only the average of which are here reported.

The amount of sporulation was likewise estimated by inspection, and recorded by indicating one or more + signs (see Table I). These signs indicate the *relative* and not the *absolute* amount of spore tissue, as compared with the amount of vegetative growth. Thus, if a 1.5 cm. mat was completely covered with spores, it was recorded $\overset{++++}{1.5}$; if a 5 cm. mat bore about 1.5 cm. of spore tissue, it was recorded $\overset{++}{5}$, since the amount of sporulation relative to vegetation is small. This method was adopted because reproduction is not an entirely separate phase, since a certain amount of vegetation is first necessary. Hence, vegetation can be considered as an absolute, while reproduction is only a relative, function.

The fungus was perpetuated in the laboratory on soaked dried peaches in deep petri dishes, autoclaved at 12 pounds pressure for 10 minutes. The growth was rapid, and gave an abundant supply of spores.

Preparation of Media.

The fruit juices were prepared by soaking the sound, commercial desiccated material in an excess of water for 24 hours, steaming at 100° for about 20 minutes, then draining and squeezing through cheese cloth. Sometimes the pulp was moistened and squeezed a second time. If the juice was for future use, it was placed in plugged Erlenmeyer flasks and sterilized at 100° for about 30 minutes.

The basal medium for all the vitamine tests consisted of Currie's mineral solution,[1] plus asparagine for a source of nitrogen, plus sucrose. Its composition was as follows: Water, 1000 cc.; monopotassium phosphate, 1.0 g.; magnesium sulfate ($7H_2O$), 0.25 g.; ferrous sulfate ($7H_2O$), 0.05 g.; 0.1 *N* sulfuric acid, 10 cc.; asparagine, 20 g.; sucrose, 80 g.

The chemicals were of the ordinary C. P. grade, the sucrose being "rock candy." Several other trial media indicated that calcium, sodium and chlorine added nothing to the value of the above medium. The 0.1 *N* sulfuric acid was added to give a certain acidity to the medium comparable to that of fruit juice. *Sclerotinia* will endure a rather wide range of reaction and still grow and reproduce normally. Indicator determina-

[1] J. N. Currie, *J. Biol. Chem.*, 31, 15 (1917).

tions showed that the cultures in these tests had P_H values between 3.8 and 4.3, which is well within the possible range of this organism. The phosphate and asparagine in the medium served as buffers.

The media were autoclaved at 12 pounds pressure for 10 minutes. This inverted about $^3/_4$ of the sucrose, and made the solution faintly yellow, but neither of these changes is detrimental to *Sclerotinia*. Some tests on the effect of high temperature on the vitamines and on fruit juices, given later, showed no detectable injuries.

Many compounds of nitrogen other than asparagine were tried, and will be indicated in their proper place. Ammonia and nitrate nitrogen can be utilized, but rather poorly; and peptone is too highly contaminated with vitamine to be of service in a basic medium.

IV. Experimental Data.

Preliminary.

In order to show the course of development of the fungus on various dilutions of fruit juices, the data in Table I are presented.

TABLE I.

The Course of Development of *Sclerotinia cinerea* on Various Fruit Juices.

Culture No.	Medium: Cc. Prune juice.	Medium: Cc. Water.	Age of the cultures in days. 3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
107	25	0	3[a] +−	3·5 +	4 +	4 ++	4 ++	4·5 ++	5 +	5 +	5 +	5 +	5·5 +
107*a*	20	5	3 +−	3·5 +	3·5 +	4 +	4 +	4 +	4·5 +−	4·5 +−	4·5 +−	4·5 +−	4·5 +−
107*b*	15	10	2 −	3 +	3·5 +	3·5 +	3·5 +	3·5 +	3·5 +	3·5 +	3·5 +	3·5 +	3·5 +
107*c*	10 Peach juice.	15	1.5 −	2 −	2 −	2 −	2 −	2.5 −	2.5 −	2.5 −	2.5 −	2.5 −	2.5 −
108	25	0	3 −	3·5 +−	4 +	4·5 ++	5 ++	5 ++	5 +++	5·5 +++	5·5 +++	6 +++	6 +++
108*a*	20	5	3 −	3 −	3·5 +−	3·5 ++	4 ++	4 ++	4 ++	4 ++	4 ++	4 ++	4 ++
108*b*	15	10	3 −	3·5 −	3·5 +−	4 ++	4 ++	4·5 +++	4·5 ++++	4·5 ++++	4·5 ++++	4·5 ++++	4·5 ++++
108*c*	10 Apricot juice.	15	2 −	2.5 −	3 +	3 +	3 ++	3 ++	3 ++	3 ++	3·5 +	3·5 +	4 +
109	25	0	4 −	4 −	4·5 −	4·5 −	5 −	5·5 −	6 −	6 −	6 −	6 −	6 −
109*a*	20	5	2 −	4 −	4 +++	4·5 +++	5 ++	5 ++	5 ++	5 ++	5 ++	5 ++	5 ++
109*b*	15	10	3 −	3·5 −	3·5 +	4 +	4·5 −	4·5 −	5 −	5 −	5 −	5 −	5 −
109*c*	10	15	2 −	3 −	3 −	3 +−	3·5 +−	3·5 −	4 −	4 −	4 −	4 −	4·5 −

[a] See Section III for explanation of figures and signs. Complete growth is 6; greatest relative reproduction is +++++.

It will be noted (1) that practically complete growth is accomplished within 10 days; (2) that sporulation begins about the third or fourth day; (3) that the amount of vegetation is not proportional to the concentration of the juice, the fungus being unable to utilize the greater amounts of nutrients in the same degree that it does the lesser; (4) that reproduction is more abundant on the peach juice than on the others; (5) that the higher concentrations are not necessarily the optimum for reproduction.

Since peach juice appeared to be the best adapted for the work at hand, it was used in most of the succeeding experiments.

TABLE II.

Efficiency of Various Compounds of Nitrogen for *Sclerotinia*, Used in the Basal Medium of Salts and Sucrose.

Culture No.	Nitrogen compound and amount used in 25 cc. of medium. G.		Age of cultures in days. 4.	7.	10.
119	NH_4NO_3	0.25	0.5	0.5	0.5
127	NH_4NO_3	0.05	0.2	0.5	0.5
120	NH_4Cl	0.40	0.2	0.5	0.8
128	NH_4Cl	0.08	0.3	0.5	0.5
121	$NaNO_3$	0.50	0.5	0.5	0.5
129	$NaNO_3$	0.10	0.2	0.5	0.5
130	Egg white	0.10	1 −	3 −	6 +++
123	Urea	0.20	Germinated, but no growth		
131	Urea	0.05	Germinated, but not growth		
124	Glycine	0.30	0.5	3 ++	3.5 ++
132	Glycine	0.06	0.3	1.5	2 ++
141	Peptone (Witte)	0.50	3 −	4.5 −	6 −
140	Peptone (Witte)	0.10	0.8	1.5 −	2 −
142	Asparagine	0.10	0.5	1 −	1.5 −

Table II presents the results of experiments designed to test the effectiveness of various sources of nitrogen used in conjunction with the mineral-sucrose basal medium. At the time these tests were made, the vitamine hypothesis for *Sclerotinia* had just been suggested to the writer. On this basis, the apparent great differences in availability of these nitrogen compounds could easily be explained by assuming a vitamine contamination in the successful ones, and the lack of it in the unsuccessful. McCollum[1] and Williams[2] found considerable vitamine B in commercial lactose, and the latter found it abundant in Witte's peptone. which examples show the possibilities of vitamine contamination in many substances.

To test this point, a series of cultures were made, using various prepara-

[1] E. V. McCollum and M. Davis, *J. Biol. Chem.*, 23, 181 (1915).

[2] R. J. Williams, *Ibid.*, 38, 465 (1919).

tions of vitamines, together with several compounds of nitrogen. The results can best be presented in the form of curves. Fig. 1 shows the effect of using glycine and prune juice, and asparagine and prune juice, singly and in combination. The growth curves show unmistakably that the nitrogen of asparagine, of glycine, or of 2 cc. of prune juice is insufficient

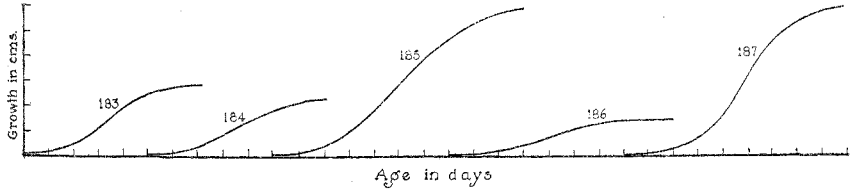

Fig. 1.—Availability of the nitrogen of glycine and of asparagine with and without the addition of prune juice.

183 25 cc. of basal medium + 2 cc. of prune juice 106.
184 " " " " " 0.1 g. of glycine.
185 " " " " " " " " " + 2 cc. of prune juice.
186 " " " " " " " " asparagine.
187 " " " " " " " " " + 2 cc. of prune juice.

for normal growth; that the vitamine content of the 2 cc. of prune juice alone, together with its small quantity of contained nitrogen, is insufficient for good growth; but that the fungus can make excellent growth on either asparagine or glycine if the growth-promoting material of the 2 cc. of prune juice is also present.

In Fig. 2 are given the results of some tests with wort used in the commercial growing of baker's yeast and with a vitamine prepared from the

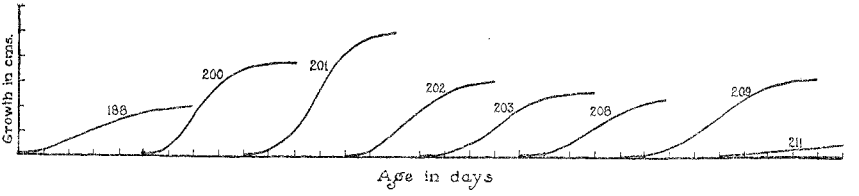

Fig. 2.—The presence of a growth-promoter in wort and in yeast vitamine, which enables *Sclerotinia* to utilize the nitrogen of $(NH_4)_2HPO_4$.

188 25 cc. of basal medium + 0.1 g. of $(NH_4)_2HPO_4$.
200 " " " " " " " " " " + 1 cc. of wort.
201 " " " " " " " " " " 5 " " "
202 " " " " " 1 cc. of wort.
203 " " " " " 5 " " "
208 " " " " " 0.1 g. of $(NH_4)_2HPO_4$ + 1 cc. of vitamine.
209 " " " " " " " " " " 5 " " "
211 " " " " " 5 cc. of vitamine.

wort by means of fullers' earth.[1] The growth curves indicate that diammonium hydrogen phosphate alone will not support growth; that the

[1] Both the wort and the vitamine were kindly furnished by Dr. R. J. Williams.

wort alone will support growth fairly well, but that the wort and the diammonium phosphate together constitute an excellent medium. Likewise, the vitamine preparation served to make the ammonia nitrogen more useful to the fungus, although the results are not striking. It should be remarked, however, that Cultures 208 and 209, containing the prepared vitamine, showed marked reproduction, a phase which will not be considered here.

Fig. 3 presents another series of cultures, using peptone. Lloyd's alkaloidal reagent, a variety of fullers' earth with a high adsorptive capacity towards alkaloids,[1] and also towards vitamine B,[2] was shaken with a solution of the peptone, in order to see whether it was a contained vitamine that gives this form of nitrogen its marked growth-promoting

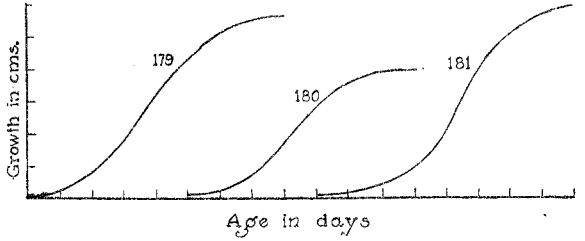

Fig. 3.—The presence of a substance in peptone (Witte) and in alcoholic extract of wheat embryo that promotes the growth of *Sclerotinia*.

179 25 cc. of basal medium + 0.1 g. of peptone.
180 " " " " " " " " " shaken with fullers' earth.
181 " " " " " " " " " " " " + alcoholic extract of wheat embryo.

properties with *Sclerotinia*. Also, a preparation of the alcoholic extract of wheat embryo was added to the "devitamized" peptone. The growth curves show that apparently there is an accessory food factor in the peptone that can be at least partially removed by shaking with fullers' earth, and that the wheat embryo extract contains a similar substance that can enhance the nutritive value of the peptone.

Accommodation of *Sclerotinia* to Low Supplies of Vitamine.

In order to test the behavior of *Sclerotinia* over long periods of time on media low in vitamine, the series shown graphically in Fig. 4 was arranged. For comparison, the growth on a typical fruit juice medium is included. It will be seen that in most cases on any medium the growth is greatest during the first week and a half; that its growth during this period is roughly proportional to the supply of vitamine; that its growth following this period is very slow. Whether this long-continued slow

[1] J. U. Lloyd, *J. Am. Pharm. Assoc.*, 1916, Apr.-May. We wish to acknowledge our indebtedness to Prof. Lloyd for our supply of this material.

[2] A. Seidell, *U. S. Public Health Report*, 31, 364 (1916).

growth represents a struggle on the part of the fungus to distribute the previously absorbed vitamine throughout a greater bulk of tissue, or whether during this period the fungus is synthesizing its own vitamine, or whether it is doing without it altogether, is not known at present. In most cultures, after the initial period of relatively rapid growth has passed, there is scarcely any visible change for the next 10 or 15 days except a

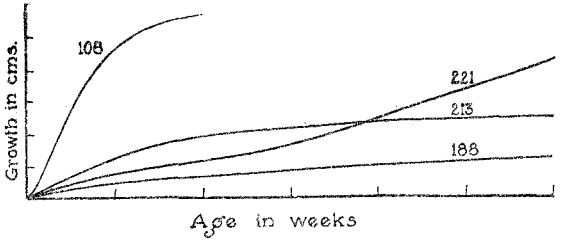

Fig. 4.—Growth of *Sclerotinia* over long periods of time on low levels of vitamine supply.

108 Peach juice medium (Table 1).

188 25 cc. of basal medium + 0.1 g. of $(NH_4)_2HPO_4$. No source of vitamine added.

213 25 cc. of basal medium + 0.1 g. of $(NH_4)_2HPO_4$ + 0.2 cc. of peach juice.

221 25 cc. of basal medium + 0.07 g. of asparagine. No source of vitamine added.

decided darkening of the mycelium. Then tufts of new, white mycelium begin to appear on the surface of the old. These slowly spread until in some cases they nearly cover the older portion. Although direct evidence is lacking, it is plausible that during this quiescent period autolytic changes are taking place in the oldest cells. This would liberate the contained vitamine into the medium or into the adjacent healthy cells, and a growth could recommence.

It is not known whether the better growth on asparagine than on diammonium hydrogen phosphate is due to a greater availability of the former or to the presence of contaminating vitamine. It should be pointed out here that in no case has the writer been sure that vitamines were entirely excluded from his cultures. As previously mentioned, in the method of inoculation used, several hundred spores are in most cases transplanted by the platinum wire in a single clump to the surface of the medium. In the case of yeast, as pointed out by Williams,[1] a single cell will show practically no division in a vitamine-free medium; whereas if many cells are present, the rate of division increases in proportion to their number. The writer has not had opportunity to make single spore inoculations. In the case of *Sclerotinia* the transfer must be made on the surface to secure normal growth, and attempts to do so with a single spore have so far been unsuccessful. It has often been observed, however, that single spores of this fungus in distilled water grow hyphae which

[1] R. J. Williams, *J. Biol. Chem.*, 38, 465 (1919).

are several hundred times the volume of the spore. How far it would develop in a nutrient medium complete except for lack of vitamine is a fact which should be ascertained.

In order to show that exhaustion of a medium and hence cessation of growth is often occasioned by the consumption of all the vitamine in the solution, the series of cultures shown in Fig. 5 was arranged. It is clear that in 223 and 224 growth ceased principally because the vitamine was consumed, and not because of an exhaustion of the nitrogen, minerals, or sugar; since when a new supply of vitamine was added the medium

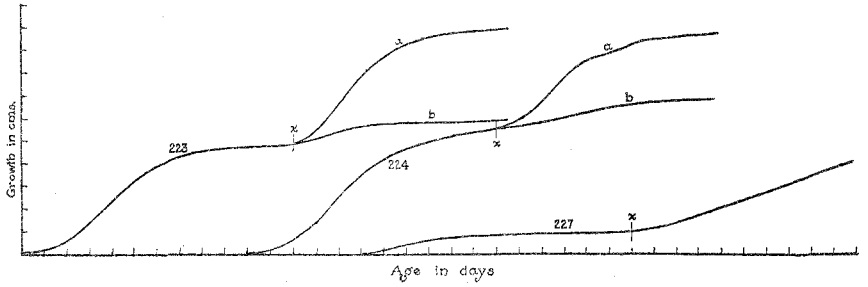

Fig. 5.—Exhaustion of the vitamine content of the medium.

223 25 cc. of basal medium + 0.07 g. of asparagine + 2 cc. of peach juice in each of 2 flasks. At *x* the fungus was removed from each flask; 4 cc. of vitamine preparation was added to *a*, and both were reinoculated.

224 is a repetition of 223.

227 25 cc. of basal medium + 0.07 g. of asparagine + 0.2 cc. of vitamine preparation. At *x* 4 cc. of vitamine was added, without removing the fungus.

could support another growth of the fungus. Number 227 brings out more vividly the reason for the failure of growth on a medium lacking in vitamine. This culture had made a growth of but one cm. in 11 days; the addition of some vitamine solution induced an immediate growth response. It is conceivable that the toxin explanation of failure of fungi to grow on "used" media, offered especially by Lutz,[1] in many cases is simply a case of exhaustion of a growth-promoting factor.

Preparation of Vitamines from Various Sources.

Since the evidence pointed to the fact that *Sclerotinia* requires a vitamine which occurs in several natural materials, which is rather heat stable, and which is adsorbed by fullers' earth, it was thought desirable to make vitamine preparations from a variety of sources, for the following reasons: (1) to obtain information concerning their possible identity with the water-soluble B; (2) to see whether a variety of sources would not furnish preparations which differed in their relative effect on growth and reproduction in *Sclerotinia*; (3) to gain some knowledge of the distribution and relative abundance of these substances in plant and animal materials; (4) and, in

[1] O. Lutz, *Ann. Mycol. von Sydow*, 7, 91 (1909).

a few instances, to obtain preliminary evidence concerning the possible role of vitamines in the phenomena of correlation in plants.

The method of obtaining the vitamines by means of Lloyd's alkaloidal reagent was used in all of these experiments. It adsorbs the vitamines from an acid solution, and releases them in an alkaline. This method was adopted because (1) it is characteristic of vitamine B, with which the writer, after a few preliminary trials, was convinced he was working; (2) it makes a more or less well defined separation of the principles studied from the other nutrients of the materials; (3) it tends to exclude the presence of toxic compounds, such as tannin, from the tests. The latter point is very important, since in one case a water extract of dried plum material inhibited the growth of the fungus entirely, while a very active vitamine preparation could be made from the material.

In the preliminary work with the juice of peaches and prunes, it was found that the presence of the pectin in the juice almost wholly inhibited the adsorptive property of the fullers' earth (see Table III, Numbers 222, 223, 227–230). When the pectin was precipitated with 2 volumes of 95% alcohol, and the alcoholic filtrate shaken with the earth, good adsorption was obtained. Williams and Seidell[1] found that acidified alcohol could be used to extract the vitamine from fullers' earth; since, however, they do not state the strength of acid or of alcohol used, it is not known whether their method and the writer's are really contradictory or not. It was decided to adopt this medium of about 65% alcohol for the preparation of the vitamines. It was slightly acid, by virtue of the natural acidity of most of the materials, and of the addition of a few drops of 1% sulfuric acid. It had certain other advantages over an aqueous medium, such as greater ease in filtration and the exclusion of starch and proteins in some of the materials.

Trials were conducted to ascertain the best conditions for the extraction, amount of Lloyd's reagent, time of shaking with it, effect of boiling the alcoholic solution with it, amount and kind of alkali to be used in liberating the adsorbed vitamine. For the last, Williams' method,[2] using saturated barium hydroxide solution, was adopted, since subsequent acidifying with sulfuric acid left no residual salt in the preparation. Table III gives the methods and results of these trials. The general procedure consisted in the precipitation and filtration of the pectin, the thorough mixing of the filtrate with the Lloyd's reagent in a mortar, shaking in a mechanical shaker for the specified length of time, filtering the fullers' earth through paper on a Büchner funnel, washing once with slightly acidified 65% alcohol, macerating the fullers' earth with the saturated barium hydroxide,

[1] R. R. Williams and A. Seidell, *J. Biol. Chem.*, **26**, 431 (1916).

[2] R. J. Williams, *Ibid.*, **38**, 465 (1919).

again shaking in the machine for 30 minutes, filtering through thin asbestos on a Büchner funnel, carefully neutralizing the filtrate to phenolphthalein with dil. sulfuric acid, then adding an excess of 5 to 10 drops of 0.1 *N* sulfuric acid to remove the barium completely and leave a faintly acid solution. This solution was now boiled for a time, in order to concentrate it and to bring the particles of barium sulfate into a filterable condition. After filtration the vitamine solution was brought to a volume corresponding to that of the original fruit juice used. The above practice of boiling for indefinite periods at atmospheric pressure was not detrimental to the vitamines. A great many trials by the writer, and the experience of almost all other workers with vitamine B, have shown that many hours of heating at 100°, either in alcohol or in water, provided the solution be acid, can be practiced with impunity.

In those cases where the treatment with fullers' earth was done in an aqueous solution of the fruit juice, the removal of the pectin was first accomplished as usual, then the alcohol removed from the filtrate by boiling. The residue was then made up to the original volume of the juice and treated with the earth.

An examination of the data in Table III reveals the following facts. (1) The first series shows that very little vitamine can be adsorbed in the presence of pectin. That the pectin itself can adsorb vitamine fairly well will be brought out later. (2) The second and third series show that boiling the alcoholic solution of vitamine with the Lloyd's reagent for 10 minutes is rather effective in removing the vitamine. Other trials, not presented here, showed, however, that this procedure is not quite so dependable as shaking at ordinary temperature, so it was abandoned. (3) Numbers 254 and 258 show that 2 hours of shaking are as effective as 5 hours in the adsorption of vitamine. (4) The third series makes a comparison concerning the amount of the fullers' earth required. Seidell[1] found that 5 g. per 100 cc. of autolyzed yeast removed practically all of the antineuritic factor. The data show that this is not the case with the materials used here. Nine g. removes appreciably more than does 4 g. Since it is very obvious that even 9 g. does not remove a very large proportion of the vitamine, 13 g. was used in all subsequent work. A larger amount was not feasible because the volume occupied by the fullers' earth and the amount of mother liquor occluded in it made comparative separations impossible. (5) Considerable variations in the amount of sporulation are noticeable in the table. This will be considered at some length in a later section.

From the above observations the following procedure was decided upon and rigidly adhered to in all the preparations of vitamine designed for

[1] A. Seidell, *U. S. Public Health Report*, **31**, 364 (1916).

TABLE III.

Efficiency of Various Methods of Preparing the Vitamine by Means of Lloyd's Reagent.

Culture No.	Material.	Kind of solution used with Lloyd's reagent.	Amt. of Lloyd's reagent per 100 cc. of solution. G.	Time of shaking. Hrs.	Activity of the various amounts of vitamine-containing solutions in 25 cc. of medium.														
					0 cc.			0.3 cc.			1 cc.			2 cc.			5 cc.		
					4 days.	7 days.	10 days.	4 days.	7 days.	10 days.	4 days.	7 days.	10 days.	4 days.	7 days.	10 days.	4 days.	7 days.	10 days.
222	Peach juice, vitamine not removed	....	..	..	0.3	0.6	0.8	0.8	2.5 —	2.5 —	...	...	...	2 —	4.5 —	5.5 —	...	...	...
227	Vitamine from 222	Aqueous, pectin present	7	10	0.3	0.6	0.8	0.3	0.5	0.8	1 —	1 +—	1 +—	...	...	...	2 ++	3.5 ++	4 ++
224	Residue from 227	....	..	..	0.3	0.6	0.8	...	...	...	...	...	...	1.5 —	4 ++	6 ++	...	...	...
248	Peach juice, vitamine not removed	....	..	..	0.3	0.5	0.7	1 —	2.5 —	4 —	0.5	3 —	4 —	...	...	...	...	...	...
250	Peach juice, same as 248, after removal of pectins	....	..	..	0.3	0.5	0.7	0.5	2.5 —	4 —	1 —	4 —	5 —	...	...	...	...	...	...
254	Vitamine from 250	Alcoholic	7	2	0.3	0.5	0.7	...	...	...	1 —	3 —	4 —	...	...	...	3 +++	4 ++++	5.5 ++++

252	Residue from 254	....	..	..	0.3	0.5	0.7	...	...	...	0.3	1.5 –	3 –	...	...	...	1 –	4.5 +–	6 +–
258	Vitamine from 250	Alcoholic	7	5	0.3	0.5	0.7	...	...	...	2 –	4 ++	4.5 ++	...	...	...	2 +–	4 +++	6 ++
256	Residue from 258	....	..	..	0.3	0.5	0.7	...	...	...	0.5	2.5 –	3 –	...	...	...	1 –	3.5 ++	6 ++
268	Vitamine from 250	Alcoholic	7	Boiled 10 min.	0.3	0.5	0.7	...	...	...	1 –	3 –	3.5 –	...	...	...	2 –	4 ++	5.5 +
266	Residue from 268	....	..	..	0.3	0.5	0.7	...	...	...	0.5	1 –	2 –	...	...	...	1 –	2.5 +–	5 –
300	Peach juice, pectin removed	....	..	..	0.2	0.4	1	...	...	...	...	...	...	2 –	5 –	5.5 –	...	...	...
296	Vitamine from 300	Alcoholic	4	Boiled 10 min.	0.2	0.4	1	...	...	...	...	...	...	1 –	2 –	2.5 +	...	...	...
297	Vitamine from 300	Alcoholic	9	Boiled 10 min.	0.2	0.4	1	...	...	...	...	...	...	1 –	2.5 –	3.5 –	...	...	...
298	Vitamine from 300	Alcoholic	4	Shaken 2 hrs.	0.2	0.4	1	...	...	...	...	...	...	0.8	1 –	2.5 –	...	...	...
299	Vitamine from 300	Alcoholic	9	Shaken 2 hrs.	0.2	0.4	1	...	...	...	...	...	...	1 +	3.5 ++	5.5 +	...	...	...

TABLE IV.

Relative Activity of Vitamine Preparations from Various Sources.

Culture. No.	Number and source of the vitamine preparation.	Activity of the various amounts of the preparations in 25 cc. of medium.											
		0 cc.			0.3 cc.			1.0 cc.			2.0 cc.		
		4 days.	7 days.	10 days.	4 days.	7 days.	10 days.	4 days.	7 days.	10 days.	4 days.	7 days.	10 days.
	Standard peach decoction (control)	0.3	0.6	0.8	1 −	2 −	2.5 −	1.5 −	3 +	4 +	1.5 −	4 +	6 ++
313	306 Peach decoction	...	...	...	0.3	1.3 −	2 −	1 −	2 +	2.5 +	1.5 +	2.5 ++	2.5 +++
370	334 Plum decoction	...	...	...	1 −	2 −	2 −	2.5 −	3.5 −	6 −	3 −	5 −	6 −
326	246 Mycelium of *Sclerotinia*	...	...	...	...	...	1.5 −	0.3	1.5 −	2.5 −	1 −	1 −	1.5 −
330	247 Mycelium of *Sclerotinia*	...	...	...	...	...	1 −	1 −	1.5 −	2 −	0.8	2.5 −	3 −
373	346 Mycelium of *Sclerotinia*	...	...	...	0.3	1 −	1 −	0.5	1.5 −	2 −	2 −	4 −	4 −
530	496 Mycelium of *Aspergillus*	...	...	...	...	0.8	...	...	1.5 +++++	...	...	2.5 ++++	...
533	506 Sphorophore of *Scleroderma*	...	...	...	...	1 −	...	...	1.5 −	...	...	4.5 −	...
376	362 Sphorophore of *Collybia*	...	...	...	1.5 +−	2.5 +−	3 +−	2 +	3.5 ++	3.5 ++	2 ++++	3 ++++	3 ++++
476	446*a* Baker's yeast, autolyzed	...	...	...	1.5 +	3 +	4 +−	2.5 ++	3.5 ++	4.5 +++	2.5 ++	5 +++	5.5 ++++

322	321 Pollen of maize	...	...	...	0.3	0.8	2	2	3.5	3.5	2	3	3.5
							−	++	+	+	+++	+++	+++
485	475 Bean sprouts	...	...	...	2	2.5	3	2	3	4.5	2.5	4	5.5
					+	−	−	+	+	−	+−	+	+
382	365 Sprouts of potato tubers	...	...	...	1	2	2.5	1	2.5	2.5	2	3	3
					−	+−	+−	+−	+	+	+	+	+
466	364*a* Wheat kernels	...	...	...	0.6	2.5	3.5	1	1.5	1.5	1.5	2	2.5
						−	−	−	−	−	−	−	−
379	364*b* Wheat kernels, sprouted	...	...	...	2.5	3.5	4	2	3.5	3.5	1	1.5	2
					−	−	−	−	−	−	−	−	−
443	364*c* Wheat kernels, sprouted	...	...	...	1.5	2	2.5	...	1	1.5	1	1.5	2
					−	−	−		−	−	+	+	+−
385	366*a* Terminal buds of bean plant	...	...	...	...	...	...	2	3	3.5	...	...	...
								+++	+++	+++			
386	366*b* Latteral buds of bean plant	...	...	...	...	...	...	2	3	3	...	...	...
								+	++	++			
387	367 Young tomato leaves	...	...	...	...	...	...	1	3	3.5	...	...	...
								+	−	−			
394	390 Rice polish	...	...	...	0.3	0.8	1.5	0.5	1	2	1	2.5	2.5
							−		−	−	+	+	+
437	415 Skim milk	...	...	...	0.7	1.3	1.5	0.8	1.5	2	1	2	2.5
						−	−		−	−	+−	+−	+−
469	447 Pancreatin	...	...	...	1	2.5	3	1.5	2.5	3	1.5	2.5	3.5
					−	−	−	+	+−	+−	−	+−	−

purposes of comparison. All quantities of materials were based on the dry matter content in grams of the sample in hand. Thus:

dry matter × 30 = volume of 65% alcoholic extract of the material,
dry matter × 4 = grams of Lloyd's reagent used,
dry matter × 32 = volume of saturated barium hydroxide solution used to extract the vitamine from the earth,
dry matter × 10 = volume of the final vitamine preparation.

In the case of a succulent material, *e. g.*, potato sprouts, a certain water content was assumed, sufficient 95% alcohol added to it to bring the resultant extract to 65%, the mixture ground with sand, enough 65% alcohol added to bring the total to the volume required by the above formula, the whole boiled for a few minutes, then filtered through paper, and washed once with a few cc. of 65% alcohol. The filtrate was then used in the manner described above, shaking with the fullers' earth for 2 hours. The final vitamine preparations were placed in bottles or test-tubes, stoppered with cotton, and sterilized at 100° for about 15 minutes.

Table IV presents the results of culture tests with the various vitamine preparations, roughly grouped according to the character of the material. Following is a description of the materials used, and a brief discussion of the results:

306. Dried apricots, soaked, steamed, and the juice expressed. This fruit, like all others of *Prunus* and *Malus*, is a natural host of *Sclerotinia*, and is a source of abundant vitamine supply. Sporulation is usually prominent in media containing vitamines from these sources.

334. Ripe plum. The material was collected in the summer of 1918, dried in an oven at 85–95°, ground to a powder, and stored in a bottle. After this rather vigorous treatment it still yields a very active vitamine preparation, at least for vegetative development.

246. Four mycelial mats of *Sclerotinia* that had grown on diluted peach juice.

247. Six mycelial mats of *Sclerotinia* grown on a synthetic medium plus vitamine. Both this and the preceding show moderate amounts of vitamine for vegetative purposes.

346. The fungus mats from Numbers 252, 256 and 258, Table III. Although these cultures had shown good sporulation, the vitamine preparation from them shows only vegetative activity. This phase will be discussed later.

496. Four mycelial mats of *Aspergillus niger*, grown on a synthetic medium free from added vitamine. This fungus grows vigorously, without any known source of vitamine, on the most highly purified of synthetic media, as Currie reports.[1] Currie, in correspondence with the writer, states that an extract of old mycelium or of vigorously sporulating tissue of *A. niger* strongly accelerates the growth of this fungus. The power of accommodation to low supplies of vitamine will be discussed later.

506. Sporophore of *Scleroderma sp.*, one of the common puff balls. The tissue was beginning to ripen. This is another material high in sporogenous tissue.

362. Sporophore of *Collybia sp.* This was selected to obtain another preparation from a spore tissue. It is high in the reproductive factor.

446*a*. Baker's yeast, autolyzed 48 hours. This is the standard source of strong preparations of vitamine B. The present preparation is seen to be very active in promoting both growth and reproduction in *Sclerotinia*.

[1] J. N. Currie, *J. Biol. Chem.*, 31, 15 (1917).

321. Pollen of maize, *Zea mays*. This was used in order to have a preparation from the spores of another plant. It is seen to be very active in producing reproduction in *Sclerotinia*; in fact, this is one of the most active preparations the writer has found.

475. Bean sprouts, species unknown, but it is the material used in "bean-sprout chop suey." The material was obtained from a Chinese shop in Chicago. According to Chick and Hume[1] the sprouting increases the vitamines in legumes in particular. Considerable vitamine is demonstrated in the present preparations.

365. Sprouts of potato tubers, *Solanum tuberosum*. The sprouts were about one cm. long.

364. Kernels of wheat, *Triticum sp.* 364*a*, dry kernels, before sprouting or soaking; 364*b*, sprouted 4 days in light, sprouts 2 cm. long; 364*c*, 8 days, sprouts (leaves) 8 cm. long. This series was designed to show whether any increase in vitamines could be demonstrated during sprouting, as has been claimed by Chick and Hume[1] for vitamine B, and by Chick and Hume and by Greig[2] for the antiscorbutic factor. In all 3 the kernel, roots and sprouts were included in the preparation. The tests with *Sclerotinia* were very irregular; so much so that the presence of some toxic material is indicated in the higher additions of vitamine. The only significance attached to these data is that they do demonstrate the existence of the vitamine, at least for vegetative purposes, in the wheat both before and after sprouting. There is some indication that reproduction is increased by the preparations from the older sprouts.

366. Scarlet runner bean, *Phaseolus multiflorus*. This plant is the classic example of correlation influences in plants. The lateral stem buds do not grow as long as the terminal bud is present and growing; if the latter is removed, the next lowest lateral bud begins to develop and becomes the growing point of the plant. It was suggested by Dr. William Crocker, of the University of Chicago, that the influence of vitamines might be concerned in these active and dormant buds. Number 366*a* is a preparation from terminal buds, 366*b* from lateral buds. Both preparations are seen to carry the vitamines in about equal amount. It is not felt that this test proves or disproves the possible controlling effect of vitamines in this plant, since only very small amounts of material were available, and since the technique of such tests with *Sclerotinia* is not as yet capable of showing positively small differences between preparations. However, the presence of vitamine in both kinds of buds is unmistakably demonstrated, with the terminal buds possibly promoting more reproduction than the lateral.

367. Young actively growing leaves of tomato, *Lycopersicum esculentum*. The positive results with this preparation are in line with the findings in animal feeding experiments that the green leaves of plants are plentifully supplied with both vitamine A and vitamine B.

390. Rice polish. This is the long-known source of vitamine B, the lack of which causes beri-beri, or polyneuritis. This preparation is only moderately active in promoting growth in *Sclerotinia*.

415. Skim milk. This is known to contain the water-soluble B in fairly high amounts, and a preparation of "protein-free milk"[3] is much used in animal feeding for this purpose. The present preparation is only moderately active towards *Sclerotinia*.

447. Pancreatin (Parke, Davis Co.). Eddy[4] has shown that pancreatic extracts contain considerable amounts of vitamine B. It is also demonstrated in the present preparation.

[1] H. Chick and E. M. Hume, *J. Roy. Army Corps*, **29**, 121 (1917).

[2] E. D. W. Greig, *Indian J. Med.*, **4**, 818 (1917).

[3] T. B. Osborne and L. B. Mendel, *Carnegie Inst. Pub.*, **156**, Pts. I and II (1911), Washington.

[4] W. H. Eddy, *J. Biol. Chem.*, **27**, 113 (1916).

The Number of Vitamines Involved and their Identity.

Several very important considerations should be pointed out in connection with the preceding experiments on vitamine preparations from different sources: (1) Every material examined, both plant and animal, supplies a vitamine active towards *Sclerotinia*. (2) Although all the vitamine preparations induce growth, only a relatively few induce reproduction. (3) The amount of reproduction in any given case is not necessarily proportional to the amount of vegetation. (4) The amount of growth is not proportional to the amount of vitamine preparation added. In fact, in some cases the higher additions promote less growth than the lower. Whether this is due to some toxic material extracted either from the material or from the fullers' earth is not known. (5) The cultures showing the greatest amount of reproduction are 322, from pollen; 376, from fungous sporophore; 385, terminal buds of *Phaseolus*; 476, yeast; 530, sporulating mycelium of *Aspergillus*; 533, sporophore of *Scleroderma*; 254 and 258, peach juice (Table III); and several of the peach juice media in Table I. It is noteworthy that most of these materials are characterized by high metabolic activity, either actual or potential. The fruit juices are not so characterized, but, on the other hand, they are the natural medium for this fungus. There is apparently some relation between the potential growth energy of a tissue and its value as a source of vitamine for the production of tissue in *Sclerotinia* with similar potential growth energy.

The above observations indicated the possible existence of two vitamine factors, one for vegetative growth and one for reproduction. There is a voluminous literature dealing with the question of vegetation *vs.* reproduction in plants. Among the factors so far discovered that control or affect this ratio are light, temperature, humidity, oxygen, age of the plants, supply of nutrients, and the ratio of certain nutrients, especially of the carbon and nitrogen. The latter factor is apparently very important for many seed plants, but has not been demonstrated to be of so much significance in the fungi. Among the latter plants light,[1] the supply of nutrients,[2] sudden reduction in the supply of nutrients,[3] and the dependence on a particular plant decoction,[4] are of importance in affecting reproduction in certain species. In the case of *Sclerotinia* these factors do not appear to operate in determining the relative proportion of the vegetative and reproductive phases, as was discussed in Section III. The controlling influences seem to be the substances of the nature of vitamines. So far as the writer is aware, no one has suggested this before for any species of

[1] G. H. Coons, *J. Agr. Res.*, 5, 713 (1916).

[2] V. I. Palladin, "Plant Physiology," trans. by Livingston (1918), Philadelphia (p. 294–299).

[3] A. J. Pieters, *Am. J. Botany*, 2, 529 (1915).

[4] G. P. Clinton, *Agr. Exp. Sta., Conn., Rpt.*, 1909–10, p. 753.

plant. Therefore, experiments were instituted to obtain information concerning this obscure phase of plant metabolism. Some of the specific problems involved are (1) the properties of the vitamine or vitamines; (2) the possible means of separating them from the materials containing them and from each other; (3) their relation to the already known vitamines; (4) their relation to the other nutritive materials of the medium.

As regards the chemical and physical properties of the vitamines in general, they have no common characteristics as a class. It is probable that water-soluble B contains nitrogen, since all preparations of it have contained nitrogen, and since Williams[1] has secured some evidence that it is an isomer of adenine. The occurrence of nitrogen in the other vitamines has not been investigated in any precise way. Fat-soluble A is destroyed by aeration at 100°;[2] the antiscorbutic C is destroyed by even lower temperatures;[3] vitamine B resists autoclaving for at least an hour,[4] 18 hours of boiling in benzene and in acetone,[5] and 24 hours of hydrolysis in 20% sulfuric acid;[6] Davis'[7] vitamine, for the influenza bacillus, withstands 100° for 1 to 2 hours; Miss Lloyd's[8] meningococcus vitamine withstands 120° for 45 minutes, and Shearer's[9] is not destroyed by conc. hydrochloric acid at 100° for 6 hours. Drying is fatal to the antiscorbutic C,[3] but not to the others, so far as tested. Vitamine B is adsorbed by fullers' earth,[10] and to a certain extent by animal charcoal[11] and by dialyzed iron;[12] vitamine C is not adsorbed by either or by a Berkefeld filter;[12] the meningococcus vitamine is adsorbed by filter paper.[8] Vitamine B is precipitated by phosphotungstic acid,[13] as are Bottomley's auximones.[14] All the vitamines so far tested are soluble in water and in aqueous alcohol, with the possible exception of fat-soluble A. All preparations of vitamine B, as well as the peat auximones,[15] give positive reactions with the Folin-Dennis color reagent for purines.[16]

[1] R. R. Williams, *J. Biol. Chem.*, **25**, 437 (1916); R. R. Williams and A. Seidell, *J. Biol. Chem.*, **26**, 431 (1916).

[2] H. Steenbock, P. W. Boutwell and H. E. Kent, *J. Biol. Chem.*, **35**, 517 (1918).

[3] A. F. Hess and L. J. Unger, *ibid.*, **35**, 487 (1918).

[4] E. V. McCollum and M. Davis, *ibid.*, **23**, 247 (1915).

[5] E. V. McCollum, *J. Am. Med. Assoc.*, **68**, 1379 (1917).

[6] E. A. Cooper and C. Funk, *Lancet*, **2**, 1266 (1911).

[7] D. J. Davis, *J. Infec. Dis.*, **23**, 248 (1918).

[8] D. Lloyd, *J. Path. Bact.*, **21**, 113 (1916).

[9] C. Shearer, *Lancet*, **1917**, 59.

[10] A. Seidell, *U. S. Public Health Report*, **31**, 364 (1916).

[11] E. A. Cooper, *Biochem. J.*, **7**, 368 (1913).

[12] A. Harden and S. S. Zilva, *Biochem. J.*, **12**, 93 (1918).

[13] R. R. Williams, *J. Biol. Chem.*, **25**, 437 (1916).

[14] W. B. Bottomley, *Proc. Roy. Soc. (London)*, **88B**, 237 (1914); O. Rosenheim, *Biochem. J.*, **11**, 7 (1917).

[15] O. Rosenheim, *ibid.*, *J.*, **11**, 7 (1917).

[16] O. Folin and W. Dennis, *J. Biol. Chem.* **12**, 239 (1912).

Thus, in the present state of our knowledge of the vitamines, we must recognize them by what they do and not by their constitution or physical and chemical properties. But since there is such a great variation in the properties of the vitamines as a class, there was hope that various treatments of the *Sclerotinia* vitamines would yield useful information.

Adsorption in Alcohol and in Water.—Although, for the reasons given previously, the writer used an alcoholic medium for the adsorption by Lloyd's reagent of the vitamines listed in Table IV, others have used a water medium for isolating vitamine B. A series of separations was made from both media for comparative studies, by making an alcoholic (65%)

TABLE V.

65% Alcoholic Compared to Aqueous Media for the Adsorption of Vitamines by Fullers' Earth.

Culture No.	Materials and treatment.	Growth at 10 days with various additions of vitamine to basal nutrient medium. 0.3 cc.	2 cc.	5 cc.
448	Vitamine prepared from fullers' earth in 65% alcoholic solution of peach juice	3·5 −	5 −	4 +++
451	Residue from 448	3·5 −	3 −	3 −
454	Vitamine prepared from fullers' earth in aqueous residue from 448	4 −	5 −	5 −
457	Residue from 454	3·5 −	6 −	6 ++
476	Vitamine prepared from fullers' earth in alcoholic solution of autolyzed yeast	4 +−	5 +++	6 ++++
479	Vitamine prepared from fullers' earth in aqueous residue from 476	2·5 −	3·5 +−	3 +−
482	Residue from 479	3 −	3·5 +	3 −
497	Autolyzed yeast	4 +++	5 +++++	6 +++++
500	Vitamine prepared from fullers' earth in aqueous solution of 497	2·5 −	3·5 +	4·5 +
503	Residue from 500	4 +−	5·5 ++	6 ++++
485	Vitamine prepared from fullers' earth in alcoholic extract of bean sprouts	3·5 −	5·5 −	6 +
489	Vitamine prepared from fullers' earth in aqueous residue from 485	2 −	3 −	3·5 +−
492	Residue from 489	2 −	2·5 −	3·5 −

extract of the vitamine-containing material, shaking with fullers' earth, then freeing the filtrate from alcohol by boiling, and using fullers' earth again on the aqueous residue; or the fullers' earth was used directly on the aqueous extract of the original. Table V presents the results of the tests.

It will be noticed in the first series that the reproductive factor is present only in the vitamine removed in an alcoholic medium, and in the residue left after all the treatments. In the second series practically all the reproductive factor is removed in the alcoholic medium, very little being in the aqueous separation or in the final residue. In the third series, the original autolyzed yeast is very rich in this factor; an aqueous treatment with Lloyd's reagent removed but a small amount; and the residue from the latter was still rich in it, showing that the failure of the aqueous treatment was not due to a lack of the vitamine. In the fourth series, the reproductive factor did not appear in any fraction in appreciable amounts, showing that there is nothing inherent in the alcoholic treatment to bring out this factor where it does not already exist.

Reference to Table III brings out the fact that in most of the trials given there, the cultures containing vitamine preparations show far more reproduction than those containing additions of the fruit juices themselves. All of these separations were made in a 65% alcoholic medium.

The above tests suggest the existence of two vitamines, one of which is adsorbed by fullers' earth more readily in alcohol, the other more readily in water.

Adsorption by Gels.—The writer had occasion to prepare two samples of pectin from peach and prune juice. When tried on nutrient media, both preparations gave indications of rather strong adsorption of vitamine, although each had been dissolved in water and reprecipitated by alcohol 3 times. Again, it has been found that Witte's peptone is heavily contaminated by vitamine. Attempts to remove it with Lloyd's reagent in either alcoholic or aqueous extract have failed. Coagulated egg white also brings down considerable vitamine with it. No differentiating action, however, towards the two vitamines by the gels has been noticed.

Resistance to Heat.—The marked resistance of the *Sclerotinia* vitamine to boiling water and to boiling alcohol has been noted. In order to make a more careful determination of this, a sample of vitamine was prepared from prune juice without subjecting it to a temperature above 65° at any stage of the process. The prunes were soaked in cool water, macerated, the juice squeezed out without heating, the pectins precipitated, Lloyd's reagent used in the alcoholic filtrate, the vitamine preparation freed from barium sulfate by centrifuging, and then concentrated *in vacuo* at 65°. This was then used for the temperature tests. The required amount of this vitamine for each culture was placed in a plugged test-tube and autoclaved for the indicated period at 12 pounds pressure. Flasks con-

taining the basal medium were sterilized separately, and then the autoclaved vitamines added to them. Table VI gives the data.

TABLE VI.

Effect of High Temperatures on the Vitamines of Prune Juice.

Culture. No.	Duration of autoclaving at 12 lbs. pressure. Min.	Growth at 10 days with various additions of vitamine to basal nutrient medium.	
		0.3 cc.	2 cc.
354	10	2.5 −	4.5 −
355	20	3.5 −	4.5 −
356	30	1.5 −	2.5 ++
357	40	3 −	3.5 +
358	50	1.5 −	4 −
359	60	1.5 −	2.5 ++
360	70	1.5 −	2.5 +
361	80	2 −	3.5 −

There is considerable immunity to the long-continued heating, although the cultures at the lower end of the series show somewhat less growth. There are also some slight indications that the reproductive factor is more abundant or active in the more strongly heated cultures.

The effect of prolonged heating on the nutritive value of a peach was tried by autoclaving a soaked peach for one hour at 12 pounds pressure. On inoculation, the fungus grew fairly well, and sporulated to a certain extent; but neither function was normal on this material. Such a test is hardly comparable to the tests on the isolated vitamine, however, because the high temperature decomposes the sugars with the formation of aldehydes, which are no doubt harmful to the fungus, and because most investigations have shown that the vitamines are less subject to injury while in the natural state than after isolation and purification.

Dialysis.—Dialysis through collodion was next tried. As a preliminary, 25 cc. of peach juice was dialyzed against running tap water for varying lengths of time. Table VII presents the results. The added vitamines represent equivalent amounts of the undialyzed original in each case.

The pectin of the juice evidently interferes with the dialysis through collodion, since there was still considerable vitamine left in Number 225 after 40 hours against distilled water. When the separated vitamine, however, is dialyzed, as in the second series, it all passes out of the dialyzing

bag during the first hour. This method apparently offers no means of differentiating between the two vitamines.

TABLE VII.

Rate of Dialysis of the Vitamines through Colloidion Treatment.

Culture. No.	Material.	Duration of dialyzing. Hours.	Growth at 10 days with various additions of vitamine to basal nutrient medium. 3 cc.	12 cc.
225	Peach juice	40	3 −	5 +−
221	Control, no vitamine added		0.8 0.3 cc.	... 2 cc.
348	Vitamine	Not dialyzed	2 −	3 +−
349	Vitamine	1	1 −	2.5 −
350	Vitamine	3	0.8	1
351	Vitamine	6	0.8	0.5
352	Vitamine	9	1	0.8

Effect of Drying.—During the summer of 1918 the writer collected a series of samples of plum fruits for proximate chemical analysis. Portions of each sample were pitted and then dried in an oven at 85–95° for 24 to 48 hours, no particular precautions as regards temperature and time being considered necessary for the purpose in mind. The material was brittle, and could easily be ground to a fine powder. These samples yielded a very active growth-promoting vitamine, as witnessed by Number 370, Table IV. Whether the apparent lack of the unproductive factor in this sample is common to the whole series has not been determined as yet.

Folin-Dennis Color Reagent.—The vitamine preparations listed in Table IV were tested with the Folin-Dennis phosphotungstic acid reagent for purines.[1] One cc. of the preparation, 1 cc. of the reagent, 2 cc. of saturated sodium carbonate solution, and 6 cc. of water were used in each case, so that a rough indication of the comparative intensities of the reaction could be obtained. Positive reactions were given by all preparations, Number 447, from pancreatin, was very weak, while Numbers 321, 362, 390 and 415 gave the strongest test. Reference to Table IV shows that of these 4, the first 2, from pollen and fungous sporophore, are very active towards *Sclerotinia* both for vegetation and for reproduction; while the latter two, from rice polish and from milk, are not very active towards *Sclerotinia*, but are conventional sources of vitamine B in animal feeding experiments. Although there is a possibility of these results indicating a lack of identity between the *Sclerotinia* vitamine and the water-soluble B, too great weight cannot be attached to them, since with the method employed the color test is not quantitative, and since the number of examples of the above correlation are too few.

[1] O. Folin and W. Dennis, *J. Biol. Chem.*, 12, 239 (1912).

Comparative Value to *Sclerotinia* and to Saccharomyces.—In order to determine whether a preparation active towards the brown-rot fungus is also active towards yeast, and in the same proportion, 4 preparations were tested out by Mr. R. J. Williams, using his method of judging the content of water-soluble B in a solution by its ability to promote multiplication in yeast.[1] Table VIII presents the results. The data for the *Sclerotinia* growths represent equivalent amounts of the preparations, and hence are comparable among themselves. The same is true for the yeast. The comparison we wish to make is that these preparations which are the more active for vegetative growth in *Sclerotinia*, Numbers 106 and 334, are the weaker towards yeast; and the two which are the more active for reproduction in *Sclerotinia*, Numbers 321 and 362, are also the more active towards yeast.

TABLE VIII.

Comparative Value of the Vitamine Preparations to *Sclerotinia* and to Yeast.

Material.	Multiplication of single yeast cell in 18 hours.	Growth of *Sclerotinia* at 10 days. Cm.
Prune juice 106 (Fig. 1).....	30	5.5 −
Vitamine 334, plum (Table IV).....	13	6 −
Vitamine 321, pollen (Table IV).....	60	3.5 +++
Vitamine 362, fungus sporophore (Table IV)...	300	3 ++++

Relative Proportion of Vitamines to Other Nutrients.

Since many plants have been shown to have their vegetative and reproductive phases more or less affected by the proportions of the elements in the nutrient medium, it was thought desirable to ascertain whether the activities of *Sclerotinia* vitamines would be modified by various proportions of the nutrient elements. A high carbohydrate to nitrogen ratio usually favors early reproduction, with scant vegetation, while a low ratio leads to vigorous vegetation and scant or delayed reproduction.

The triangle method of Schreiner and Skinner[2] for operating 3 variables against each other was used in arranging the cultures. The 3 variables chosen were the sugar, nitrogen, and vitamine contents of the medium. The sugar was furnished by sucrose alone in those cultures given prepared vitamines, and by sucrose and the sugars contained in the fruit juice when the latter was used for vitamine. The nitrogen was furnished mostly by asparagine, but partly by the contained nitrogen in the juices and in the vitamine preparation. In each case an analysis of the juice for sugar and for nitrogen, and of the vitamine preparations for nitrogen, was made and the calculated sucrose and asparagine added to bring the

[1] R. J. Williams, *J. Biol. Chem.*, 38, 465 (1919).

[2] O. Schreiner and J. Skinner, *Botan. Gaz.*, 50, 1 (1910).

totals to the desired figure. Fig. 6 indicates the constitution of the cultures in the various regions of the triangle. Many series of this kind of culture were run; some covered all the points of the triangle, others only

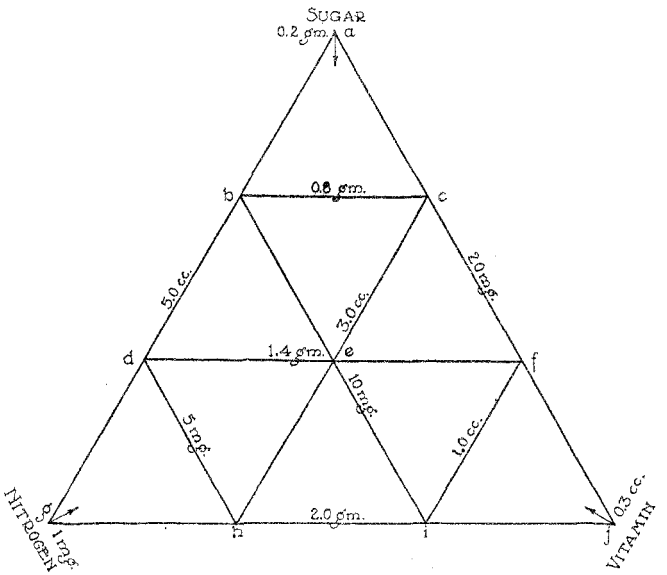

Fig. 6.—Diagram of nutrient media used to show the effect of varying the sugar, nitrogen, and vitamine contents.

certain areas. Instead of presenting all the data thus collected, Figs. 7 and 8 were constructed, representing a composite of all the cultures observed. The diagrams thus represent more or less ideal sets of cultures, although all the points on them have been verified several times. Fig. 7 represents the behavior of cultures which are supplied only with the vegetative vitamine factor; that is to say, so far as previous tests had shown, the preparations used did not show any indication of containing an appreciable amount of the reproductive factor. Fig. 8 represents the behavior of cultures supplied with preparations that had promoted both growth and reproduction in previous tests.

Considering Fig. 7 alone, first, the facts are brought out (1) that a moderate supply of all 3 factors, that is, near the center of the triangle, brings about maximum growth; (2) that vegetative growth is dependent more on the energy (sugar) supply than on the nitrogen, since point *g*, with the minimum of nitrogen, shows a larger growth than point *a*, with the minimum of sugar, and since the *area* of maximum growth is eccentric, lying towards the region of low nitrogen; (3) that the vitamine factor is intermediate in its influence, since vegetation is greater in area *f*, *i*, *j* than area *a*, *b*, *c*, but less than in area *d*, *g*, *h*.

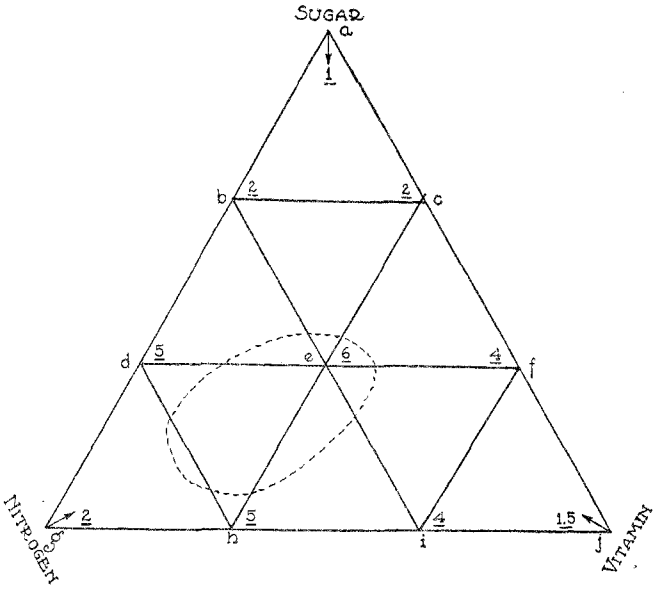

Fig. 7.—The growth of *Sclerotinia* on the media indicated in Fig. 6, when the added vitamine will promote only vegetation. The dotted line encloses the area of greatest vegetation.

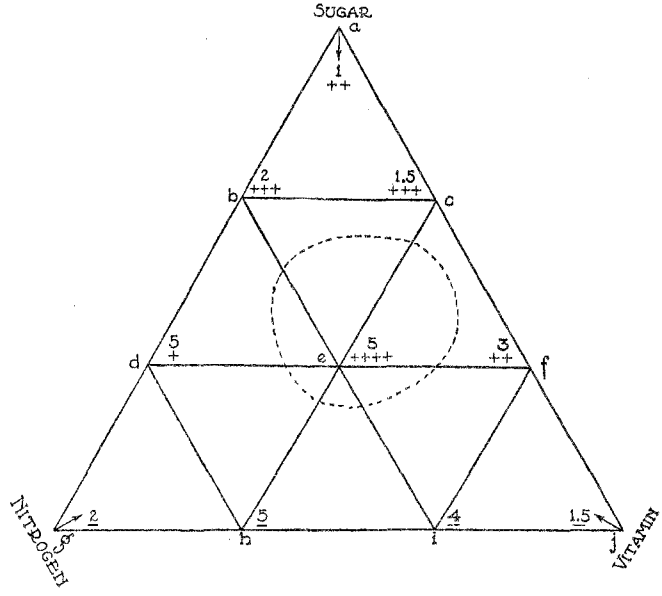

Fig. 8.—The growth of *Sclerotinia* on the media indicated in Fig. 6, when the added vitamine will promote both vegetation and reproduction. The dotted line encloses the area of greatest reproduction.

In Fig. 8, where the cultures show both vegetation and reproduction we see (1) that a moderate supply of all the factors—energy, nitrogen, and vitamine—brings both maximum vegetation and maximum reproduction near the center of the diagram; (2) that the more highly sporulating cultures lay toward the areas of higher vitamine, higher nitrogen, and hence lower sugar, the actual area of greatest reproduction being eccentric, as was the area of greatest vegetation in Fig. 7, but lying opposite to the latter; (3) that hence reproduction is more dependent on nitrogen than on sugar, whereas for vegetation the reverse is true; (4) that the same relations concerning vegetation hold as in Fig. 7, except that the points *c*, *e* and *f* show a little smaller vegetation, due to the energy demand of reproduction. These points fall in the area of lesser sugar supply; points

TABLE IX.

Effect of Varying the Nitrogen and Phosphorus Contents of the Medium on the Activities of the Vitamines.

25 Cc. of Basal Medium[a] used in Each Experiment.

Culture. No.	Constitution of media. Asparagine. G.	Vitamine. No.	Salt added.	Growth at 8 days with various additions of vitamine. 0.3 cc.	2 cc.
508	0.05	334		2.5 −	5.5 −
510	0.02	334		3 −	5.5 −
512	0.05	334	0.07 g. KH_2PO_4	3 −	5 −
514	0.05	362		2 −	3.5 +++++
516	0.02	362		3 −	3 ++++
518	0.05	362	0.07 g. KH_2PO_4	2.5 −	4 ++++
520	0.05	321		2.5 −	4 ++
522	0.02	321		2.5 −	4.5 ++
524	0.05	321	0.07 g. KH_2PO_4	5.5 −	5.5 +
526	0.05	0.3 cc. 334 + 0.3 cc. 321			2 −
527	0.05	2.0 cc. 334 + 2.0 cc. 321			4 −
528	0.05	2.0 cc. 334 + 0.3 cc. 321			5 −
529	0.05	0.3 cc. 334 + 2.0 cc. 321			4.5 ++

[a] The basal medium contained salts (including phosphate) and sugar, but no nitrogen. The indicated additions of asparagine, therefore, constitute the sole source of nitrogen; but the indicated additions of KH_2PO_4 are in addition to that already present, which is 0.025 g. per culture.

b and *d* do also, but here the greater vitamine supply promotes greater utilization of the sugar.

Still further evidence was obtained that exaggerations in the proportions of nutrients can bring about only a quantitative and not a qualitative difference in the relative amount of vegetation and reproduction in *Sclerotinia*. Three preparations of vitamine were selected that showed very pronounced tendencies in the direction of their activities. Numbers 321 and 362 were very active in promoting reproduction, and Number 334 had showed good growth-promoting powers, but no power for reproduction. The attempt was made to reverse these activities by suitable proportions of nitrogen, phosphorus, and sugar in the medium. Table IX gives the composition of the media and the results. It will be seen that each vitamine was tested in two different concentrations of asparagine, and then with high additional phosphate. Vitamine 334, which in all previous tests had shown only vegetative activity, could not induce reproduction with any change in the nitrogen and phosphorus supply that was tried. The other vitamine preparations, which had previously been shown to promote reproduction, could not be made to lose this activity by varying the nitrogen and phosphorus supply. When combinations of two preparations were tried, the reproductive factor was suppressed in all cases except in that in which the reproductive vitamine was in excess.

Discussion.

It has been conclusively demonstrated that *Sclerotinia cinerea* cannot thrive on a medium that does not contain at least a certain proportion of the extract from some plant or animal material. These materials do not supply the previously recognized nutrient factors, such as salts, nitrogen, and energy. The facts point to the existence in the plant and animal extracts of a substance similar in nature to the now well-recognized vitamine B of the higher animals. Further examination reveals the fact that this substance has at least one characteristic in common with vitamine B: its adsorption by fullers' earth in an acid medium, and its release again in an alkaline medium. Furthermore, preparations from the conventional sources of vitamine B are found to be potent towards *Sclerotinia*. These coincidences point to the possible identity of the vitamine for the animals and the vitamine for the fungus.

The tests shown in Table IV of the vitamine preparation from the leaves and buds of plants, seeds, seed sprouts, tuber sprouts, rice polish, sporophores and mycelia of fungi, the mycelium of *Sclerotinia* itself, pollen of maize, fruit juices, wort, autolyzed yeast, milk, and pancreatin, show two outstanding features: One is that every material examined furnishes a substance which activates the growth of *Sclerotinia*. The other is that most of the materials enable only vegetative growth to take place, while

a few enable the fungus to sporulate as well. These results, together with the properties of the vitamine as described in the preceding pages, leads to the consideration of two distinct lines of evidence: one for the existence of two vitamines, and one for the existence of only one.

The following points can be enumerated in favor of the two-vitamine hypothesis: (1) There is a considerable body of evidence to show that the reproductive factor is more readily adsorbed by fullers' earth in an alcoholic medium, while the vegetative factor is more readily adsorbed in an aqueous medium. Materials demonstrated to give rise to both activities, *e. g.*, autolyzed yeast and peach decoction, will yield preparations stronger in the one activity or in the other, depending on the medium used in the preparation (Table V). (2) There is some evidence that prolonged high temperatures destroy the vegetative factor more quickly than the reproductive (Table VI). (3) A vitamine preparation which, on the ordinary medium, induces only vegetative growth, cannot induce reproduction also by changes in the proportions of the nutrients, at least so far as the latter have been tried. And a preparation that exhibits both factors on the normal medium has not been made to lose the reproductive factor by changes in the medium, provided the latter will still allow fairly good vegetation to take place (Table IX, Figs. 7 and 8).

From the standpoint of the presence of two vitamines, it is seen that, although growth can apparently take place with only the vegetative factor present, reproduction cannot take place unless both factors are present, since a certain amount of mycelium is necessary to support the reproductive tissue. This is clear from an inspection of Table IX, Cultures 514–518; it is also shown in a large number of other cultures throughout the experiments, by the appearance of reproduction on the larger additions of vitamine more frequently than on the lower additions. The amounts of vegetation indicated in the two columns of Table IX do not differ greatly; but the greater additions of vitamine were necessary to support the heavy reproduction activities. The growths throughout the 0.3 cc. column are about the same, but the growths in the 2 cc. column are noticeably smaller in the 3 middle cultures where the heavy reproduction took place. Although in both amounts of added vitamine the 2 factors are in the same proportion, it is conceivable that the vegetative factor must build up a certain amount of mycelium, with presumably a certain storage of materials, before the reproductive factor can come into play. If the latter factor is absent, vegetative growth will continue until some limiting factor enters in—it may be nitrogen, sugar, its own vitamine, or even old age. If the reproductive factor is present, it constitutes a limiting factor on vegetation by making heavy demands on the mycelium for materials with which to build the spores. Coons[1] draws a

[1] G. H. Coons, *J. Agr. Res.*, **5**, 713 (1916).

vivid picture of the struggle that takes place in *Plenodomus* between the vegetative and the reproductive tendencies; he calls vegetation static, because it stores energy, and reproduction dynamic because it releases energy. Klebs always speaks of reproduction and vegetation as being opposed to each other. Coons, to his knowledge, was not speaking in terms of vitamines, nor was he dealing with them, but the phenomena in the two fungi are similar. There is just so much material, carbon, and nitrogen material in the medium available for purposes of construction; whether this is converted into the one kind of tissue or the other in *Sclerotinia* may depend upon the relative activities of the two vitamines involved. The ease with which one or the other vitamine can carry on its own activity depends to a certain extent on the total and relative supplies of nutrients, but *the differences are quantitative and not qualitative.*

The above experiments furnish evidence that the shuffling of the nitrogen and sugar constituents of the medium will not of itself determine the occurrence or non-occurrence of reproduction in *Sclerotinia*. Both vitamine factors must be present if reproduction is to occur. However, there also must be a proper balance of the other constituents, or sporulation may not take place, probably because the mycelium must have a certain minimum storage of reserve food before it can support reproductive tissue.

On the other hand, certain of the above experiments and observations are more explainable on the hypothesis of a single vitamine. In the first place, since yeast growth is activated both by those preparations which promote only vegetation in *Sclerotinia* and by those which promote both vegetation and reproduction, but is activated more by the latter than by the former, it appears as if a single vitamine were active in all the preparations, although in different degrees.

In the second place, the materials which furnish the reproductive factor in greatest abundance are pollen, fungus sporophores, yeast, peach juice, and the terminal buds of *Phaseolus multiflorus* (Table IV). With the exception of the fruit tissue, these are all tissues characterized by high metabolic activities. In the case of the pollen and of the fungus spores, they are capable of great independent growth; and the yeast, when provided with proper nourishment, has the power of extremely rapid cell division and growth. Now, the one phenomenon which is common to all living tissues and which varies in intensity according to what we are wont to turn "high" or "low metabolic activity," whether the tissue be secretory, growing, muscular, or what not, is respiration. All the materials examined for vitamine were, at one time at least, living materials; and as such must have exhibited respiration in greater or less degree. The tissues which must have been characterized by the greatest respiratory activity, either actual or potential, are those which are found

to contain the most vitamine—pollen, fungus spores, yeast, terminal buds. Osborne and Mendel[1] have found that liver, heart muscle, kidney, brain, and skeletal muscle contain vitamine B in decreasing quantities in the order named; and that the leaves of spinach, alfalfa, clover, cabbage, and timothy are fairly high in the vitamine. Voegtlin and Myers[2] recently showed that the embryos of the wheat and maize kernels contain all the vitamine, the endosperms containing none. Dutcher[3] found that the catalase content of the organs of pigeons fell very low during polyneuritis, but rose again very rapidly when vitamine was fed.

All the above observations point to the conclusion that there is some direct relation between the respiratory activity of a tissue and its content, and, probably, requirement also, of vitamine.

With this in mind, we may think of *Sclerotinia* as having its life phases governed to a certain degree by the supply of vitamine at its disposal. With a meagre supply, vegetation only will take place; with a more plentiful supply, reproduction as well will be possible. Reproduction in this case is simply a modified vegetation. A vegetative hypha grows vertically from the mycelium, and is pinched off into a chain of spores containing a concentrated supply of reserve food. These spores are capable of great independent growth, that is, they contain sufficient reserve supply to enable them to germinate in distilled water and produce hyphae which aggregate several hundred times the volume of the original spores. We can readily believe that to do this the spore must have considerable vitamine stored within it. Hence the mother hypha must have had at its disposal abundant vitamine; and hence, also, the more abundant the vitamine in a medium, other factors being suitable, the more abundant will be the sporulation.

This hypothesis of a single vitamine leaves unexplained, of course, the failure to secure qualitative differences in the vegetation-reproduction ratio by changes in the proportions of nutrients; and it fails to accord with the different degree of adsorbability on fullers' earth in alcoholic and in aqueous media. Nevertheless, in the light of all the facts available, both in these experiments and in those of others, the idea of a single vitamine for normal metabolism in *Sclerotinia* is more plausible than that of two vitamines.

As regards the identity of this vitamine, the evidence so far is not very clear. It can probably be found in almost all plant and animal materials. Vitamine B, the only well-known vitamine that bears any similarity to the one in question, has been found in a great many plant and animal

[1] T. B. Osborne and L. B. Mendel, *J. Biol. Chem.*, **34**, 17 (1918); **37**, 187 (1919).

[2] C. Voegtlin and C. N. Myers, *Am. J. Physiol.*, **48**, 504 (1919).

[3] R. A. Dutcher, *J. Biol. Chem.*, **36**, 63 (1918); R. A. Dutcher and F. A. Collatz, *ibid.*, **36**, 547 (1918).

tissues, although in some of them, notably in polished rice and in the endosperm of wheat, it is in very small amounts, or even absent. This apparent universality of vitamine B and of the *Sclerotinia* vitamine, their behavior towards fullers' earth, and the fact that materials very high in the B factor (yeast, pollen,[1] barley malt) have also furnished some of the most active preparations for *Sclerotinia*, argue for the identical nature of the two vitamines. It is on these bases that Williams believes that his yeast vitamine is also vitamine B. However, we cannot come to a definite conclusion at present concerning the identity of the vitamine for *Sclerotinia*. A much wider search for sources of the vitamines, and more effective separations of them, must first be made.

The writer wishes to acknowledge at this place the helpful criticism and suggestions of Professor William Crocker, under whose direction the foregoing work was done.

VI. Summary.

A review of the existing literature on vitamines reveals the probability of their universal occurrence in the organic world. Phenomena of vitamine-like origin have been recorded for the mammals, the birds, the seed-plants, the fungi, and the bacteria. Many other phenomena of animal and plant life, especially of the parasitic fungi, not as yet connected with vitamines, lend themselves to a vitamine explanation.

Sclerotinia cinerea, the brown rot fungus of peaches and plums, cannot grow on a medium made up of sucrose, salts, and asparagine. The addition to this medium of small amounts of plant decoctions, especially of the fruits of plums and peaches, induces growth. The experiments in the foregoing paper show that the factor furnished by the plant decoction is not one of mineral, nitrogen, or energy requirements, but is of the nature of a vitamine.

By means of adsorption onto fullers' earth, vitamine preparations were made from a large number of widely scattered sources, both plant and animal. All of these preparations were active in promoting growth in *Sclerotinia*. A few of them would promote reproduction as well.

Experiments designed to show whether two separate vitamine factors are involved in these two phases, vegetation and reproduction, in the life history of *Sclerotinia* yielded some evidence favoring this view: (1) the reproductive element is adsorbed by fullers' earth more readily in an alcoholic, and the vegetative element more readily in an aqueous, medium; (2) some sources yield preparations which predominate in the vegetative factor, others yield preparations that promote both activities; (3) preparations which promote only vegetation on a normal medium have not been induced to promote reproduction by changes in the proportions of the nutrients in the medium; and preparations that, on normal media

[1] R. A. Dutcher, *J. Biol. Chem.*, 36, 551 (1918).

promote both activities, have not been made to lose entirely the reproductive element, thus indicating that the influence of the nutrients in affecting the ratio of vegetation to reproduction is quantitative and not qualitative.

On the other hand, the hypothesis of the existence of but a single vitamine for *Sclerotinia* is more plausible, according to much of the experimental evidence. It is very probable that reproduction in *Sclerotinia* is simply a different manifestation of the same activities as characterize vegetation. The single activity that is apparently most dependent on a vitamine supply is respiration. Respiration is common to all the materials which have yielded the vitamine; and the degree of metabolic, and hence respiratory, activity in these materials is proportional to the activity of the vitamine prepared from them. Thus the evidence is accumulating in favor of the view that there is a close connection between respiration in a cell and its vitamine content, and also its vitamine requirement. Just which cells in the plant world can synthesize this vitamine is still an open question. The *Sclerotinia* vitamine is possibly identical with the water-soluble B of the higher animals; and since the latter cannot synthesize this vitamine, it becomes an important point to know which plant organs can.

ST PAUL, MINNESOTA.

THE MECHANISM OF THE REACTION BETWEEN ETHYLENE AND SULFUR CHLORIDE.[1]

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I. Introduction.[2]

When ethylene gas is led into rapidly agitated sulfur monochloride at a temperature of 40° to 60°, β,β-dichloro-ethylsulfide and sulfur are formed. This may be represented by the following equation:

$$2CH_2 = CH_2 + S_2Cl_2 \longrightarrow (CH_2ClCH_2)_2S + S.$$

The following mechanisms of the reaction are possible: (1) the reaction may take place by the simultaneous action of two molecules of ethylene and one of sulfur monochloride; (2) one molecule of sulfur monochloride may react with one molecule of ethylene to give the intermediate compound $CH_2ClCH_2S_2Cl$, which then may react further with ethylene to give dichloro-ethylsulfide and sulfur, or a disulfide, $(CH_2ClCH_2)_2S_2$,

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[2] The work presented in this paper was incidental to an investigation of an important war problem. As other matters were of greater moment, an exhaustive study of the mechanism of this reaction was impossible. It is believed, however, that the present incomplete results are of sufficient interest to warrant publication. An investigation of the same general problem from a different angle is planned.