highly improbable. Other investigators, for instance, Jenkins, have proved that the nitrate content undergoes only an insignificant decrease,[1] and still others, as Fesca and Behrens, assert that the nitric acid disappears completely, probably by reduction. These contradicting statements may be due to the great variations occurring in the nitrate percentage. A small amount might disappear completely while a larger amount decreases but little, although in both cases the absolute amount disappearing may be the same.

The writer examined, qualitatively, both fresh and fermented leaves from the same farm near Quincy, Fla., and found a moderate amount of nitrate in both. The samples of tobacco examined by Jenkins contained from 1.89 to 2.59 per cent of nitric acid (N_2O_5) at a water content of 23.5 to 27.5 per cent, while Behrens's samples contained only 0.2 per cent of this acid in the dry matter. From the disappearance of such a small amount of nitric acid, it can not be inferred that larger quantities would disappear entirely. In one of Jenkins's samples the amount of nitric acid diminished from 2.59 to 2.35, that is, a diminution corresponding to 0.4 per cent calculated for dry matter of the wrapper leaves. Dambergis observed variations of nitric acid in Greek tobacco of commerce of from 0.5 to 3.37 per cent of the dry matter. Not only the mode of manuring, but also the nature of the soil and the weather, influence the nitrate content of the plants, hence large differences can not be a matter of surprise.

The principal changes which take place during the sweating or fermentation process, as found by various investigators, may be summed up as follows:

(1) Decrease of nicotine.
(2) Increase of ammonia.
(3) Increase of alkaline reaction.
(4) Disappearance of sugar.
(5) Decrease of nitrate.
(6) Improvement of flavor and aroma.

THE COLD SWEAT, AGING, OR AFTER-FERMENTATION.

The cold sweat which unfermented tobacco undergoes, and which corresponds with the aging of wines, may be intentionally carried on for as long as two years, where the main fermentation process has to be shortened for any reason or is not thoroughly completed. Fully and perfectly fermented leaves do not require this cold sweat, and the manufacturers of a good product prevent after-fermentation by giving such a degree of dryness in packing that further changes are stopped, as after-fermentation might finally lead to great differences in the product, which should be uniform. The interior of the piles or cases would naturally become warmer, and the leaves would change more

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[1] Conn. Expt. Sta., Ann. Rept. 1892. To Jenkins belongs the credit of having first compared the fermented with the unfermented leaf in regard to the chemical changes.

than those near the sides. In many cases, however, a further change is found necessary, and slightly moistened sponges are placed in the cases with the tobacco in order to maintain a certain degree of moisture. After-fermentation carried on for too long a time might finally destroy all good qualities by further oxidations.

THE PETUNING OF THE TOBACCO.

The petuning is an operation first practiced in Cuba, and consists in spraying a liquid on the leaves during or after the sweating process. The fillers only, and not the wrappers, are petuned, the intention being to give them a darker color, an improved flavor, and the appearance and character of a strong tobacco. The composition of the petuning liquid used in Cuba is kept secret, and indeed each planter claims to have something known only to himself. It is generally believed that one method of preparing the petuning fluid is by pouring organic fluids yielding ammonium carbonate over crushed tobacco stems, and letting this mixture digest. This liquid is, of course, very liable to putrefy, and consequently a most luxuriant growth of bacteria may be expected within a few days in the warm climate of Cuba. It is no wonder then that on the surface of Havana tobacco various bacteria are found, although it may be doubted whether they live long on these fermenting leaves. The ammonium carbonate contained in the petuning liquids increases the alkaline reaction already present in the fermenting leaf, and thus supports the energy of the oxidizing process which brings on the dark color frequently desired for the filler leaves.

One might naturally suppose that the ammonium carbonate would dissolve some resinous matter from the stems which would lead to an improvement of the aroma of the fillers if the hypothesis is correct that this aroma depends to a great extent upon the resin content of the tobacco plant, but this the writer holds is doubtful.

Petuning is practiced in some parts of the United States also, but the opinion of tobacco manufacturers whom the writer has consulted upon the subject is that the effect of the treatment is overrated. By the most intelligent growers a hot solution of ammonium carbonate is left to act upon the stems of Havana tobacco. This extract is prepared anew every day for use, which easily accounts for the fact that the tobacco leaves thus treated do not show any bacterial flora on their surface. The petuning liquid often has a different composition. The tobacco stems are extracted with water containing rum, molasses, or sour wine, consequently these liquids may swarm with bacteria after they stand for a while. The molasses is supposed to disguise the bitter taste derived from the stems.

Related to the petuning is the so-called "conditioning" of the tobacco, consisting in the spraying with a 2 per cent solution of glycerin. This operation is carried on only with chewing, plug, and cigarette tobaccos, and is intended to keep these products moist and pliable, as perfectly dry tobacco would easily crumble to a powder.

THE BACTERIAL FERMENTATION THEORY OF SUCHSLAND.

It has long been recognized that the main feature of the processes going on in the sweating, or the so-called fermentation, of tobacco consists in oxidations. These are accompanied by certain decompositions liberating ammonia, and are the source of the striking development of heat in the fermenting piles. Now, what is the cause of these powerful oxidations? Nessler, as well as Schlösing, asserts that it is merely the common oxygen of the air that attacks certain compounds in the cells with great ease, no other cause being required. Schlösing admits bacterial action only for initiating the elevation of temperature, but not for the main processes later on. On the other hand, Suchsland attributes all the oxidations and the development of heat to the action of certain bacteria, which are specific for different kinds of tobacco and which impart to each of them a specific aroma.

Nessler's and Schlösing's views must assume substances of an unusual affinity for oxygen, if the rather indifferent atmospheric ogygen could exert such a powerful result without the intervention of any activifying principle, hence Suchsland's view seemed more probable and soon found many followers. He prepared pure cultures of microbes found upon different kinds of tobacco,[1] and by transferring those obtained from Havana tobacco to German tobacco he expected to develop the Havana aroma in the German tobacco, but thus far no new developments have startled tobacco growers. Davalos described mold fungi and microbes occurring upon fermenting tobacco leaves in Havana, but without proving their importance for the fermentation process (see Petuning). Vernhout observed only one kind of bacterium upon fermented tobacco leaves. This developed at 50° C. (122° F.) upon agar plates, and was a thermophile kind of the group of *Bacillus subtilis*.[2] It developed also in decoctions of tobacco and was capable of decomposing proteids with development of ammonia. Vernhout, however, leaves it entirely undecided as to whether this microbe plays any important part in the fermentation process. Also Koning[3], described several kinds of bacteria from fermenting tobacco leaves which he found to be identical with those occurring also on the green tobacco leaves. Besides the known bacteria, *B. mycoides* and *B. subtilis*, he described five aerobic new kinds, called *B. tobacci* Nos. I, II, III, IV, and V, of which *B. tobacci* III seemed to have most influence on the aroma. These statements may well be doubted, as a direct microscopical investigation of the surface of the fermenting leaves is wanting.

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[1] Ber. d. Deut. Bot. Ges., Vol. IX, 1891.

[2] How some authors can assert that the fermentation is caused by *anaerobic* bacteria when it is a known fact that the most important changes going on consist of *oxidations* remains difficult to understand.

[3] Zeitschr. für Unters. der Nahrungs and Genussmittel, 1898, No. 3. It may also be mentioned that this author claims to have discovered the bacteria causing the mosaic disease of tobacco, while the most careful researches of Bejerinck have proved that bacteria are not the cause of it.

The writer has repeatedly tried to scrape off bacteria from the surface of freshly fermented Florida tobacco leaves, but has searched in vain with the highest magnifying power for the millions of microbes naturally to be expected if they really play a part in raising the temperature of the fermenting heap and bringing on powerful chemical changes. These *fermenting* leaves are, however, exceedingly *smooth* and *clean*, and the scrapings obtained from them consist almost exclusively of particles of the epidermis. Only here and there, by application of staining methods, some small globules become visible which might represent spores or cocci. Certainly so few microbes could never be held responsible for the action in the fermenting heap, but, on the contrary, colonies of luxuriant growth, as seen spreading profusely upon potatoes or agar, ought to be expected. It is very instructive that Behrens in his attempt to isolate bacteria from the surface of cured tobacco leaves, obtained two spore-forming microbes, *Bacillus subtilis* and a *Clostridium*. One can not suppress the supposition that both kinds have been present only as spores and as such would remain inactive during the so-called fermentation process, as there is not sufficient water to bring on their germination.

It is evident that for the proper examination of fermenting tobacco leaves one must *avoid petuned* leaves, upon which all kinds of microbes can be found when a putrefying petuning liquid is applied. But this liquid is not at all essential for starting the fermentation process. The fermenting Florida tobacco leaves the writer had under examination were not petuned, and he most emphatically declares (1) that there are no bacteria in the cells of the tobacco leaf, and (2) that the surface is remarkably clean and is not covered by a bacterial coating. This observation was made also by Mr. Albert F. Woods, of the Division of Vegetable Physiology and Pathology, two years ago in his study of spots on fermented leaves.

The chief object and pride of the tobacco manufacturer is to produce a cigar leaf of faultless quality. This would be impossible were bacteria to develop their activity promiscuously on the surface, as their first step would be to reach the nourishing material in the interior of the cells, otherwise they would be incapable of multiplying except for a short time. In gaining entrance to the cells the cellulose walls would have to yield, or, in other words, the surface of the leaves would be attacked.

We have here quite a different case from that of the fermentation of sauerkraut, which contains over 92 per cent of water and a proportion of cellulose to water as 1 to 62. In the fermenting tobacco leaf the amount of water is generally below 25 per cent and the proportion of cellulose to water is generally less than 1 to 1.5. In fact, the water present merely suffices to impregnate the cellulose walls and contents of the cells, and is entirely insufficient to bring organic matter from the interior of the cells to the surface, where bacteria might feed upon it.

It is indeed a matter of interest to observe how the tobacco leaf becomes less fit to support bacterial life after being cured and fermented. While the expressed juice of the fresh tobacco leaf exposed to the air at the ordinary temperature teems with myriads of bacteria within twenty-four hours, the equally concentrated extract of cured or fermented leaves will remain perfectly clear for many days.[1] On the fresh tobacco leaf, as everywhere in nature, numerous kinds of microbes occur, but these seem to die off when cured leaves are fermented, as will be seen from the following experiment by the writer with Florida tobacco leaves: Into about 15 cc. of sterilized beef broth, contained in three test tubes closed with cotton plugs, were introduced, with all necessary precautions, (1) a small scrap of fresh leaf, (2) a small scrap of cured leaf which had been packed two months waiting fermentation, and (3) a scrap of fermented leaf. The tubes were kept at from 15° to 18° C. for several days. No. 1 was turbid after one day, when a scum formed and the liquid became very turbid; the liquid swarmed with bacteria, thick and thin rods and cocci being revealed by the microscope. Nos. 2 and 3 remained perfectly clear, and after eight days merely a trace of flocculi was seen at the bottom, in which a few cocci (Sarcina (?)) could be recognized. Indeed, the juice of the fermented tobacco leaf acts as an antiseptic upon the ordinary bacteria of putrefaction. When a slice of meat is wrapped in a fresh tobacco leaf, and another in a moistened, fermented tobacco leaf, it will be seen after a few days that the former slice is rotten and the latter not.[2] This property of course disappears upon considerable dilution of the juice, as will be seen from the following experiments: Ten grams of fermented and well-dried tobacco leaf were pulverized and extracted with 250 cc. of boiling water. A part of the filtrate received an addition of sugar and another part an addition of peptone. The well-sterilized flasks were infected with small chips of fermented tobacco leaf and some of them kept at 50° C. (122° F.) for three days, and some at from 18 to 20° C. (64.4° to 68° F.). There was more or less development in all the flasks, but further tests, as the inoculation in peptone solution or on potatoes, revealed as the only organism a bacillus resembling *B. subtilis*. The development of the colonies, the mode of growth on the surface of peptone solution and on potato, and the spore-forming threads left no doubt on this point. This result is then in full accordance with the observations of others—that is, that this bacillus can be cultivated from fermented tobacco leaves. But as the most careful searching of the surface of the fermented leaves for the bacillus itself proved vain, it must be assumed that it exists on these leaves only in the form of spores.

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[1] It may be mentioned, however, that a diluted (1 per cent) solution of a neutral nicotine salt will permit a bacterial growth.

[2] Southern manufacturers assert that the workmen in tobacco factories better resist epidemics than those not so employed.

When by accident leaves are too much moistened before they are subjected to the sweating, they will soon lose their coherence and show spots and finally holes. The water content, the writer found, in one such case amounted to 36 per cent. Here, then, is a true action of bacteria, which can develop under these conditions, as the high percentage of water admits an abundant exit of organic compounds from the interior of the cells to the surface and the formation of a diluted solution. Now, it is the experience of every tobacco manufacturer that the product will invariably spoil when the water content is increased to such a point as to permit an exit of soluble organic compounds from the cells. Here, then, begins the parallelism to the fermentation of sauerkraut or ensilage, but not before. The objection that certain kinds of thermophylic bacteria might be capable of developing on the leaves in the presence of a smaller percentage of water can not be sustained, as they require liquid food as well.[1] And how will they reach the interior of the cells without eating through the cellular walls, that is, without ruining the product? The claim that it is not the bacteria but the enzyms they produce that enter can not hold good, as the latter must be dissolved before they can migrate into the interior of the cells, and hence a water increase is again required. The conclusion that must invariably be reached, therefore, is that the bacteria found upon the fermenting tobacco leaves do not participate in any way in the fermentation process, but that they are accidentally present and probably only in the form of spores.

THE OXIDIZING AGENCY IN THE FERMENTING TOBACCO LEAF.

After showing that the bacterial theory of Suchsland is erroneous, as there exists no bacterial coating on the leaves, the question naturally presents itself, what is the cause of the oxidizing action? The assumption of Nessler and of Schlösing that the contact with the atmospheric oxygen would suffice can not be correct for the following reasons: (1) The substances undergoing oxidation (tannin, nicotine, etc.) do not show such powerful affinities for oxygen as to account for the considerable development of heat; and (2) neither curing nor fermentation sets in when the fresh leaves are killed by direct application of steam, although those organic matters which become oxidized in the fermentation process are not changed at all thereby.

Neither the tannin nor the nicotine of the leaves can be energetically oxidized by the molecular oxygen of the air without assistance or stimulation of some sort. In the same way dilute alcohol can not be oxidized into acetic acid by the common molecular oxygen of the air except through the intervention of certain bacteria or platinum black.[2]

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[1] Cohn made investigations on the growth of thermogenic micrococci in the refuse from the cotton purifier. However, he had to add a fair percentage of water to start the development.

[2] Müller-Thurgau declares (l. c., p. 508) that "In the beginning of the curing the changes consist in an increased respiration, but later on, after the cells have died, in other (*'anderweitigen'*) oxidation processes," but he gives no explanation of the cause of these latter ones.

The oxidations in the treatment of tobacco commence with the curing process and are continued in the fermenting process. In the latter case, but not in the former, the aid of bacteria has been invoked for explanation. But when oxidations can go on in the *curing* without bacterial aid, even after the death of the cells, then it might be supposed that the same cause would also lead to oxidation later on during the fermenting process. Now, what is the true cause of these phenomena? There remains, in fact, as the only explanation the writer's suggestion that an *oxidizing enzym* is the final cause of the energetic oxidizing action after death of the cells as it is capable of instigating certain compounds to take up the molecular oxygen of the air.

The formation of enzyms is a physiological necessity for every living organism. Various enzyms come into action especially in the development of shoots, as well as in the inanition state of the plants. Green plants, as well as lower fungi, prepare enzyms, which may act on protein, polyanhydrids of glucoses, glucosides, or fat, splitting or dissolving these bodies and thus making them more easily accessible to the protoplasm.[1] The list of enzyms has been enlarged in recent years by the oxidizing enzyms or the oxidases, which were brought to our knowledge first by French savants, as Gabriel Bertrand, Bourquelot, Gouirand, Cazeneuve, and others. The most thorough investigations on this subject are those by Professor Bertrand, who has shown their wide distribution through the vegetable kingdom.[2]

The best reaction for oxidizing enzyms consists in the production of a blue color with the tincture of guaiac, which reaction can be obtained with various vegetable objects. This blue coloration is produced in many cases only upon addition of peroxide of hydrogen, in which case it was formerly considered as a reaction upon diastase. While the crude diastase of malt gives this blue reaction in a very marked degree, the diastase of certain fungi (*Aspergillus oryzæ*) will, as the writer long since ascertained, yield this reaction either only slightly or not at all, although this diastase has very energetic qualities and produces glucose from starch. Raciborski and other authors also have proved that this blue reaction is not characteristic of pure diastase, but only of an admixture of an oxidase.

The brown, black, or reddish coloration of freshly prepared juices of potatoes, turnips, etc., setting in when exposed to the air, the brown color of the falling leaves in autumn, and similar phenomena are generally due to the action of the oxidases. The oxidation of tannin by oxidases plays an important part in certain fruits ripening or overripe.

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[1] A trophic irritation is exerted when rapidly developing cells or cells in a state of inanition require nourishment, and this stimulus leads to the production of enzyms by the nuclei—an interesting case of physiological adaptation. Well-nourished cells killed in the full vigor of life often give only slight indications of amylolytic and proteolytic enzyms.

[2] Further contributions have been published by Grüss and by Raciborski (Ber. d. Deut. Bot. Ges., Vol. XVI, Nos. 3 and 5, 1898).

The blackening of bananas some time after they are gathered and the brown color on the surface of a slice of apple may also be mentioned as due to these agents.

Oxidizing enzyms also occur in animal organisms, as the investigations of Pieri, Abelous, Bougault, Salkowski, Yamagiva, Linossier, Jaquet, and Schmiedeberg have revealed. Such enzyms were found in various organs, and are capable of easily oxidizing not only guaiac tincture,[1] but also certain aldehydes, such as salicylic aldehyde. Spitzer has determined the amount of oxygen liberated by different organs from peroxide of hydrogen, and has observed that various poisons, such as potassium cyanide, hydroxylamin, etc., small quantities of acids and alkalies, and a temperature of about 70° C. (158° F.) destroy or diminish the oxidizing action of this animal enzym. Water extracts the enzym from the organs, and highly diluted acids, cautiously added, precipitate it with all its original properties. (Abelous could, however, prepare clear solutions only by application of potassium nitrate.) It has the character of a nucleo-proteid and contains from 0.19 to 0.23 per cent of iron. On the other hand, Bertrand and Villiers have found a small amount of manganese in the vegetable oxidases.

That oxidations also can proceed in certain cases without the aid of oxidizing enzyms is a well-known fact. But this is only the case with substances of a specific kind showing a great chemical energy, and even in such cases the presence of oxidizing enzyms will cause such a powerful increase of intensity that the difference becomes most striking, especially when chromogens consisting of certain derivatives of polyvalent phenols are present. The colored product (brown, red, or black) formed by oxidation will appear much sooner and in much greater quantity in the presence of certain oxidizing enzyms than in their absence.[2] On the other hand, oxidizing enzyms can bring on oxidations with certain compounds, as, for example, tyrosin, which under ordinary circumstances would not be oxidized at all by the indifferent oxygen of the air.

Bertrand has characterized different oxidases. While the oxidase of *Rhus vernicifera*, the Japanese lac tree, oxidizes mainly benzene derivatives containing at least two hydroxyl or two amido groups in ortho or para position, another oxidase, isolated from certain green plants, as well as from fungi, acts easily in tyrosin, which the former can not affect, therefore he distinguishes the latter as tyrosinase from the former as laccase. It is the laccase which acts upon the laccol in the

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[1] This blue reaction can be obtained not only by the action of oxidizing enzyms, but also by that of powerful oxidizing chemicals. Such bodies (nitrous acid, free chlorine, etc.) are usually absent when the test for oxidizing enzyms is made. Any intelligent chemist will be able to decide at once by control experiments whether he can trace the reaction rapidly setting in to oxidases or not.

[2] The attempt to explain such rapid oxidations by the assumption that certain salts or ordinary albuminous matter would activify the oxygen, must be considered a failure.

juice of the lac tree and converts it by oxidation into a black substance. Laccase is killed at 75.5° C. (168° F.) and gives the guaiac reaction without the aid of hydrogen peroxide. Like laccase, the oxidases of *Senecio vulgaris*, *Lactuca sativa*, and *Taraxacum dens leonis* fail to attack tyrosin. In certain objects, especially in fungi, however, laccase and tyrosinase occur simultaneously.

The oxidation of polyvalent phenols by laccase leads not only to organic acids, but even to the production of carbonic acid. Bertrand observed in one case that for 23.3 cc. absorbed oxygen as much as 13.7 cc. of carbonic acid were produced.

Gouirand has observed in certain spoiled wines an oxidase[1] which oxidizes the coloring matter, the tannin, and the alcohol of the wine, with production of carbonic acid. This oxidase is destroyed in plain aqueous solution at 72.5° C. (162.5° F.), while in the wine a temperature of 60° C. (140° F.) suffices. Very small doses of sulphurous acid will also kill it. It is supposed to be derived from the fungus *Botrytis cinerea*, which frequently grows upon ripening grapes,[2] while Martinand and Tolomei observed an oxidase in ripe grapes. As to the Florida tobacco leaf, the writer has demonstrated the presence of a relatively large amount of oxidases in it.

These oxidizing enzyms belong, like other enzyms, to the protein compounds, forming a special group of labile proteins, i. e., proteins containing much chemical energy, which on the one hand is the cause of their activity and on the other of their changeability to indifferent proteids by heat, acids, and poisons. They are, as it is expressed, easily killed. The labile, active atomic groups in the molecules change thereby, the atoms migrating into more stable position.

Views on the physiological functions of the oxidizing enzyms.—As to the physiological function of the oxidizing enzyms, no perfectly satisfactory explanation has thus far been proposed. Some authors suppose that they are important agencies in the respiration process and that even respiration itself is caused by them when they are supported by certain properties of the living protoplasm. This is, however, improbable for several reasons: (1) Not every plant contains oxidizing enzyms; (2) many plants contain them only in certain stages; and (3) carbohydrates and fat, the materials which by their combustion serve for support of the respiration and for the production of energy, are not attacked by the oxidizing enzyms, but are attacked very energetically by the protoplasm.

Portier believes that the oxidase of the blood, of which he made a special study, serves to augment the vitality of the leucocytes, which prepare the oxidase and finally deliver it up to the blood when they die. This hypothesis will certainly not find support. The suggestion has also been advanced that the oxidizing enzyms play in plants the

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[1] This œnoxidase is supposed by Bertrand to be identical with laccase.

[2] See also Cazeneuve, Compt. rend., Vol. CXXIV.

same part that the hæmoglobin does in animals, but neither is this view justified, as the oxidizing enzyms are not carriers of molecular oxygen, but simply instigators of oxidation.

The writer's view on this subject is that as the living protoplasm can oxidize carbohydrates and fat, but does not attack or attacks only with difficulty compounds of the benzene group, and, on the other hand, as just the opposite takes place with the oxidizing enzyms, it may be inferred that there exists between the protoplasm and the oxidizing enzyms a certain division of labor, the former oxidizing the compounds of the methan series and the latter those of the benzene series. The former provides for the kinetic energy of the cells; the latter destroys by partial oxidation noxious by-products. The oxidations in the former case are generally complete, but in the latter only partial.

The oxidizing action of enzyms might be compared to that of platinum black. In both cases chemical energy is conveyed to certain organic compounds, which are thus rendered capable of taking up the oxygen directly from the air. The further inference might also be justified that just as platinum black brings on not only oxidations, but also reductions under certain circumstances, the same may be possible for the oxidases; for example, if platinum black is added to a mixture of glucose and potassium nitrate in aqueous solution, a reduction of nitrate to ammonia takes place by aid of hydrogen atoms in the sugar, while the oxygen of the nitrate is thrown upon the glucose and organic acids thereby formed. When the analogy of action of the oxidase to platinum black is justified, there will be a simple explanation for the disappearance of a certain portion of the nitrate and also of a certain portion of the glucose during the fermentation process of tobacco. Preliminary qualitative experiments by the writer have indeed proved the formation of ammonia under these conditions. A full account of quantitative tests will follow in a later bulletin.

The oxidizing enzyms may occur in various parts of the plant—in young and active as well as in dormant tissue. Grüss has observed that there occurs frequently, but not always, a coincidence between the transformation of starch and increase of oxidase. Whether the amount of oxidase augments with the ripening of fruits has not been thoroughly investigated. Tolomei observed that in olives it does increase during the ripening process.

The juice of the fresh tobacco leaf soon turns dark upon exposure to air and gradually forms a sediment, but if boiled this dark coloration does not set in, the oxidase having been killed.

The tobacco oxidase and peroxidase.—There exist, evidently, two kinds of oxidizing enzyms in the Florida tobacco leaf. The first kind oxidizes guaiaconic acid (the characteristic reactive in the guaiac resin) to guaiac blue without the aid of peroxide of hydrogen, but the second kind oxidizes it only when this substance is present. Both kinds of oxidizing enzyms, which may be distinguished as tobacco oxidase and