served, yet their formation takes place very readily. If silver nitrate is added to a neutral solution of quinoline or pyridine methyl iodide in water or alcohol, the precipitate formed is not pure silver iodide, but a mixture of that with the double salt of silver iodide and the pyridine or quinoline salt. No attempt has been made to prepare these double salts of the pyridine and quinoline salts in a pure state as they are very insoluble in all reagents.

A number of open chain ammonium salts, as, *e. g.*, benzyldimethylphenylammonium chloride, and tetramethylammonium iodide, in nitrobenzene solution, were shaken varying lengths of time with molecular silver. Not the slightest indication of any reaction could be detected.

A further study of the salts of pyridine and quinoline is now being carried on. It is not deemed advisable to write structural formulas for these salts till a larger number of their derivatives have been studied, and some definit evidence has been obtained as to which carbon atom is the center of their basicity.

Summary.

It has been shown in this paper that the halogen salts of phenylacridol, pyridine and quinoline react with silver to form silver halides and unsaturated compounds similar to triphenylmethyl, which unsaturated compounds readily absorb oxygen just as does triphenylmethyl. It has also been shown that open-chain ammonium salts do not react in this way.

It was concluded that the salts of acridine, pyridine and quinoline are probably quinocarbonium salts because of their analogy to the derivatives of the triphenylcarbinols and the xanthenols, and not ammonium salts as hitherto assumed.

Hitherto, undescribed silver chloride double salts of the salts of the acridols have been prepared and studied and it has been shown that silver iodide forms double salts with the iodides of pyridine and quinoline.

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BIOCHEMICAL AND BACTERIOLOGICAL STUDIES ON THE BANANA.

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In an earlier paper[1] compiled analyses were presented to show the composition of ripe bananas and that the essential change during ripening is a transformation of starch into soluble carbohydrates with a decrease in the total carbohydrates. Using the relative amounts of soluble and insoluble carbohydrates present in a given fruit as an index to the degree of ripeness attained, experimental evidence was presented to show the effect of various abnormal conditions upon the ripening process. Fruits

[1] *J. Biol. Chem.*, 1, 4 and 5, 1906.

submerged in an inert substance (oil) or treated with inert coatings (paraffin) or placed in an atmosphere devoid of available oxygen (carbon dioxide, hydrogen, illuminating gas, vacuum) or in an atmosphere of accumulated respiratory products, did not ripen, showing that oxygen is essential to normal maturation processes. A few attempts to detect the more specific agencies by which ripening takes place, *i. e.*, enzymatic agencies, were not successful.

In the work herein described,[1] the study of enzymes has been given more extended attention. As an essential adjunct to this, a bacteriological examination of the fruit, at different stages of ripeness, was made to determin how great is the likelihood of microörganisms being responsible for biochemical changes which could be mistaken for transformations of an enzymatic nature. While it is true that all tests for enzymes were carried out under conditions of strict asepsis, it is not inconceivable that, in tests made with solid tissue, bacteria enclosed therein might remain for a time unaffected or might completely withstand the action of the antiseptic substance.

In addition to this, the types of carbohydrates to be found in the banana were briefly studied to emphasize the significance of the presence or absence of the carbohydrate enzymes investigated, and, if possible, to throw some light upon the mechanism of the starch transformation.

Bacteriological Studies.[2]

The literature concerning the bacterial diseases of fruits is very abundant. Little has been done, however, to determin whether or not normal, healthy fruits regularly have a bacterial flora.

Tellarico,[3] in his careful study of the enzymatic changes taking place during maturation of bananas, has taken pains to determin the bacteriological condition of the normal fruit. His efforts to cultivate bacteria in steril boullion, agar and gelatin, with banana pulp as the source of infection, were so generally negative in result as to lead him to the conclusion that, normally, the fruit is steril. Occasional growths which he did observe were attributed to abrasions on the surface of the peel through which infection took place.

Experiments.

In 1906–7, and again in 1909–10, series of experiments were conducted to determin if possible the bacteriological condition of bananas which

[1] Work set forth in a thesis presented for the degree of Doctor of Philosophy, Yale University, 1910, forms the basis of this paper.

[2] This work was done in the Laboratory of Bacteriology and Hygiene, Sheffield Scientific School.

[3] Tellarico, G., "Gli Enzimi idrolytici e catalizzanti nel processo di maturazione delle frutta," *Archivio .ci Farmacologia sperimentale e Scienze affini*, **7**, 27–68 (1908). For translating the essential parts of this paper I am indebted to Dr. William Verdi, of New Haven, Connecticut.

were sound and normal, so far as outward appearance enabled one to judge. In the course of these trials a considerable number of individual fruits were examined, representing the product of different localities under varied seasonal conditions.

The fruits examined were in different stages of maturation, varying from the unripened to the fully ripened condition. One examination was made of a fruit in advanced decay. As it will appear later, this consideration of the sterility of banana tissue naturally led us to the further consideration of the efficiency of the banana peel as a means of protection against bacterial invasion.

Technic.—The experimental technic was as follows:

Fruits were chosen which showed no abrasions of the surface of the peel. They were cut from the hand in such a manner as not to expose the pulp, and the cut ends were at once seared over with a hot rod, or thoroughly flamed. The peel was then cleaned by scrubbing with a brush, after which it was rinsed off with corrosive sublimate solution. The work table and the hands of the manipulator were also carefully sterilized. The operation of sectioning the fruit and inoculating culture tubes was carried out in a sterilized glass chamber, open only in front, and large enough to permit of free manipulation. Above all was a glass canopy to prevent contamination from falling organisms and from infected air currents exhaled by the operator. Cut surfaces of the fruit were flamed as soon as exposed, to guard against mechanical distribution of infection inward towards the center of the pulp.

Culture Media.—Dextrose-boullion was generally used as the culture medium. A few cultures were tried, however, in a medium of steril banana infusion. The dextrose-boullion was prepared as follows:

To 300 cc. of distilled water were added 1 gram of Liebig's extract of beef, 3 grams of "Witte peptone," 1.5 grams of salt (NaCl), and 1.5 grams of glucose (Merck's). The mixture was boiled until complete solution took place, after which the reaction was corrected to faint acidity to litmus (sufficient to turn blue litmus violet). The solution was filtered, tubed and sterilized in an autoclave for 5–6 minutes.

The banana infusion was prepared as follows:

One hundred grams of ripe fruit were macerated in a mortar with sufficient sand, (about 50 grams), to make a thin paste. After thoroughly grinding, 200 cc. of distilled water were added and the suspension incubated at 30°–35° for 24 hours with chloroform as an antiseptic. The suspension was then filtered through cheese cloth and the turbid filtrate passed through an asbestos mat on a Buchner filter. Finally, this filtrate was passed through a paper filter and a clear solution obtained which, after being tubed and sterilized in an autoclave for 12 minutes, remained perfectly clear.

The Sterility of Banana Tissue.—Triplicate cultures for both aerobic and anaerobic growths were prepared from ripe pulp, unripe pulp and scrapings from the inner surfaces of both ripe and unripe peel. The tubes were inoculated with approximately 1 cc. of material and then placed at an incubation temperature of 35–37°. The trials extended over periods of from ten to twenty-one days, examinations being made at certain intervals, and transplantations to agar, gelatin, litmus milk and egg-meat mixture made as conditions suggested. When growths

occurred, only such examinations were made as were sufficient to establish whether they were occasioned by contaminating forms, or whether they might reasonably be attributed to organisms contained in the fruit tissue with which the tubes were inoculated.

The preliminary trials (1906–7) were conducted in four series representing different individuals from different bunches of fruit. The results indicated, in general, that neither the ripe nor the unripe pulp harbored aerobic or anaerobic organisms. The few growths which did occur were usually attributable to contaminating air forms. Growths were obtained with greater frequency, however, when the scrapings from the inner surface of the peel were used, and for these growths anaerobic conditions seemed most favorable.

When in the later investigation (1909–10) this work was duplicated the results were found to agree substantially with those of the preliminary trials. Both aerobic and anaerobic cultures of fruit pulp remained almost uniformly clear. Again the regions of the inner peel appeared to harbor organisms, which did not this time, however, exhibit a preference for anaerobic conditions. Streptococci, curved bacilli—accompanied by the production of putrefactive odor—and a motil bacillus—probably a contaminating form——were the types of organisms found. The plan of experiment and the details thereof are set forth in Table I.

The Efficiency of the Banana Peel as a means of Protection against Bacterial Invasion.—If the fruit normally harbors a varied bacterial flora, the question naturally arises as to how the infection originates. Two possibilities present themselves prominently. It might occur while the fruit is still attached to the tree, through the diffusion of infected plant juice, or microörganisms might penetrate the protective covering, *i. e.*, the peel, in which case infection might occur at any time.

It would be of much interest in this connection to examin the circulation of the banana tree. We have had no opportunity to do this, but in view of the chances for the infection of the plant juice through the agency of various pests[1] it is very probable that the juice is not steril.

With regard to infection through the peel, the following experiment was tried to gain some knowledge as to the permeability of this tissue to bacteria:

Unripe and ripe fruits were selected, the peels of which were free from abrasions or other imperfections. After cleaning and bathing in corrosive sublimate solution, both the stem and flower ends were sealed over with paraffin for a distance of about two inches. Two baths were prepared, one of tap water and the other of distilled water into which had been poured an active culture of the bacillus of malignant edema. In each of these baths an unripe and a ripe fruit were submerged. Suitable control cultures were made at once from fruits taken from the same hand and bunch. After twenty-four hours the submerged fruits were taken from the baths, rinsed with water

[1] Hubert, P., *Le Bananier*, Paris, 1907.

TABLE I.—BACTERIOLOGICAL EXAMINATION OF BANANA TISSUES.

Series 1, Aerobic Cultures.

	Oct 4.	Oct. 7.	Oct. 9.	Oct. 11.	Oct. 13.	Oct 15.	
1–A.	Ripe pulp	Transplanted	Subculture clear	Clear	Clear	Clear	There was a slight sediment at bottom of the tubes. An examination showed no bacilli or cocci present. The deposit was probably due to prolonged incubation of the culture medium.
B.	Ripe pulp	Transplanted	Subculture clear	Clear	Clear	Clear	
C.	Ripe pulp			Transplanted	Clear	Clear	
2–A.	Unripe pulp	Transplanted	Subculture clear	Clear	Clear	Clear	
B.	Unripe pulp	Transplanted	Subculture clear	Clear	Clear	Clear	
C.	Unripe pulp			Transplanted	Clear	Clear	
3–A.	Inner ripe peel	Transplanted	Subculture cloudy (Streptococci)				
B.	Inner ripe peel			Transplanted	Cloudy (Streptococci)		
C.	Inner ripe peel	Transplanted	Subculture cloudy (Motil bacilli)	Agar stroke—pearly growth Gelatin—slight liquefaction			Agar stroke—growth extended over much of the surface. Gelatin—pronounced liquefaction. Satisfactory colonies were not obtained. Appeared to be a member of proteus group.
4–A.	Inner unripe peel	Transplanted	Subculture cloudy (Streptococci)				
B.	Inner unripe peel	Transplanted	Subculture cloudy (Streptococci)				
C.	Inner unripe peel			Transplanted	Cloudy (Streptococci)		

Series 2, Anaerobic Cultures.

	Oct. 4.	Oct. 7.	Oct. 11.	Oct. 14.
1–A.	Ripe pulp	Transplanted	Subculture cloudy (Streptococci)	
B.	Ripe pulp	Transplanted	Subculture clear	Clear
C.	Ripe pulp		Transplanted	Clear
2–A.	Unripe pulp	Transplanted	Subculture clear	Clear
B.	Unripe pulp	Transplanted	Subculture clear	Clear
C.	Unripe pulp		Transplanted	Clear
3–A.	Inner ripe peel	Transplanted	Subculture cloudy, no odor (Streptococci)	
B.	Inner ripe peel	Transplanted	Subculture cloudy, no odor (Streptococci)	
C.	Inner ripe peel		Transplanted	Cloudy
4–A.	Inner unripe peel	Transplanted	Subculture clear	Cloudy
B.	Inner unripe peel	Transplanted	Subculture cloudy, no odor (Streptococci)	
C.	Inner unripe peel		Culture cloudy, putrefactive odor	Transplanted { 1. Aerobic subculture cloudy (Streptococci). 2. Anaerobic subculture lost. (Curved bacilli were observed in the original culture).

and then with corrosive sublimate, after which they were dried by flaming. Cultures were then made according to methods already described. Aerobic conditions were used for the cultures of fruits submerged in tap water and anaerobic conditions for those submerged in water containing the bacillus of malignant edema.

The observations upon the various cultures, transplantations and subcultures extended over a period of eighteen days. In the aerobic cultures no organisms were found, although streptococci were observed in two of the controls. In the anaerobic cultures strepto-bacilli and small motil bacilli were found in two of the experimental trials—cultures of inner ripe peel. Small motil bacilli were also observed in one of the controls of this series. None of the cultures developed any odor of putrefaction.

There was no evidence of any invasion by organisms which the bathing fluids contained, and since the conditions imposed were more severe than would be encountered naturally, the probability of infection through this channel is very slight.

Bacterial Flora of Decayed Banana Tissue.—A study of the flora of a decayed banana led to some interesting results. Aerobic and anaerobic cultures were made and incubated at 35–37°. In an aerobic culture of portions of the inner peel, an abundant growth was obtained in forty-eight hours. After transplanting to steril dextrose-bouillon, decided cloudiness appeared in twenty-four hours, and an examination of the medium revealed the presence of extremely motil bacilli. Subcultures on agar stroke, gelatin stab and in litmus milk were made and examined after thirty-six hours at room temperature. The agar tube showed a decided growth, confined to the path of the stroke but spreading at the bottom of the tube. The growth was raised, pearly, soft and distinctly chromogenic (green-yellow). The gelatin stab showed a growth chiefly confined to the surface. Liquefaction did not occur until after five or six days. After three days the litmus milk became curdled and its reaction was acid.

The organism had the general characteristics of a pseudomonas, presumably being a member of the bacillus fluorescens group, although the flourescent character was not so marked as in the laboratory stock cultures of members of this group.

Several of the cultures produced a luxuriant growth of mold which was identified as Aspergillus, although possessing some irregular characters.

Artificial Infection of Various Fruits.—In order to study the effect of the pseudomonas and the mold upon various fruits, steril sections of apple, orange and banana were inoculated in Petri dishes and incubated at 35–37°. After five days the apple and orange sections had developed no indications of decay, the only change noted being slight desiccation due to the prolonged incubation. The banana section, however, showed

in the region of inoculation, a yellowish, moist area which was much less firm in structure than the surrounding tissue. Control sections incubated for a similar length of time remained sound and showed only desiccation. Upon inoculating steril dextrose-boullion with material from this infected area, the organism was again isolated, and found to possess all of its original characters. It was demonstrated repeatedly that banana tissue was the most susceptible to inroads by this organism.

The mold grew luxuriantly upon both the banana and orange, but neither apple nor pear proved to be a favorable habitat for its growth.

When unsterilized ripe banana tissue was inoculated with the pseudomonas or with streptococci no injurious effect was observed. This may be explained by the reasonable assumption that normal fruit tissue exerts a deterent action toward infecting organisms which the process of sterilization destroys. Furthermore, natural decay begins only after the fruit reaches a state of relaxed enzymatic activity, *i. e.*, after those biochemical changes which accompany maturation are complete. The conditions then become favorable for the multiplication of whatever bacterial flora may be present.

Conclusions.

From the foregoing experiments it appears that the inner portions of the pulp of sound bananas are practically steril, but that the regions of the inner coats of the peel may be sparsely inhabited by bacteria, which, during normal ripening processes, are held in check but subsequently find conditions favorable to growth. The resistance of the protective covering of the fruit to invasion by bacteria points to the circulation of the plant juice as a more probable channel of infection, and suggests that infection occurs while the fruit is still on the tree.

The limited experiments with the organism of decay hardly justify the conclusion that it is specific for banana tissue. It can be said, however, that the bacillus readily produces decay, and, to the extent of the trials herein described, exhibits a marked tendency toward a specific character.

Biochemical Studies.[1]

Scattered experiments have demonstrated or indicated the presence of various enzymes in bananas.

In the course of his work on "The Proteases of Plants," Vines[2] observed certain enzymatic agencies in banana tissue. By means of the tryptophane test he obtained evidence of autodigestion when 10 grams of fresh pulp were digested with 40 cc. of water for five and a half hours, both with and without hydrocyanic acid as an antiseptic. He also found that the tissue digested "Witte-peptone" but did not digest fibrin. It

[1] This work was done in the Laboratory of Physiological Chemistry, Sheffield Scientific School.

[2] "Proteolytic Enzymes in Plants," *Ann. Bot.*, 17, 244–5 (1903).

exhibited some action towards the caseinogen of milk and the ripe tissue was found to contain peroxidase.

Vinson[1] has found the inverting action of bananas to be much less intense than that of dates, and he suggests that, as in the case of dates, there may be cane sugar and invert sugar varieties of bananas.

Mierau[2] discovered that an accurate determination of sugar in the banana cannot be made unless the invertase (sucrase) is first destroyed by heat. Omitting this precaution, extraction for five hours at 54–57° resulted in the inversion of all the sucrose in the fruit and considerable sucrose which he added as an experiment. He observed also that freshly prepared water extracts of banana were dextrorotatory and that they became levorotatory on standing.

The amyloclastic activity of bananas has been demonstrated by Jähkel.[3] He has not investigated the other enzymes present.

In his very complete work on the catalytic processes occurring in ripening fruits, Tellarico[4] has corroborated the presence in the banana of many enzymes detected, in isolated experiments, by other investigators. His observations extended over a series of fruits from the very green to the over-ripened condition represented by "coefficients of ripeness"[5] from 0.85 to 2.8, respectively. He has demonstrated the presence of invertase, amylase, protease (gelatin-liquefying) and catalase. His results for tyrosinase are not conclusive and no lipoclastic enzyme was detected at any stage of maturation. He has stated his methods in considerable detail and we have given them careful attention in the experiments to be described later.

The power of plant juices to effect or facilitate the oxidation of tincture of guaiacum, phenol and related substances has been very widely observed. In 1856, Schönbein[6] showed that this property is not peculiar to plant juices, but is possessed by animal secretions as well and that it is of an enzymatic nature. The numerous attempts to explain this phenomenon have resulted in the postulation of so many different enzymes that the mechanism of the reaction has come to appear much involved.

Moore and Whitley[7] have conceived that the reaction is due to a single enzyme. They consider that fruit tissues contain preformed peroxides

[1] *Bot. Gaz.*, 43, 397 (1907).

[2] *Chem. Ztg.*, 55, 1002 (1893); 56, 1021–22; 71, 1283.

[3] "Ueber Anatomie und Mikrochemie der Bananerfrucht und ihre Reifungserscheinungen," Diss., Univ. of Kiel, 1909.

[4] *Archivio ci Farmacologia sperimentale e Scienze affini*, 7, 27–68 (1908).

[5] As the banana ripens the peel becomes thinner and decreases in weight, consequently the number representing the ratio, weight of pulp: weight of peel, increases. This ratio, numerically expressed, is taken as the "coefficient of ripeness."

[6] *Z. Biol.*, 3, 325.

[7] *Biochem. J.*, 4, 3 and 4, 136.

thus eradicating the necessity for an "oxygenase" which plays an important role in the theory of Bach and Chodat[1] and other theories. Tissues which do not react until hydrogen peroxide is supplied have, according to the authors' views, become deprived of their original organic peroxide. In their experimental studies with various tissues they found that the inner surfaces of banana peel rapidly blued tincture of guaiacum to which hydrogen peroxide had been added but that the pulp reacted more slowly. When the reagent was applied to cut surfaces of the fruit the coloration appeared to follow the course of the vessels.

Experiments.

General Methods.—The appropriate preparation of material to be tested for enzymatic activity is of great importance. Extracellular enzymes may be removed from tissues with comparative ease, but endoenzymes (intracellular) present much greater difficulty. Obviously the tissue cells must be ruptured, and this can be accomplished either by great pressure, by thorough grinding in presence of some mechanical agent, (*e. g.*, sand) or by a combination of both processes.

It must be continually borne in mind that enzymes, like colloidal substances in general, are readily adsorbed, and for this reason, as Vernon[2] and others have observed, kieselguhr is objectionable as a mechanical agent, and, furthermore, attempts to render an enzyme solution clear by filtration through paper pulp or ordinary filter paper, are disastrous for the same reason.

In the work herein described three methods of preparation were employed:

I. The fresh material was reduced to a comminuted condition by grinding in a ball-mill. Sufficient sand or paper pulp was then added to make a stiff paste, the mass wrapped in stout cloth and subjected to pressure of about 3000 lbs. per square inch in an hydraulic press. The expressed juice was then filtered through cheesecloth and the turbid filtrate used for the various tests to be described. The solution thus obtained was a light yellow, opalescent fluid which darkened considerably on standing.

II. Ten parts by weight of the fresh material were thoroughly comminuted in a mortar. Sufficient sand was added to make a paste and the grinding continued. One hundred parts by volume of 25% glycerol were next added and the mixture well stirred. The turbid solution obtained by filtering the suspension through cheesecloth was used for tests as in I.

III. Ten parts by weight of the fresh material were thoroughly comminuted in a mortar—without sand if ripe, with sand if unripe fruit was used,—and then suspended in 100 cc. of distilled water. When sand was used it quickly settled and permitted the supernatant suspension to be decanted. Aliquot portions of this suspension were employed for the trials as required. At the end of the experiment the suspended matter was removed by filtration, or, in quantitative work, the whole was made up to a definit volume and filtered. The volume occupied by the suspended matter in the aliquot used—generally 10 cc.—was negligible.

[1] *Biochem. Centr.*, 1903. Referred to by Moore and Whitley, *Biochem. J.*, 4, 3 and 4, 136.

[2] "Intracellular Enzymes," London, 1908.

Preparations made by method III, were very satisfactory and often exhibited activity which could not be demonstrated conclusively by the other methods.

Fresh fruit was employed for each series of trials, thus enabling the investigator to encounter the variety of conditions necessarily existing in different shipments of fruit, in different bunches of the same shipment and, indeed, in different individual fruits of the same hand and bunch.

If it is desirable, however, to exactly duplicate any particular observation at any time, an enzyme solution cannot be relied upon on account of its rapid deterioration. The stock preparation, therefore, must be in a dry state. Various methods are in use for obtaining dry material which retains its enzymatic activity, but on account of a high water content and a considerable amount of sugar—in the ripe fruit—the banana offers peculiar difficulties in this particular. Evaporation at a low temperature is a tedious process. The acetone-ether treatment, as applied to yeast and fungus enzymes, is ineffective in the case of the ripe fruit and otherwise undesirable on account of the doubtful immunity of certain enzymes to acetone (Gruss).[1] Although temporarily inhibited, they are not destroyed, however, by low temperatures and the drying method as described by Shackel[2] was found to be admirably adapted to the problem at hand.[3] The process, which consists in thoroughly freezing the material and then drying over sulfuric acid in acid *in vacuo*, was carried out in the following manner:

The fruit pulp was cut in thin slices and plunged into liquid air. About 100–125 grams of tissue were thus solidly frozen. The frozen material was then transferred to a carefully lubricated and tested Hempel desiccator, a triangular support of glass serving as an insulator. The desiccator was then quickly evacuated by means of a Geryk pump, a vacuum of 0.25 mm. being obtained. After fifteen hours of desiccation, the material was readily reduced to a fine powder by grinding in a small mortar. A longer period of drying (forty-eight hours) had no advantage as regards the ease with which the material could be reduced to the powdered condition.

The resultant powder was yellow in case of the ripe, and white in case of the unripe fruit. It retained all the enzymatic properties which could be demonstrated for the fresh fruit. No exact observations were made, however, as to the comparative degrees of activity. Glycerol extracts or water suspensions of this powder were made as desired.

In general the experiments were conducted at a temperature of from 30–35° approximating the optimum temperature at which bananas are ripened in commercial practice. Since the maximum rapidity of satis-

[1] *Z. Pflanzenkrankheiten*, [2] **17**, 65–79; [4] **17**, 193–223.

[2] *Am. J. Physiol.*, **24**, 325 (1909).

[3] Every convenience for carrying out the technic of this method was placed at my disposal through the courtesy of Dr. Thomas B. Osborne of the Connecticut Agriculture Experiment Station, and of Dr. Lynde Wheeler of the Department of Physics in the Sheffield Scientific School.

factory ripening is secured with this degree of heat, it is presumably the temperature most favorable to enzymatic action.

Amylase.—In the trials herein described, expressed juice, turbid glycerol extracts and water suspensions of the fruit were used, and both soluble starch and banana starch were employed as substrates.

Banana starch was prepared as follows:

The peel was removed from the unripe fruit and the pulp grated to a pasty condition. The paste was then rubbed up in a mortar, a little acetic acid being added, to "cut" the mucilaginous material and free the starch. The fruit tissue was then removed by filtering through cheesecloth and the cloudy filtrate allowed to settle out in a tall glass cylinder. The starch thus precipitating was washed with water by repeated decantations.

The following procedure was employed to detect amyloclastic action in the fruit at various stages of ripeness:

From 2 to 5 cc. of banana preparation were placed in contact with 2 cc. of 1% starch solution or paste. Toluene was used as an antiseptic and the reaction of the medium was the natural reaction of the fruit. The duration of the trial and the temperature at which it was conducted was varied as shown in the table (II). Controls were made which duplicated the experiments exactly except that the banana preparations were previously boiled. Amyloclastic action was detected by changes in the iodine reaction.

The results of these trials are presented in Table II.

TABLE II.—THE ACTION OF BANANA PREPARATIONS UPON STARCH.

Preparation.	Coef. of ripeness.	Substrate.	Temperature.	Duration of experiment. Hours.	Color with iodine.
1. Unripe fruit, expressed juice	0.9	Soluble starch	30–35°	12	Blue
2. Unripe fruit, expressed juice	0.9	Soluble starch	30–35°	40	Blue-violet
3. Unripe fruit, expressed juice	0.9	Banana starch	55°	1	Blue
4. Ripe fruit, expressed juice	2.3	Soluble starch	30–35°	12	Colorless
5. Ripe fruit, expressed juice	2.3	Banana starch	55°	1	Red
6. Ripe fruit, expressed juice	2.3	Soluble starch	18°	40	Colorless
7. Unripe fruit, glycerol extract	1.0	Soluble starch	30–35°	36	Blue
8. Unripe fruit, glycerol extract	1.5	Soluble starch	30–35°	36	Red
9. Ripe fruit, glycerol extract	2.8	Soluble starch	30–35°	36	Colorless
10. Over-ripe fruit, glycerol extract	4.0	Soluble starch	30–35°	36	Colorless
11. Ripe fruit, water suspension	2.8	Soluble starch	30–35°	36	Colorless
12. Ripe powder, water suspension	...	Soluble starch	30–35°	36	Colorless
13. Ripe fruit, expressed juice filtered clear	2.3	Soluble starch	30–35°	36	Violet

Judging by the color produced with iodine, amyloclastic action is slight in the very green fruit. It increases as maturation proceeds and is still very marked when the fruit has reached the over-ripe condition. Starch-hydrolyzing power was retained by the powdered tissue but the clear-filtered expressed juice was especially inert.

In the earlier studies referred to on page 1706, the tests for amylase were

very uncertain and did not warrant the assertion of the presence of the enzyme. This result is now explained by the fact that the banana preparations were then filtered clear, by which process the enzyme was largely removed, and the duration of experiment was not sufficiently long for the clear filtrate to exhibit whatever activity it may, possibly, have retained.

Sucrase.—The action of banana preparations upon sucrose was easily demonstrated by means of the polariscope. On account of the reducing sugars present in the preparations themselves, qualitative tests with Fehling's solution were valueless. These interfering sugars may, however, be removed by dialysis without inhibiting the activity of the enzymes, as will be demonstrated later.

The general procedure to detect sucrose-hydrolyzing action was to establish, first, the rotation of a definit amount of substrate, *viz.*, sucrose solution; second, the rotation of an equal volume of sucrose plus a definit amount of banana extract or other banana preparation; third, the rotation of such a mixture after complete hydrolysis with hydrochloric acid; fourth, the rotation of such a mixture after standing under antiseptic conditions for a given time at a given temperature; and fifth, the behavior of suitable controls. With these data obtained, it was possible to determin the rotation of the sugars present in the banana preparations used and the decrease in rotation due to the hydrolyzable sugars contained therein. Also, by comparison with the rotation resulting on complete acid hydrolysis the extent of enzymatic hydrolysis was approximated.

The solution to be examined was transferred to a 50 cc. flask, a few drops of basic lead acetate added and made up to volume. After filtering, readings were taken at 20° in a 200 mm. tube. An approximately 0.5 *N* solution of sucrose was the substrate employed.

The behavior of sucrose in the presence of banana preparations is shown by the following data. Toluene was used as an antiseptic unless otherwise stated.

It appears from the following table (Series I) that there is in the banana extract a dextro-rotatory sugar which, upon acid hydrolysis, increases the levo-rotation of the mixture about 0.3–0.4° beyond that due to the added sucrose. Sucrose in presence of boiled extract suffered no change. The alteration of the substrate when in contact with unboiled extract was very slight when the behavior of the extract itself is considered.

From trials 9 and 10 (Series II) it is seen that in one hour at 55° a very marked action on the added sucrose took place and in three hours at the same temperature (trial 6) over 80% was inverted. In trial 16 the hydrolysis was practically complete. The unripe fruit in water suspension exhibited hydrolyzing action (trials 14 and 15) but the activity of chloroform water extracts was very much less intense.

TABLE III.—THE ACTION OF BANANA PREPARATIONS UPON SUCROSE.

Series I.

Description of experiment.	Polariscopic reading, Ventzke degrees.
1. Ten cc. sucrose	+ 9.9
2. Ten cc. sucrose, 10 cc. water extract ripe fruit (coeff. 2.5)	+11.0
3. Ten cc. sucrose, 10 cc. water extract ripe fruit, hydrolyzed with HCl	— 3.6
4. Ten cc. sucrose, 10 cc. water extract ripe fruit, 15 hrs. at 30–35°	+ 8.4
5. Ten cc. sucrose, 10 cc. water extract ripe fruit *boiled*, 15 hrs. at 30–35°	+11.2
Series II.	
6. Ten cc. sucrose, 10 cc. suspension ripe fruit, 3 hrs. at 55°	— 0.9
7. Ten cc. water, 10 cc. suspension fruit, 3 hrs. at 55°	± 0.0
8. Ten cc. water, 10 cc. suspension ripe fruit read at once	+ 2.0
9. Twenty cc. sucrose, 10 cc. suspension ripe fruit, 1 hr. at 55°	+15.8
10. Twenty cc. sucrose, 10 cc. suspension ripe fruit *boiled*, 1 hr. at 55°	+21.9
Series III.	
11. Ten cc. sucrose, 10 cc. $CHCl_3$-water extract unripe fruit, read at once	+10.4
12. Ten cc. sucrose, 10 cc. $CHCl_3$-water extract unripe fruit, 6 hrs. at 55°	+ 9.8
13. Duplicate of 12	+10.1
14. Ten cc. sucrose, 10 cc. water suspension unripe fruit, 6 hrs. at 55°	+ 7.2
15. Duplicate of 14	+ 7.2
16. Ten cc. sucrose, 10 cc. water suspension over-ripe fruit, 6 hrs. at 55°	— 3.5

A series of experiments to study the influence of the reaction of the medium upon the activity of the sucrase indicated that for the amounts of enzyme and substrate used in the foregoing trials, the natural acidity of the extract or other banana preparation was quite as favorable to hydrolysis as a medium rendered more distinctly acid by the addition of a few drops of dilute acetic acid. Alkalinity by sodium carbonate exerted a retarding influence.

Raffinase.—The polariscopic method for the detection of raffinase was similar to that already described for the detection of sucrase. Raffinose hydrolyzes into equal molecules of glucose, fructose and galactose. Its value for α_D is 104.5°, hence any hydrolyzing action is accompanied by a decrease in rotatory power.

Preliminary trials made with both unripe and ripe banana preparations and a very dilute raffinose solution indicated positive action but the actual readings were so small that they were scarcely outside the limits of reasonable experimental error. A new series of experiments was therefore tried with a stronger solution of substrate (5%). Its specific rotation was found to be 106°. The details of the experiments can best be presented in tabular form. Toluene was employed as an antiseptic.

On acid hydrolysis the raffinose solution underwent a change in rotation of 5.7° (+6.1 to +0.4). The banana suspension suffered an enzymatic hydrolysis during the experiment represented by a total change in rota-

tion of 2.4° (+1.4 to —1.0°). Similarly, the glycerol extract underwent a total change of 1.1°.

TABLE IV.—THE ACTION OF BANANA PREPARATIONS UPON RAFFINOSE.

Description of experiment.	Polariscopic reading, Ventzke degrees.
1. Ten cc. raffinose, read at once	+6.1
2. Ten cc. raffinose, hydrolyzed with HCl	+0.4
3. Ten cc. glycerol extract ripe fruit, read at once	+0.5
4. Ten cc. water suspension ripe fruit, read at once	+1.4
5. Ten cc. raffinose, 10 cc. suspension, 10 cc. water, 96 hrs. at 30–35°	+1.7
6. Ten cc. water, 10 cc. suspension, 10 cc. water, 96 hrs. at 30–35°	—1.0
7. Ten cc. raffinose, 10 cc. glycerol extract, 10 cc. water, 96 hrs. at 30–35°	+3.6
8. Ten cc. water, 10 cc. glycerol extract, 10 cc. water, 96 hrs. at 30–35°	—0.6

In trial 5, the initial rotation may be assumed to be +7.5. After ninety-six hours at 30–35°, the rotation had decreased to +1.7°, which is an actual decrease of 5.8°. Deducting the change undergone by the suspension alone, *viz.*, 2.4°, the decrease attributable to the hydrolysis of the substrate is 3.4°.

It appears above that acid hydrolysis of a raffinose aliquot equivalent to that employed in trial 5, effected a change of 5.7°, which closely approximates complete decomposition of the substrate. The percentage hydrolysis then in trial 5 may be represented by the ratio 3.4/5.7 or nearly 60%. Similarly, it may be shown that in trial 7 over 33% of the substrate has been decomposed.

This observation was corroborated by experiments with a dialyzed banana preparation. Sugar-free material was obtained by dialyzing 100 grams of macerated ripe fruit pulp for ten days. Raffinose aliquots were allowed to stand in contact with portions of this dialyzed preparation for four days at 30–35°, after which time suspended tissue was filtered off and qualitative tests for reducing sugars made with Fehling's solution. Copious reduction was obtained which suitable controls demonstrated to be due to a raffinose-hydrolyzing enzyme in the dialyzed suspension.

The enzymatic decomposition of raffinose by banana tissue is therefore conclusively demonstrated, but whether it is due to a specific enzyme-raffinase—or to the alleged adaptability of sucrase (Hudson)[1] cannot be concluded with certainty.

Maltase.—The effect of banana preparations upon maltose solutions was studied in three series of experiments with fruits varying in coefficients of ripeness from 2.0 to 3.0. The suspension was prepared as described in the general methods, page 1715. The trials were conducted at 30–35° in presence of toluene as an antiseptic and the reaction of the medium was the natural faintly acid reaction of the suspension employed.

[1] U. S. Dept. Agr., Bur. of Chem., *Circ.* 50, 1910.

The preparation of the solution for polariscopic observations was the same as described for the detection of sucrase, page 1718.

The accompanying table describes the experiments in full:

TABLE V.—THE ACTION OF BANANA PREPARATIONS UPON MALTOSE.

Description of experiment.	Polariscopic reading, Ventzke degrees.
Series I.—Coefficient of Ripeness 2.0.	
1. Ten cc. 20% maltose, 10 cc. suspension, 20 cc. water, read at once.....	+30.9
2. Ten cc. 20% maltose, 10 cc. suspension, 20 cc. water, after 96 hrs.....	+27.8
3. Ten cc. water, 10 cc. suspension, 20 cc. water, read at once.....	+ 1.4
4. Ten cc. water, 10 cc. suspension, 20 cc. water, after 96 hrs.....	— 0.6
5. Ten cc. 20% maltose, 10 cc. suspension *boiled*, 20 cc. water, after 96 hrs.	+30.4
Decrease in rotation between 1 and 2.....	3.1
Decrease in rotation between 3 and 4.....	2.0
Decrease due to alteration in substrate.....	1.1
Series II.—Coefficient of Ripeness 2.6.	
6. Ten cc. 10% maltose, 10 cc. suspension, 20 cc. water, read at once.....	+16.0
7. Ten cc. 10% maltose, 10 cc. suspension, 20 cc. water, after 4 weeks....	+12.4
8. Ten cc. water, 10 cc. suspension, 20 cc. water, read at once.....	+ 1.0
9. Ten cc. water, 10 cc. suspension, 20 cc. water, after 4 weeks.....	— 0.2
10. Ten cc. 10% maltose, 10 cc. suspension, *boiled*, read at once.....	+16.0
11. Ten cc. 10% maltose, 10 cc. suspension, *boiled*, after 4 weeks.....	+16.0
Decrease in rotation between 6 and 7.....	3.6
Decrease in rotation between 8 and 9.....	1.2
Decrease due to alteration in substrate.....	2.4
Series III. Coefficient of Ripeness 3.0.	
12. Ten cc. 20% maltose, 20 cc. suspension, 20 cc. water, read at once.....	+38.0
13. Ten cc. 20% maltose, 20 cc. suspension, 20 cc. water, after 72 hrs.....	+27.6
14. Ten cc. water, 20 cc. suspension, 20 cc. water, read at once.....	+ 6.9
15. Ten cc. water, 20 cc. suspension, 20 cc. water, after 72 hrs.....	— 0.6
Decrease in rotation between 12 and 13.....	10.4
Decrease in rotation between 14 and 15.....	7.5
Decrease due to alteration in substrate.....	2.9

Considering that 10 cc. of 20% maltose solution, when completely hydrolyzed, made up to a volume of 50 cc. and examined in a 200 mm. tube at 20°, should show a decrease in rotation of about 19°, and, making reasonable allowance for experimental error, the action upon the substrate as indicated by the foregoing results is not unquestionable. In the absence of further experimental evidence, it can only be observed that if banana tissue possesses maltose-hydrolyzing power it is very slight. It is considered (Vinson)[1] that starch in the banana and certain other fruits undergoes conversion to cane sugar without passing through the maltose stage, the changes being effected by some enzyme as yet unknown. The above results combined with the fact that maltose could

[1] *Bot. Gaz.*, **43**, 397 (1907).

not be detected in the fruit (page 1729) seems to substantiate rather than to refute such an opinion.

Dextrinase.—Qualitative tests and polariscopic examinations were made to detect this enzyme. The commercial dextrin used had a slight reducing action upon Fehling's solution, which was not perceptibly increased, however, after standing in contact with dialyzed banana suspension for four days at 30–35°.

The rotatory power of the solutions remained practically unchanged.

Lactase.—No evidence of a lactose-hydrolyzing enzyme could be obtained. A lactose solution after ninety-six hours contact with banana suspension at 30–35° showed a decrease in rotation of 2.9° which was practically the change due to the suspension itself. Obviously, hydrolysis of this substrate would result in an increased rotatory power.

Protease (Peptonizing).—In examining the banana for protease the first experiments were made to detect gelatin-liquefying action.

The expressed juice of both the unripe and the ripe fruit were mixed with 10% "gold seal" gelatin containing 0.5% phenol, and solid tissue was embedded in the same medium. The trials were conducted both at room temperature and at 30–35° and extended over periods of time from twenty-four hours to three weeks. The results were uniformly negative.

In order to simulate Tellarico's[1] experiments more exactly, a water extract of ripe fruit was prepared according to his method. Four cc. of phenol-gelatin were then mixed with 2, 5 and 7 cc. of extract and incubated. The results were again negative.

By increasing the strength of the gelatin to 20% it was possible to increase the ratio extract: gelatin without preventing the ready solidification of the mixture. Although this ratio was made as great as 2.5 : 1 no evidence of liquefaction was observed even on prolonged incubation.

As a control experiment pineapple tissue—which contains an active gelatin-liquefying enzyme—was embedded in the same phenol-gelatin. Tests were repeated at the same time with ripe yellow and ripe red banana tissue for comparison. The preparations were allowed to stand at room temperature. Pineapple tissue effected marked liquefaction in an hour and in two hours the process was complete. In forty-eight hours slight but decided liquefaction had taken place around the peel of the yellow fruit preparation. After two or three weeks the liquefied area was still very small. Tubes of steril dextrose-bouillon and of banana infusion were inoculated with loops from the liquefied area as well as with fragments of adjacent tissue in order to determin if bacteria were responsible for the phenomenon. No indication of bacterial growth could be detected in any of the cultures. The preparation of ripe red fruit tissue remained firm after three weeks.

[1] *Archivio ci Farmacologia sperimentale e Scienze affini*, **7**, 27–68 (1908).

Many attempts to duplicate this action with yellow fruit tissue were unsuccessful. Only once more was any suggestion of liquefaction observed. Inasmuch as the peel had appeared to be the seat of liquefying activity, preparations of the peel of unripe, ripe and over-ripe fruits were made. After ten days at room temperature, a test-tube preparation of the peel of ripe fruit showed a slight depression on the surface of the gelatin some distance removed from the tissue itself. Subsequently, a reddish streak was observed extending from the tissue to the point of depression at which distinct liquefaction had now taken place.

While it is difficult to believe that the positive results obtained were due to bacteria, especially since efforts to detect microörganisms by cultures resulted negatively, it is nevertheless conceivable that, locally in the tissue, on account of the chemical action of peroxidase on phenol, antisepsis was destroyed to such an extent as to no longer completely inhibit the activity of bacteria which appear to be sparsely distributed through the banana, and which our experiments show are resident especially in the inner coats of the peel.

The discrepancy between this experience and that of Tellarico may be explained by the fact that his experiments were upon a different variety of fruit. In the highly cultivated varieties upon which the tests herein described were made, it is perhaps true that some characters and properties have been lost or modified.

The observation of Vines[1] with regard to the digestion of milk by banana tissue has been mentioned. This suggested an investigation of the behavior of the fruit toward casein. After forty-eight hours at 30–35° the digestion of casein powder (according to Hammarsten) by ripe fruit tissue was demonstrated by positive tryptophane tests. These tests in case of the unripe fruit were undecisive.

No evidence of the digestion of fibrin could be obtained by trials with both unripe and ripe tissue. Biuret tests were uniformly negative with one exception in which a faint test was obtained. This may have been due to proteoses in the banana preparation itself.

Protease (Peptoclastic).—The next phase of proteoclastic action investigated was the behavior of fruit tissue and extracts towards various proteoses.

The experiments with extracts were unsatisfactory and almost uniformly negative.

The behavior of the fruit tissue was then tried, the general procedure being as follows:

One-half of a gram of "Witte peptone" and 5 grams of comminuted fruit tissue were placed in 20–30 cc. of water with toluene and incubated at 30–35° for seventy-two hours. Evidence of peptoclastic action was obtained by means of the tryptophane test with chlorine water.

[1] *Ann. Bot.*, 17, 244–5 (1903).

The results are given in the following table:

TABLE VI.—THE ACTION OF BANANA TISSUE UPON WITTE PEPTONE.

Description of experiment.	Tryptophane test.
1. 5 grams peptone, 5 grams ripe red banana pulp, 25 cc. water....	Positive
2. 5 grams peptone, 5 grams ripe red banana pulp *boiled*, 25 cc. water.	Negative
3. No peptone, 5 grams ripe red banana pulp, 25 cc. water.....	Positive (faint)
4. 5 grams peptone, 5 grams ripe yellow banana pulp, 25 cc. water...	Positive
5. 5 grams peptone, 5 grams ripe yellow banana pulp *boiled*, 25 cc. water.....	Negative
6. No peptone, 5 grams ripe yellow banana pulp, 25 cc. water.....	Positive (faint)
7. 5 grams peptone, 5 grams unripe yellow banana pulp, 25 cc. water	Negative (?)
8. 5 grams peptone, 5 grams unripe yellow banana pulp *boiled*, 25 cc. water.....	Negative
9. No peptone, 5 grams unripe yellow banana pulp, 25 cc. water....	Negative

These results indicate that the fruits, except in the unripe stage, underwent autodigestion but also digested the added peptone as shown by the increased intensity of the tryptophane test. Other proteoses such as caseose and the proteoses resulting from the digestion of egg-white were likewise digested.

The evidence then upon the nature of the protease of banana appears to justify its classification with the erepsin-like enzymes. It does not attack the native proteins except casein, does not liquefy gelatin—except with the irregularity shown—but does digest proteoses with readiness.

NOTE.—During the progress of the tests to detect peptoclastic action it was continually observed that banana tissue in presence of "Witte peptone," caseose and egg proteoses produced a distinct brown-red color in the solution similar to that produced by the action of the tissue upon phenol and hydroquinone which will be described when the subject of peroxidase is considered. Both the unripe and ripe tissue produced the coloration and the ripe red fruit pulp most intensely of all. Color was not produced when the peptone or other proteose was omitted nor did it appear when the fruit tissue was previously boiled. The phenomenon was not observed in the case of pineapple tissue which is actively peptoclastic but which, moreover, does not effect the oxidation of phenol or hydroquinone. The same result was obtained with banana tissue and a variety of proteoses, marked coloration being produced with (1) proteose from wheat embyro prepared without enzyme, (2) albumose from egg by papäin and (3) Grübler peptone. Fainter color was produced with (4) fibrinose prepared by autolysis in chloroform but practically none with (5) albumose from self-digestion of purified gelatin and (6) albumose from Brazil nut. The color production appears to depend rather upon the source of the proteose than upon the method of preparation, *i. e.*, whether with or without enzyme.

These observations suggest the presence, in "Witte peptone" and other proteose products, of phenol-like substances which undergo enzymatic change accompanied by the production of color.

Lipase.—The first experiments made to detect lipoclastic action in bananas were conducted as follows:

1. One cc. of ethyl butyrate, 2 cc. of glycerol extract of ripe pulp (coefficient of ripeness 2.3), 3 cc. of water and a few drops of toluene were placed in a flask and made neutral to phenolphthalein with 0.05 *N* sodium hydroxide. 2. This trial was similarly pre-

pared except that the extract used was previously boiled. 3. In this trial distilled water was substituted for banana extract, otherwise the conditions were the same as in experiment 1.

The three preparations were then incubated at 30–35° and at intervals of twenty-four, forty-eight and seventy-two hours the acidity developed was neutralized with 0.05 *N* NaOH.

The total acidity developed in experiment 1, after seventy-two hours was equivalent to 1.1 cc. of 0.05 *N* alkali. In experiments 2 and 3, 0.6 cc. and 0.45 cc., respectively, were required. Trial 1 developed a greater amount of acidity than either of the others but it is evident that the decomposition of the substrate was very slight.

A glycerol extract of an over-ripe banana was then prepared. The peel of this fruit was black but the pulp was still sound. The odor of esters which constitutes the characteristic aroma of the properly ripened fruit was very pronounced and suggested the possibility of more striking results as regards lipoclastic activity. After sixty hours, only 0.35 cc. of 0.05 *N* NaOH was required to neutralize the acidity developed. In this experiment litmus was used as an indicator.

Since fruit suspensions have been found to exhibit greater enzymatic activity than any other preparations employed, a new series of trials was arranged using suspensions of fresh fruit in various degrees of ripeness and of unripe and ripe banana powder as well. Butyrin (Merck's) was substituted for ethyl butyrate as a substrate, and, for comparison, the action of glycerol extracts upon this ester was studied. The general plan of the experiments was similar to that already described. Tenth-normal NaOH was employed and titrations were made at twenty-four hour intervals. The duration of the experiments was from five to fourteen days.

As was anticipated, the suspensions showed a more decided action. The decomposition of the substrate was effected by the unripe and the ripe fruit both in the fresh and the powdered condition. Glycerol extracts were no more effective toward butyrin than toward ethyl butyrate. Prolonged incubation had no appreciable effect upon the ester as shown by trial 9. The slight acidity developed in the banana preparations themselves (trials 6 and 8) was due probably to gradual diffusion of fruit acids retained in the fragments of tissue in the suspension and also to the saponification of the natural esters contained therein.

In the following table acidity developed at the conclusion of the experiments is given in terms of 0.1 *N* sodium hydroxide.

The total acidity produced in experiment 5 after fourteen days was equivalent to 11.8 cc. of 0.1 *N* sodium hydroxide. Deducting the amount produced in the corresponding blank (6), 1.4 cc., there remain 10.4 cc., which is attributable to the decomposition of the ester which was added. The acidity resulting upon complete decomposition of 0.25 cc. of butyrin

is equivalent to 26.08 cc. of 0.1 *N* sodium hydroxide,[1] hence the extent of saponification effected in the experiment in question may be represented by the ratio 10.4/26.08 or nearly 40%. Similarly, the saponification in trial 7 was approximately 30%.

TABLE VII.—THE ACTION OF BANANA PREPARATIONS UPON BUTYRIN.

Description of experiment.	Cc. of 0.1 *N* NaOH.
1. 0.25 cc. butyrin, 10 cc. glycerol extract unripe fruit (168 hrs.)	2.0
2. 0.25 cc. butyrin, 10 cc. glycerol extract ripe fruit (168 hrs.)	1.6
3. 0.25 cc. butyrin, 10 cc. suspension unripe fruit (168 hrs.)	5.4
4. 0.25 cc. butyrin, 10 cc. suspension ripe fruit (168 hrs.)	4.2
5. 0.25 cc. butyrin, 10 cc. suspension ripe fruit (336 hrs.)	11.8
6. No butyrin, 10 cc. suspension ripe fruit (336 hrs.)	1.4
7. 0.25 cc. butyrin, 10 cc. suspension unripe powder (336 hrs.)	8.2
8. No butyrin, 10 cc. suspension unripe powder (336 hrs.)	0.6
9. 0.25 cc. butyrin, 10 cc. distilled water (168 hrs.)	0.3
10. 0.25 cc. butyrin, 10 cc. suspension ripe *red* fruit (168 hrs.)	3.0
11. 0.25 cc. butyrin, 10 cc. glycerol extract ripe peel (120 hrs.)	0.6
12. 0.25 cc. butyrin, 10 cc. suspension ripe fruit *boiled* (216 hrs.)	0.7

Peroxidase.—During the progress of the experiments to detect a gelatin-liquefying enzyme, it was observed that the trials in which phenol was used as an antiseptic rapidly acquired an intense brown-red color indicating an oxidation of the phenol. This suggested an investigation of the behavior of extracts and of banana tissue towards the usual oxidase reagents such as phenol, pyrogallol, hydroquinone, tincture of guaiacum and hydrogen peroxide.

Water extracts of both unripe and ripe fruit produced an oxidation of phenol (0.5% solution) which was indicated by the production of a red color. They also effected the oxidation of pyrogallol and hydroquinone, but only after the addition of hydrogen peroxide. No color was produced in any case if the extract was previously boiled and the action of hydrogen peroxide upon the several reagents was either negative or negligible.

The same phenomena were observed when fruit tissue was immersed directly in the various reagents except that the oxidation of pyrogallol and hydroquinone was accomplished without the addition of hydrogen peroxide, but the presence of this reagent intensified both reactions.

Sections of unripe and of ripe fruit immersed in tincture of guaiacum were apparently without oxidizing effect. Upon the addition of hydrogen peroxide the ripe pulp turned blue locally producing a mottled appearance, but in the unripe fruit sections the blue color was confined almost entirely to the inner layers of the peel.

[1] The specific gravity of butyrin is 1.052 (Merck's Index, 1907, p. 109), hence 0.25 cc. = 0.2625 gram = 0.2295 gram of butyric acid = 26.08 cc. 0.1 *N* NaOH. Working on 0.25 cc. of substrate, each cc. of 0.1 *N* NaOH required represents an hydrolysis of approximately 3.8% (3.83%).

The decomposition of hydrogen peroxide was studied by allowing the fruit preparations to act upon this reagent (Oakland brand) diluted with an equal volume of water, the reaction being corrected to neutrality to litmus if necessary. The experiments were conducted in fermentation tubes, the Hill tube being convenient when solid tissue was employed. Decomposition of the substrate was demonstrated by an accumulation of gas in the closed arm. Peroxidative action was exhibited at all stages of maturation.

Conclusions.

The experimental evidence with regard to the various enzymes investigated may be summarized as follows:

Amylase is present in the early stages of ripening and persists even in the over-ripe fruit. Its action could not be satisfactorily demonstrated previous to the first heating which initiates the ripening process.

Sucrase is present in the unripe stage but is much more intense in the ripened fruit. Alkalinity retards or inhibits its action.

The hydrolysis of raffinose by banana tissue is conclusively demonstrated. The specificity of the enzyme effecting this hydrolysis has yet to be established.

The slight change undergone by maltose solutions under the influence of banana preparations does not warrant the unqualified assertion of the presence of maltase.

No evidence of the presence of dextrinase or of lactase was obtained.

A protease is present which appears to be of the erepsin type.

Lipoclastic action was exhibited by both the unripe and ripe fruit.

Peroxidase was found at all the stages of ripeness investigated.

Carbohydrate Transformations.

General Types of Carbohydrate Transformations.—The antecedents of reserve carbohydrate in plant tissues as well as its subsequent transformation products have been the subject of much investigation. With regard to starch, Sachs[1] attributed its formation to the interaction of carbonic acid, water and fruit acids under the influence of light on chlorophyll. Reinke[2] believed that another carbohydrate precedes starch in the metabolic process and his opinion was shared by Meyer[3] who demonstrated that starch free leaves of various plants formed starch in carbonic acid free air from nutrient solutions of glucose, fructose, galactose, sucrose, mannite, maltose and glycerol but not from lactose, raffinose or inosit.

Sachs observed that in very young vegetable organs the parenchyma cells became filled with starch grains, and subsequently with sugar, not only when the reserve is starch but also when it is sucrose (root of beet),

[1] "Physiology of Plants."

[2] "Lehrbuch der Botanik," p. 70 (1880).

[3] *Bot. Z.*, 5–8, (1886).

inulin (tubes of dahlia), cellulose (date) or even fat (germinating seeds). He has pointed out that the carbohydrate transformations in the sugar beet are, beginning with the leaf store of starch, a conversion into reducing sugar (glucose) which may be detected in the petiole and an elaboration into cane sugar in the root. In a similar manner, the transitory starch of potato is translocated in the form of glucose, and, on reaching the tuber, is re-elaborated into starch.

Girard[1] has demonstrated that transitory starch is preceded by cane sugar in the juices of the plant. Brown and Morris,[2] after a careful study of carbohydrate metabolism in foliage leaves, conclude that the initial carbohydrate formed is sucrose, and that, when the supply of this substance becomes greater than is required for the immediate needs of the tissues, starch is elaborated as a more stable reserve. They consider that starch and sucrose are translocated as maltose and invert sugar, respectively.

Against the accumulated evidence that sucrose is the initial carbohydrate in the carbohydrate metabolism of plants, Vinson[3] raises the physical objection in the instance of the date that the partial osmotic pressure of cane sugar would inhibit flow in that direction before the observed percentage of that sugar could be reached. There is, however, a physiological reason that makes it difficult to understand why sucrose would be the first formed product in the assimilative process. This sugar although soluble and diffusible belongs, like starch and inulin, to the class of reserve materials, and is not directly assimilable. It seems reasonable to expect that the initial carbohydrate would be of a nature to supply at once the requirements of tissue building and not to require modification before it can be so utilized. Browne and Blouin[4] have shown that in the very young sugar cane an active invertase is present to render the sucrose containing juice of the mother cane available to the needs of the young plant. Later on in its development the plant shows a preponderance of sucrose, and, of the reducing sugars present, glucose is present in excess of fructose. This indicates to the authors that the latter sugar is used more extensively than the former for the purpose of tissue building.

Vinson's conception of the translocation of sugars from stems and leaves to fruits is that sucrose or a mixture of simple sugars enters the fruit and when osmotic equilibrium is reached, starch or invert sugar or both are formed, whereby osmotic pressure is reduced. The nature of subsequent transformations depends upon the catalytic agencies involved during the ripening process.

[1] *Bull. soc. chim.*, [2] **42**, 290; *Compt. rend.*, **108**, 609 (1882).
[2] *J. Chem. Soc.* (*London*), **63**, 604 (1893).
[3] *Bot. Gaz.*, **43**, 397 (1907).
[4] Louisiana State Univ., Agr. Expt. Sta., *Bull.* 91 (1907).

Carbohydrate Transformations in the Banana.—The banana belongs to that class of fruits in which the reserve material is starch. The types of carbohydrates resulting from the action of enzymes during ripening have been studied by analyses of desiccated banana powder prepared, as previously described, from the unripe and the ripe fruit.

The method of analysis was as follows:

Ten grams of desiccated material were extracted with absolute ether. The residue was then extracted thoroughly with boiling 95% alcohol, thereby extracting sucrose, invert sugar and maltose, if present, and at the same time inhibiting the action of sucrase. The alcoholic solution was evaporated to dryness at a temperature of 60° and the residue taken up in a definit amount of water and examined for reducing sugar both before and after hydrolysis.

The alcohol-extracted residue was then extracted with cold water for several hours with repeated agitation. The separation of the insoluble material involved a difficult filtration which was satisfactorily accomplished by passing first through cheesecloth and then refiltering the turbid solution through paper. This filtrate contained dextrins and related carbohydrates.

The residue was next boiled over asbestos for 15–20 minutes with constant stirring to gelatinize the starch. After cooling to 55° it was digested with saliva. The digestate was filtered and the filtrate made up to definit volume. Glucose (due to starch) was determined after hydrolyzing with 2% hydrochloric acid.

The residue from the salivary digestion was hydrolyzed with 2% hydrochloric acid and reducing sugars due to xylose and related pentoses then determined.

All copper reductions were made by the uniform method of Munson and Walker.[1]

The results given in the subjoined table are expressed in terms of glucose (except in case of starch) and the figures have been calculated for the original fresh pulp on the basis of 75% water content.

TABLE X.—THE CARBOHYDRATES OF UNRIPE AND RIPE BANANA PULP.

	Unripe fruit.		Ripe fruit.	
	As analyzed. Per cent.	Original. Per cent.	As analyzed. Per cent.	Original. Per cent.
Reducing sugars before hydrolysis (as glucose)...	0.30	0.08	16.82	4.21
Increase after hydrolysis (as glucose).....	0.59	0.15	23.26	5.82
Dextrins, etc. (as glucose).....	2.00	0.50	13.70	3.43
Starch (as such).....	55.97	13.99	2.83	0.71
Xylose and other pentoses, (as glucose).....	8.82	2.21	3.00	0.75
Total.....	67.68	16.93	59.61	14.92

It appears that the essential change during ripening is a conversion of starch into soluble carbohydrates which consist principally of cane and invert sugars and dextrins. Maltose is not present. An examination of the alcohol soluble sugars by means of the osazone test failed to produce any maltosazone crystals.

Galactans[2] were not found in the unripe or ripe pulp or in the peel of the ripe fruit. The method used was that described by Bauer.[3]

[1] U. S. Dept. Agr., Bur. of Chem., *Bull.* **107**.

[2] The examination of the banana for galactans is due to Miss Jessamine Chapman.

[3] *Z. physiol. Chem.*, **51**, 158 (1908).

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ENZYMES PRESENT IN ALFALFA SEEDS, ALFALFA INVESTIGATION, IV.[1]

BY C. A. JACOBSON.

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Any investigation aiming at a study of the life processes and metabolic changes of a plant will necessarily lead back to those changes which take place in the sprouting of the seed. The chemical changes involved in sprouting are conditioned not only by temperature and moisture, but by certain chemical complexes known under the name of enzymes.

These substances are frequently found in seeds, and it was the purpose of this investigation to characterize certain ones that might be present in the seeds of the alfalfa.

A good grade of *Medicago sativa* seeds was obtained from the *Skane Experiment Station*. To about 100 grams of the finely ground seed meal, 500 cc. of water were added, and the material allowed to soak for about 2 hours. The infusion was then filtered through muslin and the emulsion obtained used for the following tests, and will be spoken of as the seed extract.

Green,[2] Weis[3] and others have described the existence of proteolytic enzymes in germinating seeds, and Vines,[4] in his extensive researches, records their presence in many varieties of plants.

The alfalfa seed extract, mentioned above, was subjected to qualitative tests for protease, not only with the tryptophane reaction,[5] but by the digestion of casein and Witte-peptone, titrating the resulting amino acids according to the method of Sörensen.[6] All the tests showed the presence of a proteolytic enzyme, which will be discussed in detail further on.

[1] The present investigation was carried out in the laboratory of Prof. S. G. Hedin at Uppsala, Sweden; and I gratefully acknowledge Prof. Hedin's valuable counsel, as well as the use of his laboratory equipment. Professors Hammarsten and Mörner have also contributed new thoughts and suggestions.

[2] *Phil. Trans.*, **178B**, 58 (1887).

[3] *Comptes-rendus des travaux du Laboratoire de Carlsberg*, **5**, 133 (1903).

[4] *Annals of Botany*, years 1902–1906.

[5] *Ibid.*, **16**, 13 (1902).

[6] *Biochem. Z.*, **7**, 45.