fluorescent materials in the organism affect the activity of diastase and other enzymes. In the instance of luciferesceine, we have a strongly fluorescent material normally present in considerable amounts in an organism; it can here evidently exert no deleterious action upon the organism containing it. Most, but not all of the organisms containing it, however, are nocturnal in habit, and their activities depressed by exposure to light. It is of interest to note in this connection, that according to the theory of fluorescence as a "step-down" process of radiation, and the law of Stokes expressing this theory, luciferesceine should fail to show fluorescence in the emitted light of the insect, since all of the light emitted by the insect is less refrangible than the longest wave length in the fluorescent light of luciferesceine; however, Nichols and Merritt[1] have shown that Stokes' law fails to hold good for many common fluorescent substances, and my own experiments indicate that any light within the visible spectrum of shorter wave length than the yellow-orange, will excite a visible fluorescence in solutions of luciferesceine. On the whole it seems that this substance is contained in a defensive secretion of the insect, and that its fluorescence is a property dependent on its chemical constitution and having no reference to the life processes of the organism or possibly to its defensive function either.[2]

In conclusion I wish to express my indebtedness to Dr. Wm. W. Coblentz, of the Bureau of Standards, the original discoverer of luciferesceine in the Lampyridae, to Dr. Carl L. Alsberg, of the Bureau of Plant Industry, and to Dr. W. H. Schultz and other workers in the Hygienic Laboratory, for assistance and advice in this work, and to Dr. E. A. Schwarz and Mr. H. S. Barber, of the U. S. National Museum, for entomologic information.

THE PREPARATION AND PROPERTIES OF AN OXIDASE OCCURRING IN FRUITS.

By H. P. Bassett and Firman Thompson.

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Within recent years there have been numerous reports on the existence of a class of enzymes which are capable of promoting various oxidizing processes, and which appear to be very widely distributed in both plants and animals. To this class of enzymes the generic name of oxidases has been given.

[1] Nichols and Merritt, *Physic. Rev.*, 16, 18–36 (1904).

[2] Jordan (*Botanical Gaz.*, Chicago, 27 (1899)) arrived at a somewhat similar conclusion in regard to the fluorescent pigment of *Bacillus fluorescens liquefaciens*. Although this pigment is yellow, with a green fluorescence, it shows some analogies to luciferesceine; it is soluble in water, but not in carbon tetrachloride, chloroform, ethyl alcohol or ether, and does not appear to be precipitated by lead acetate or ammonium sulfate.

Among the first of these enzymes to be discovered was laccase, occurring in the sap of the lac tree, by Yoshida.[1] Some years later Yoshida's results were confirmed by Bertrand,[2] who also showed that it was able to oxidize certain aromatic compounds which contain at least two NH_2 or OH groups, *e. g.*, hydroquinone to quinone, pyrogallol to purpurogallin, etc. He has also reported its occurrence in many other plants of widely different families.

Martinand[3] reports the presence of a similar, if not identical, enzyme called oenoxydase in ripe grapes, and which is probably the cause of a peculiar disorder of wines known as "la casse."

Lindet[4] has also investigated a similar enzyme occurring in the juice of the apple, which causes the oxidation of various aromatic compounds, among others that of pyrogallol to purpurogallin.

In carrying out some analyses of fruits in connection with the Department of Plant Pathology of the Delaware Experiment Station, it was noted by one of the writers that the tannin, as determined by Carpene's ammoniacal zinc acetate method, showed widely divergent results on samples of the same fruits taken at the same time but under slightly different conditions. While the method is not all that could be desired for this determination, it was shown by the analysis of solutions of known tannin content, that the discrepancies could not be entirely attributed to deficiencies of the method.

The results seemed to show a marked and rapid increase of tannin on injury of any kind which allowed access of air. For instance, a sample was taken by dropping the fruit into boiling water immediately after plucking and the tannin determined at once. At the same time another sample of fruit on the same tree was injured by repeated puncturing of the stem and fruit with a pin and allowed to remain on the tree for 48 hours, when the tannin was determined. The tannin in the injured fruit was about three times that of the uninjured. In another case apples that had fallen from the tree showed about twice as much tannin as those freshly plucked.

From these and other similar results the conclusion was drawn that tannin or a tannin-like substance (since this method probably includes compounds other than true tannic acid) was formed comparatively rapidly on exposure of the injured fruit or fruit pulp to the air. It was thought if this were the case that it would probably be shown by a decrease in the soluble nitrogen of the juice due to the precipitation of the soluble proteins by the tannin formed. Accordingly, juices were pre-

[1] *J. Chem. Soc.*, 43, 472.
[2] *Compt. rend.*, 118, 1215.
[3] *Ibid.*, 121, 512.
[4] *Ibid.*, 120, 370.

pared from a number of different fruits by first grinding through an ordinary meat chopper and pressing through fine Canton flannel. The materials used were green walnut hulls, ripe apples, green apples and pears.

The juices were sampled immediately after pressing out and every 24 hours thereafter, the samples being filtered through asbestos, using suction. In the case of the walnut hulls the juice was divided into two portions, one of which was raised to boiling temperature and kept there for thirty minutes. The results in the following table are expressed as grams of soluble nitrogen in 50 cc. samples of the juice.

	Fresh juice.	22 hours.	46 hours.	94 hours.
Walnut hulls, natural	0.2646	0.2372	0.2365	0.2219
Walnut hulls, boiled	0.2646	0.2604	0.2632	0.2478
Ripe apples	0.0074	0.0072	0.0074	..
Green apples	0.0106	0.0076	0.0038	..
Pears	0.0151	0.0105	0.0130	..

The sampling was discontinued in each case when fermentation was apparent. It will be noted that while the boiled walnut juice showed practically no decrease in soluble nitrogen the unboiled juice showed a gradual decrease amounting to 16 per cent. in 94 hours. Unripe apples showed the greatest decrease, amounting to 64 per cent. in 46 hours.

This series of experiments would seem to indicate that there was a compound formed, which was probably of the nature of a tannin, having the power to convert the nitrogen into an insoluble form.

A similar series of experiments was then arranged in which the fruits were ground with calcium carbonate as suggested by Appleman's work on catalase.[1] The results are given in the following table expressed as grams of nitrogen in 50 cc. as before.

	Fresh juice.	24 hours.	48 hours.	72 hours.
Walnut hulls, natural	0.2202	0.2666	0.2565	0.2311
Ripe apples	0.0034	0.0021	0.0021	..
Green apples	0.0083	0.0084	..	..
Pears	0.0113	0.0130	..	..

As before the sampling was discontinued when fermentation was plainly evident. It will be noted that there was practically no change in the soluble nitrogen in the time covered by the experiments. There is at present no adequate explanation of the increase in soluble nitrogen shown by the walnut juice during the first 48 hours. Fermentation was apparent in about thirty-six hours after preparation, which was a considerably shorter time than in the previous experiments.

From these results it was concluded that there was an oxidizing enzyme present which was only active in acid solutions and which has been prepared in the following manner from pears.

[1] *Botan. Gaz.*, **50**, 182.

The fruit is ground with calcium carbonate in an ordinary meat chopper and the juice expressed through Canton flannel and allowed to settle in a cool place over night. The supernatant liquid is then drawn off the top and somewhat more than an equal volume of 95 per cent. alcohol added to it. The precipitate which is produced is then allowed to settle, the supernatant liquid siphoned off and the precipitate collected on a Buchner funnel. This precipitate contains the enzyme and may be prepared for use by suspension in water.

It having been found by Bertrand in working with laccase and by Lindet with an oxidase found in cider and wines that these enzymes possessed the property of oxidizing certain polyatomic phenols, *e. g.*, hydroquinone to quinone and pyrogallol to purpurogallin, an attempt was made to study the properties of the enzyme by preparing artificial solutions of gallic acid and albumin and measuring the rate and extent of formation of any tannin-like compounds by the decrease of the soluble nitrogen. The following series of solutions were accordingly prepared.

No. 1. 200 cc. 1 per cent. gallic acid solution and 300 cc. water.

No. 2. 200 cc. 1 per cent. gallic acid solution, 100 cc. albumin solution, 200 cc. water.

No. 3. 200 cc. 1 per cent. gallic acid solution, 50 cc. enzyme suspension, 250 cc. water.

No. 4. 200 cc. 1 per cent. gallic acid solution, 100 cc. albumin solution, 50 cc. enzyme suspension and 150 cc. water.

No. 5. 50 cc. enzyme suspension, 100 cc. albumin solution and 350 cc. water.

No. 6. 50 cc. enzyme suspension, and 450 cc. water.

No. 7. Same as No. 1 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

No. 8. Same as No. 2 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

No. 9. Same as No. 3 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

No. 10. Same as No. 4 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

No. 11. Same as No. 5 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

No. 12. Same as No. 6 with addition of 50 cc. of 3 per cent. hydrogen peroxide.

The albumin used was a solution of egg white in water and contained 1.36 grams of nitrogen per liter. The enzyme suspension was prepared in the manner indicated above, 50 cc. containing 0.00448 gram nitrogen.

In about an hour after the addition of the enzyme, a very heavy, flocculent precipitate had formed and settled in the flasks containing all three constituents, *viz.*, gallic acid, albumin and enzyme. Those containing gallic acid and enzyme without albumin had turned a rich wine-red color, presumably from the oxidation of the gallic acid.

Samples of 50 cc. each were taken after 15 hours and every 24 hours thereafter until there was no longer any decrease in the nitrogen or until the solution showed signs of fermentation. The results in the following table are expressed as grams of soluble nitrogen in 50 cc.

No.	Nitrogen present.	15 hours.	39 hours.	63 hours.	87 hours.	137 hours.	161 hours.
1	0.0013	0.0016	0.0013	..	..	..	..
2	0.0149	0.0146	0.0146	0.0143	0.0147	0.0146	..
3	0.0017	0.0018	0.0017	..	..	..	..
4	0.0153	0.0113	0.0106	0.0102	0.0077	0.0044	0.0032
5	0.0140	0.0138	0.0146	..	..	..	..
6	0.0004	0.0013	0.0010	..	..	..	..
7	0.0013	0.0018	0.0018	..	..	..	..
8	0.0149	0.0153	0.0145	0.0149	0.0151	..	..
9	0.0017	0.0017	0.0017	..	..	..	..
10	0.0153	0.0081	0.0048	0.0037	0.0035	..	..
11	0.0140	0.0147	0.0149	..	..	..	..
12	0.0004	0.0020	0.0014	..	..	..	..

Three days after the experiment had been started, tannin determinations were made by precipitation with hide powder, titrating before and after the precipitation with tenth-normal permanganate. In the following table the results are expressed as the number of cc. of tenth-normal permanganate required by 10 cc. of the liquid.

No.	Before precipitation.	After precipitation.	Difference.	No.	Before precipitation.	After precipitation.	Difference.
1	15.1	15.2	—0.1	7	28.5	24.3	4.2
2	18.0	17.8	0.2	8	29.1	27.0	2.1
3	18.0	15.0	3.0	9	30.5	27.7	2.8
4	18.1	16.3	1.8	10	31.5	28.6	2.9
5	5.8	6.0	—0.2	11	13.5	11.5	2.0
6	5.6	5.8	—0.2	12	13.3	13.4	—0.1

The differences in titration, which would represent the tannin precipitated by the hide powder are insignificant except in the cases where the enzyme and gallic acid were present together in the series containing no hydrogen peroxide. Where hydrogen peroxide was present there would appear to have been some oxidation due to it alone, as important differences were shown in almost every case.

Each of these solutions was also tested for tannin by Oliver's ammonia-nitric acid test as given in Cohen's "Tests and Reagents" with the following results:

No. 1. No precipitate
No. 2. No precipitate
No. 3. Heavy precipitate
No. 4. Heavy precipitate
No. 5. No precipitate
No. 6. No precipitate
No. 7. No precipitate
No. 8. No precipitate
No. 9. No precipitate
No. 10. No precipitate

Since it had been previously observed that the juices that had been prepared by grinding with calcium carbonate and in which there was apparently no action of the enzyme, fermented much sooner than the juices that had been prepared by grinding without calcium carbonate, it was thought that the substances formed in the reaction might have

some germicidal properties. Accordingly, six days after the gallic acid series of experiments had been started, cultures on agar-agar, gelatin and beef tea were made from the solutions Nos. 2, 3, 4 and 5.

Ninety-six hours after the setting of the cultures the following results were noted:

No.	Agar-agar.	Gelatin.	Beef tea.
2	Heavy fungus growth	Heavy fungus growth	Cloudy
3	One colony	One colony	Clear
4	Slight growth	liquefied	Slight cloudiness
5	Heavy growth	liquefied	Cloudy

In both cases where the gallic acid and enzyme were not present together there was a heavy fungus growth and in both cases where they were present there was only a very slight growth in any of the media.

The conclusion would seem to be justified that the substance formed by the action of the enzyme and gallic acid shows a marked inhibitive effect on fungus and bacterial growths.

From the almost instantaneous appearance of the precipitate and from the figures obtained for soluble nitrogen it was inferred that the action was comparatively rapid and accordingly an experiment was planned to test this point.

A solution consisting of 100 cc. of albumin solution containing 1.52 grams of nitrogen per liter, 200 cc. of 1 per cent. gallic acid solution, 50 cc. of enzyme suspension and 50 cc. of 3 per cent. hydrogen peroxide was diluted to 500 cc: Another solution which was the same in every respect with the exception of the hydrogen peroxide, which was omitted, was prepared at the same time. Samples of these two solutions were taken every fifteen minutes for about two hours and the soluble nitrogen determined.

The solutions could not be obtained clear on filtering and no flocculent precipitate separated out as in the previous series.

The determinations of nitrogen are given in the following table, the figures representing the number of grams of nitrogen in 50 cc. as before.

	N present.	15 min.	45 min.	75 min.	105 min	165 min.
With H_2O_2.....	0.0152	0.0158	0.0144	0.0147	0.0147	0.0143
Without H_2O_2....	0.0152	0.0151	0.0141	0.0154	0.0148	0.0127

As will be noted there was very little, if any, action taking place in this series of experiments and accordingly 60 cc. more of the enzyme suspension was added in each case. Immediately a flocculent precipitate formed, which settled very rapidly. Samples taken ten minutes after the addition of the enzyme and filtered as rapidly as possible showed the following amounts of soluble nitrogen in 50 cc.

With H_2O_2.....	0.0090
Without H_2O_2....	0.0076

The same experiment was then repeated with the constituents in the same proportion as before except the quantity of the enzyme suspension which was increased to 150 cc. Samples were taken every fifteen minutes for the first hour and at longer intervals thereafter up to four and one-half hours. The determinations of soluble nitrogen are given in the following table:

	15 min.	30 min.	45 min.	1 hour.	1½ hrs.	2 hours	4½ hrs.
With H_2O_2....	0.0112	0.0119	0.0120	0.0120	0.0125	0.0115	0.0109
Without H_2O_2	0.0122	0.0123	0.0123	0.0123	0.0122	0.0116	0.0123

The nitrogen present at the beginning of the experiment was 0.0159 gram in each case. It will be noted that in the first case there was a decrease in soluble nitrogen amounting to about 30 per cent. and about 23 per cent. in the second with practically no further change up to four and one-half hours, when sampling was discontinued. From these results it was concluded that the action of the enzyme was very rapid and that it takes place only when the concentration is above a certain minimum.

The enzyme as prepared in the manner previously indicated deteriorates rapidly in slightly acid solutions and can not be dried. It can be preserved for a considerable time on ice, in chloroform water and in 5 per cent. alcohol. It produces an intense blue color with an alcoholic solution of guaiacum when one or two drops of acetic acid are added to the solution. A neutral solution gives a dirty green precipitate.

The freshly prepared juice also produced this blue color when it was slightly acid.

By this means an attempt was made to study the localization of the enzyme in the fruit. Using Kieffer pears, it was found that the blue color developed first around the core and immediately under the peel, but was finally developed uniformly over the freshly cut surface.

Several normal fruits were injured by passing a sharp instrument through them from side to side and allowing them to remain on the tree for forty-eight hours. A section was then made through the injury and the guaiacum solution was applied. The blue color developed first quite strongly around the walls of the injury and gradually developed in the other parts of the pear.

The ability of the enzyme to liberate oxygen from hydrogen peroxide was tested, using the apparatus described by Appleman in his work on catalase.[1] No liberation of oxygen was noted in any case.

As cold weather approached the pears were removed from the tree and stored in a cool, dry place, by which means it was hoped that the work might be continued for some time. An attempt to prepare some of the enzyme from these pears eight days after their removal from the tree

[1] *Botan. Gaz.*, 50, 162.

resulted in a preparation which had lost practically all of its power.

On testing the freshly cut surface with guaiacum solution no blue color was developed excepting rather faintly around the core.

While it is appreciated that the work as it now stands is incomplete and requires further confirmation and study, the termination of the fruit season has brought it to a close for the present and it was thought that the results obtained thus far would justify a preliminary report. It is proposed to continue the study during the coming season, when it is believed much more striking results will be obtained from the immature fruit. The present work has been done on fruit that was practically mature but there are indications that the enzyme is much more active in green fruit.

From the results now at hand we believe that the following conclusions can be drawn at least tentatively:

1. That there is present in fruits, in general, an oxidizing enzyme capable of producing a tannin-like substance having the power to precipitate protein nitrogen.

2. That this enzyme is active only in slightly acid solutions and when the concentration is above a certain minimum.

3. That this tannin-like substance or substances does not exist in the normal fruit on the tree but is rapidly formed on injury or removal of the fruit from the tree.

4. That the function of this substance or substances is to inhibit fungus and bacterial growths on injury of the fruits, in part, probably, by the conversion of the protein into an insoluble form and in part by the germicidal action of the substance itself.

It should also be stated that there are indications of another enzyme in the calcium carbonate precipitate, which is very active in liberating oxygen from hydrogen peroxide and is believed to be of the nature of a catalase. However, there has not yet been opportunity for the thorough study of this phase of the question.

We desire to express our thanks to Dr. C. F. Dawson, of this Station, who kindly prepared the cultures and offered valuable suggestions during the progress of the work.

DELAWARE COLLEGE AGRICULTURAL EXPERIMENT STATION.

[FROM THE STORRS AGRICULTURAL EXPERIMENT STATION.]

THE OCCURRENCE OF TYROSINE CRYSTALS IN ROQUEFORT CHEESE.

BY ARTHUR W. DOX.

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While engaged in some coöperative investigations looking toward the introduction of foreign methods of cheese manufacture into this