LXXX. THE ENZYMES OF THE LATEX OF THE INDIAN POPPY (*PAPAYER SOMNIFERUM*).

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THIS paper must be considered in the nature of a preliminary note. The work done has simply been qualitative and pressure of other work has prevented a more detailed treatment hitherto. In view of certain interesting results obtained and as it is possible the work may have to be discontinued it is deemed worth while to put it on record.

When poppy capsules are lanced for opium¹ the latex which immediately exudes varies considerably in colour from capsules in the same field and from the same pure race of poppy and even at times from capsules on the same plants. It may be pure white, smoky grey, light pink or deep pink in colour. It rapidly darkens to some shade of brown, either light chestnut or deep mahogany and at times it is almost black. When this opium is dried at air temperature and powdered the resulting powder may vary from a pale straw colour to almost black.

Moreover in a field sown with a single pure race of poppy, samples of opium collected on the same day at the same time under apparently identical conditions, on being subsequently air dried and powdered vary considerably in colour, *i.e.* from pale straw colour to almost black.

It would appear probable that the change is due to oxidation and one would expect to find powerful oxidising enzymes in the latex.

That the change is due to oxidation seems proved by the following experiments.

A. Some of the fresh latex diluted slightly with water to make it more liquid was drawn into the bulb of a Lunge nitrometer over mercury. The tap was turned off, thus leaving some of the latex exposed in the cup of the nitrometer. The remainder of the latex was effectually sealed up by the mercury out of contact with air. The latex exposed to air rapidly darkened. That sealed up after three months still shows no sign of darkening.

B. Portions of fresh latex were placed in small distillation flasks which were exhausted with a Geryk pump and sealed off. After three months these samples still show no sign of darkening whereas latex in similarly sealed flasks which were afterwards cracked rapidly darkened.

¹ For details of method of lancing see Annett [1921].

Latex kept in closed vessels over alkaline pyrogallol darkens because the complete absorption of oxygen by this reagent is a slow process unless the vessel is vigorously shaken and this is not feasible in the presence of the latex.

These preliminary experiments would appear to show that the darkening of the poppy latex is due to an oxidation process. It therefore appeared of interest to examine the latex for the presence of oxidising enzymes.

(1) Some fresh latex was shaken with water and divided into portions (a) and (b). The flask containing (a) was placed in boiling water for two minutes. (a) and (b) were then both filtered. Next day the filtrate from (b) was much darker than the filtrate from (a), thus indicating that the boiling had destroyed the oxidising enzyme.

(2) Latex mixed with water and fresh guaiacum tincture gave no blueing but when the test was repeated in presence of H_2O_2 a strong blue colour was immediately produced. It would therefore appear that a peroxidase is present in the latex. Spence [1908] working with rubber latex obtained a similar result but on dialysis with water for 24 hours he obtained both oxidase and peroxidase.

(3) Some fresh latex was immediately tested for oxidising enzymes with the following results:

(a) Latex and water + guaiacum tincture gave an immediate green colour but when the test was repeated in presence of H_2O_2 no reaction was obtained.

(b) A similar test with benzidine gave a deep colour immediately in absence of H_2O_2 , but when the test was repeated in presence of H_2O_2 no reaction was obtained.

(c) A similar test with tyrosine suspension in absence of H_2O_2 gave darkening within 15 minutes and next day the liquid was very dark. On repeating the test in presence of H_2O_2 no darkening was obtained.

(d) Pyrogallol gave similar results, *i.e.* oxidation in absence of H_2O_2 but no reaction in its presence.

This result is directly opposed to that obtained under (2) above. The latex used under (3) was, however, used within 20 minutes of the lancing of the capsules while that used under (2) was probably two hours old. Some of the same latex used for (a), (b), (c) and (d) above tested about an hour later gave similar results except in the case of benzidine with which reagent a strong reaction was obtained both with and without H_2O_2 .

In these cases controls were done with boiled liquids and all gave negative results.

This would indicate that the relative freshness of the two lots of latex used had nothing to do with the different results under (2) and (3).

(4) 10–15 g. of latex, collected for experiments in (3) above, were rubbed up in a mortar with distilled water and poured into a cleaned goat's stomach. This was allowed to diffuse in a vessel through which a slow stream of tap water was passed. The tap water was passed through thymol before reaching the diffusing vessel. Next day the walls of the goat's stomach were quite

black, indicating the presence of tyrosinase in the latex. 100 cc. of the dialysed liquor, the total volume of which was about 300 cc., were removed for enzyme tests. It was very faintly acid in reaction to litmus paper. 40 cc. of the liquid were boiled in a water-bath for five minutes. The table sets out the results of experiments then performed with the boiled and unboiled dialysed liquid. 3 cc. of the liquid were taken for the test in each case: “—” indicates no reaction, “+” a reaction, and “++” a very powerful reaction. Where H_2O_2 was used one drop of the 10 volume reagent was employed.

Reagent	Unboiled dialysed liquid		Boiled dialysed liquid	
	No H_2O_2	With H_2O_2	No H_2O_2	With H_2O_2
Guaiacum	++	—	—	—
Benzidine	++	—	—	—
Pyrogallol	{ ++ next day }	—	—	—
Tyrosine	{ ++ next day }	—	—	—

After another two days more dialysed liquid was removed from the goat's stomach. It was then quite neutral to litmus. The above tests were repeated with exactly similar results except that the unboiled liquid gave a very faint reaction with benzidine in presence of H_2O_2 .

Another portion of the dialysed liquid was filtered and tested as above. Almost identical results were obtained. That is to say reactions were obtained with guaiacum, benzidine and tyrosine in absence of H_2O_2 . In the case of guaiacum and tyrosine no such reaction was obtained in presence of H_2O_2 , but in the case of benzidine a very faint reaction was obtained in the presence of H_2O_2 .

(5) Some latex was collected and tested within 10 minutes of lancing with guaiacum and a deep green colour was immediately obtained. The test when repeated in presence of H_2O_2 gave an even more intense reaction. This is again a contradictory result.

The rest of this latex was shaken with water and divided into two portions *A* and *B*. *A* was rendered faintly alkaline with NaHCO_3 and one-half of it, *A'*, was boiled in a water-bath for five minutes. One-half of *B* was boiled similarly and is called *B'*. *A*, *A'*, *B* and *B'* were then tested with guaiacum, benzidine, and tyrosine with and without addition of H_2O_2 .

The boiled solutions gave no reaction in any case. The reaction with guaiacum in case of both *A* and *B* solutions was very doubtful probably owing to the latex having been too largely diluted.

With benzidine both in the case of *A* and *B* a very strong reaction was obtained in absence of H_2O_2 , the reaction being distinctly stronger in the alkaline solution. In the presence of H_2O_2 a faint but positive reaction was obtained with both solutions *A* and *B* but the reaction was much weaker than in absence of H_2O_2 .

With tyrosine a practically similar result was observed namely a strong reaction in absence of H_2O_2 in both solutions *A* and *B*, but it was not more

marked in the alkaline solution. In presence of H_2O_2 a very faint but positive reaction was obtained in the case of both *A* and *B* solutions.

(6) Some of the latex collected for tests under section (3) was immediately after collection rubbed up with water and poured into a tall cylinder. Toluene was poured on the surface and the cylinder allowed to stand undisturbed. The insoluble portions of the latex settled out at the bottom of the cylinder. It will be remembered that when tested under section (3) a strong reaction was given with both guaiacum and benzidine in absence of H_2O_2 but there was no reaction in presence of H_2O_2 .

After the cylinder had stood for eight days some of the liquid was drawn up from the bottom with a pipette. It contained pieces of the insoluble matter of the latex. This liquid reacted with guaiacum both in presence and absence of H_2O_2 the reaction being slightly stronger in the latter case. It reacted with benzidine in absence of H_2O_2 but not when H_2O_2 was present.

(7) It appeared of interest to test old powdered opium for oxidising enzymes.

Some opium powder two years old was rubbed up with water and dialysed. The dialysed liquid was then tested with guaiacum and benzidine in presence and absence of H_2O_2 . Guaiacum gave no reaction in either case; benzidine reacted strongly in absence of H_2O_2 but in presence of H_2O_2 there was no reaction.

Tests for other enzymes in the latex.

We have been unable to find any reference to work on the enzyme content of poppy latex, with the exception of a statement that it contains only a small amount of protease [Czapek].

Fifty capsules were lanced and the latex which exuded was immediately scraped off. It was macerated with sand and 100 cc. water. After straining through coarse cloth the filtrate was divided into two portions *A* and *B*. The latter was boiled. The usual tests for enzymes were then carried out on the following substances: 1 % starch solution, 1 % cane sugar solution, 1 % α -methyl glucoside solution, 1 % urea solution and white flour extract (10 %). The result of these tests indicated the complete absence of amylase, invertase, maltase and urease. The test for protease indicated the possible presence of protease in small quantity as a very faint positive result was obtained.

Emulsin was tested for with amygdalin as in Armstrong and Horton's test, and a negative result obtained.

There was one interesting observation we made in all these tests, namely that the liquids in the tests with unboiled latex all darkened distinctly with reference to the control experiments carried out with boiled latex. This is another indication of the presence of oxidising enzymes.

CONCLUSIONS.

1. The darkening of the latex of the Indian Opium Poppy is a process of oxidation.

2. The latex has a powerful oxidising action on guaiacum tincture, pyrogallol, benzidine and tyrosine in the absence of H_2O_2 . In almost all the experiments tried it was found that H_2O_2 inhibited these reactions.

Chaudat and Staub [1907] found that hydrogen peroxide retarded the action of tyrosinase.

Bach [1906] and also von Fürth and Jerusalem [1907] have found that though hydrogen peroxide in certain amounts retards the action of tyrosinase, yet minute quantities accelerate the action of the enzyme.

3. The dialysed latex both before and after filtration also oxidises guaiacum tincture, pyrogallol, benzidine and tyrosine in absence of H_2O_2 but the reaction is inhibited in the presence of this reagent.

4. The actions on benzidine and tyrosine were particularly powerful. In the case of tyrosine the darkening always appeared as a surface film on the liquid and gradually diffused downwards.

5. Opium powder stored for three years has been shown to possess an oxidising enzyme which acts on benzidine.

The author has recently shown that dry opium powder loses a considerable proportion of its morphine on storage. This may be due to the action of oxidising enzymes.

6. The following enzymes were tested for in the latex and not found, viz. amylase, invertase, maltase, emulsin and urease. There were indications of a weak proteolytic activity.

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