the rate of 649 feet per minute. The summer of 1924 was a cold summer so that the surface temperature never reached as high as 22° C.

FRANK A. STROMSTEN
IOWA LAKESIDE LABORATORY

SPECIAL ARTICLES

RETARDATION OF THE ACTION OF OXIDASES BY BACTERIA

IN a variety of plants and animals there are found substances which are capable of accelerating certain oxidations. These substances, in the majority of cases, resemble the hydrolyzing enzymes in the minute quantities in which they are effective and in their instability towards heat. The discovery of these oxidizing enzymes we owe to Schönbein, who employed guaiac as a means of detecting them. According to Bach and Chodat,[1] the oxidizing enzymes which occur in living tissues in reality consist of two parts: the one part, oxygenase, acting as the carrier of the oxygen; the other part, peroxidase, facilitating the transfer of the oxygen to the material undergoing oxidation. Onslow[2] believes that in plant tissues there are two separate enzymes acting as above.

It has long been known that milk would turn blue in the presence of guaiac, showing the presence of oxidases. The writers have found that stale milk would have no effect on a solution of guaiac. The staleness of milk is due to the growth of bacteria. This paper is an attempt to correlate the number of bacteria present in the milk with the destruction of the oxidases. It is an attempt to show that the presence of bacteria will hinder and finally stop all oxidations, through oxidizing enzymes.

In all the observations the following method was employed. Fresh Grade B milk was obtained at the store. Two hundred cc were put into a sterile bottle; corked with cotton and kept in an icebox at a temperature of 12° C. for further tests. From this stock supply there were daily drawn 5 cc of milk by means of a sterile pipette and tested as follows. To 2 cc in a sterile test tube was added 1 cc of a 1 per cent. solution of guaiac and the time determined for the blue color to disappear. The remaining 3 cc were treated in the following way in order to determine their bacterial count.

Four dilutions were made up: 1 to 100; 1 to 10,000; 1 to 1,000,000 and 1 to 100,000,000. One of each of the last three dilutions was plated out into a separate petri dish, and agar-agar added. The dish was rotated so as to form an emulsion between the milk and the agar. They were then kept at a temperature of 37.5° C. for a period of five days and then the colonies of bacteria counted. This was done daily at the same hour until the milk taken from the stock bottle failed to give a blue color with the guaiac solution. All apparatus used was sterilized in an autoclave, with live steam for two hours, and then allowed to cool before using.

The results are given in Tables 1 and 2. They show that at first the increase in bacteria accelerated the action of the oxidases as shown by the increasing length of time it takes for the blue color of the guaiac to disappear. But as the bacteria continue to increase the color of the guaiac disappears more and more quickly until finally there can be gotten no color with the guaiac. In the observations there is quite an agreement between the number of bacteria present and the time taken for the color of the guaiac to disappear.

TABLE I

SAMPLES OF 2 CC OF MILK PLUS 1 CC OF GUAIAC. TIME IN MINUTES IS GIVEN FOR THE BLUE COLOR TO DISAPPEAR

Sample of Milk	Number of Days 1	2	3	4	5	6	7	8	9	10
A	29	33.5	37.5	41	40	28	20	*	...	.....
B	51	55.0	59.0	40	21	19	17	15	...	.....
C	.....	48.0	45.0	40	20	18	*	.....	...	.....

* No blue color obtained.

TABLE II

BACTERIAL COUNT IS GIVEN IN MILLIONS OF BACTERIA PER CC OF EACH SAMPLE OF MILK USED

Sample of Milk	Number of Days 1	2	3	4	5	6	7	8
A	.06	.41	1.5	2.54	500	.....	600	1,100
B	.05	.25	2.5	18.6	400	1,100	22,000	37,000
C	.....	.60	7.0	12.0	900	.....	.....	.....

It will be seen that the three observations taken on the fourth day are practically equal in respect to time, although the bacterial count (Table 2) varies. Is this failure of the oxidases to change guaiac due to an accumulation of bacterial toxins? This phase of the problem will be determined in our next paper.

Summary: The staleness of the milk prevents the action of these oxidases to oxidize this solution of

[1] Bach and Chodat, Centr. f. Biochem, 1903, 1, pp. 417 and 457.

[2] Onslow, *Biochem. Jour.*, 1920, 14, pp. 535, 541.

guaiac to guaiac blue. This prevention is due to the number of bacteria present. Up to about three millions of bacteria per cc the action of the oxidases is accelerated and from then on their action is retarded.

IRVING KUSHNER
ALEX. S. CHAIKELIS
COLLEGE OF THE CITY OF NEW YORK,
LABORATORY OF PHYSIOLOGY

CULTIVATION OF THE VIRUS OF TOBACCO MOSAIC BY THE METHOD OF OLITSKY

RECENT publications of Olitsky[1] on the cultivation of the virus of mosaic disease of tobacco and tomato attracted unusual attention. The intense but fruitless search which has been made by numerous workers for the causal agent of this pathological condition has made it evident that the problem presents many difficulties. Perhaps no type of plant disease has been more seriously studied by pathologists during recent years than mosaic. It is not surprising, therefore, that Dr. Olitsky's announcement of artificial cultivation of the virus should receive immediate and enthusiastic consideration.

The objective aspect of Olitsky's experiments is extremely simple and should be easily duplicated by any one caring to make the test. The bearing of positive results in this connection on future studies of the general problem of mosaic would undoubtedly be very great, and any effort to verify the findings reported is fully warranted. With this in view, an exact repetition of the experiments described was undertaken. The method followed is essentially as follows. Eighty grams of young tomato tissues were minced and then mortared to a pulp. This was mixed with 250 cc of sterile, distilled water. The mixture was centrifuged for one hour at 1,500 to 2,000 revolutions per minute. The supernatant liquid was passed successively through two Berkefeld N size filters and disposed in 3 to 5 cc portions in small test tubes. This, if it was found to have a pH value between 5.3 and 6.0, constituted the "culture" medium. This medium was held at 28 to 30 degrees C. for seven days to insure sterility. The inoculum used at first consisted of Berkefeld V filtrate from inoculated tobacco and tomato extract. Later sap was drawn directly from the stems of infected plants by means of capillary glass tubes and placed at once into the culture medium. Each culture tube received either 0.1 to 0.2 cc of the infectious filtrate or 0.01 cc of the sap as an inoculum. Succeeding transfers were made by putting 0.1 to 0.2 cc from the first culture into a second as a subplant and so on indefinitely. This procedure, of course, made a series of dilutions of the original inoculum, and Olitsky concludes that growth must have taken place if a decrease of infectiousness did not accompany the succeeding transfers. Every detail of Olitsky's procedure was carried out as completely as possible with one single exception, namely, the use of tobacco instead of tomato plants as tests of the infectiousness of the various cultures. This should not, however, influence the results, as tobacco is quite as susceptible to mosaic as are tomatoes. An additional check (not used by Olitsky) was introduced by the use of sterile, distilled water as a "culture" medium. All dilutions or transfers were made at the same time and in the same manner in both the water and tomato extract. Ten plants were inoculated with each dilution in each of three series; so that the figures given below represent the number of infections in a population of 30 plants for each transfer number. The results of the three separate series of experiments, including more than 260 plants, are given in summary form here.

NUMBER OF PLANTS INFECTED IN THIRTY INOCULATIONS

Transfer No.	Water.	Extract.
1	5	5
2	3	3
3	1	1
4	3	0
5	1	0

The original undiluted filtrate which was used as an inoculum gave 21 infections in 30 inoculations. It is clear that so far as these results are concerned Olitsky's findings are not confirmed, for there is no indication of an increase of the virus as the transfers proceed. The water cultures gave a rate of infection slightly higher than those made in tomato extract in the higher dilutions. These data are, no doubt, too meager to establish conclusions contrary to those reached by Olitsky, but they suggest the desirability of greater accumulation of experimental evidence, and are given here in hopes that they may assist in keeping the question open until the facts are fully established. It appears to the writer not impossible that Olitsky's results may have an interpretation other than that indicated in his articles.

MAURICE MULVANIA

[1] Olitsky, Peter K., "Experiments on the cultivation of the active agent of mosaic disease of tobacco and tomato." SCIENCE, Vol. LX, No. 1565, 1924, p. 592; "Experiments in the cultivation of the active agent of mosaic disease in tobacco and tomato plants." *Jour. of Exp. Med.*, Vol. XLI, No. 1, pp. 129–136, 1925.

NORTH CAROLINA ACADEMY OF SCIENCE

THE twenty-fourth annual meeting of the North Carolina Academy of Science was held at State College, Raleigh, May 1 and 2, 1925. The academy is making an especial effort to help the cause of science