LXXXIV. OBSERVATIONS ON CERTAIN REDUCING AND OXIDISING REACTIONS IN MILK.

By PAUL HAAS and THOMAS GEORGE HILL.

From the Botanical Department of University College.

(*Received July 16th, 1923.*)

I. The reduction of nitrate to nitrite.

In 1904 Kastle and Elvove [1904] first described the reducing action of a substance contained in the sap of the potato tuber upon sodium nitrate in the presence of acetaldehyde and other less potent activators. During the course of an investigation of this and similar reducing reactions of certain plant extracts, comparative experiments were made with cow's milk, since this substance has long been known to exert a reducing action upon methylene blue, both in the presence[1] and in the absence of aldehydes. It was then found that milk also possesses the property of reducing nitrate to nitrite in the presence of acetaldehyde, but it was subsequently ascertained that this observation had been previously recorded by Bach [1911] who had concluded that the action was due to an enzyme which appeared to be present in very small quantity. The experimental evidence here brought forward does not support this view. Later Bach [1912] found that a similar reducing reaction is given by an extract of calves' liver, an observation which was subsequently made anew by Harris and Creighton [1912].

The reduction of sodium nitrate to sodium nitrite by milk is easily demonstrated as follows:

Exp. 1. To 10 cc. of fresh cow's milk, 3 cc. of 4 % sodium nitrate are added, together with two drops of 10 % acetaldehyde; the tube is placed in a thermostat at 50° for ten minutes, then removed, cooled under the tap, and tested for nitrite by the addition of a few drops of Griess-Ilosvay reagent. If nitrite be present a pink colour will appear very quickly and rapidly increase to its maximum intensity, whilst a control in which the nitrate solution is replaced by an equivalent volume of water gives a negative reaction. The intensity of the colour varies with the amount of nitrite present; the normal range is from pale pink to magenta; if in excess, the magenta colour quickly becomes brown.

[1] Schardinger reductase.

Although acetaldehyde is not essential for the process, it is requisite if time is a factor of any importance, since the amount of nitrite produced in its absence is very small even after some hours, and in dealing with milk, with its generally large bacterial flora, the sooner an experiment is completed the better; antiseptics such as toluene, lysol, and chloroform may be employed in prolonged experiments.

Acetaldehyde may be replaced by other aldehydes such as benzaldehyde and cinnamaldehyde; formaldehyde, however, in our experience has a retarding and sometimes an inhibiting action.

The reducing agent is destroyed by boiling, the critical temperature being about 72°, but much depends upon the time of exposure to the high temperature, a fact already commented on by previous authors for enzymes of milk, Schardinger's reductase for instance [Rullmann, 1913, 1914; Seligmann, 1906]. The following typical observations show the importance of the time factor and also that the Schardinger reductase is more sensitive to heat than is the nitrate-reducing agent.

Table I.

Temp. ° C.	Time in minutes	Schardinger reductase	Nitrate reducing agent
70°	17	+	+
72°	5	+	+
	10	+	+ weak
	15	–	+ very weak
75°	15	–	+ very weak
80°	10	–	–

With the object of preparing from milk a clear solution containing the nitrate-reducing agent freed from fat or protein, the milk was coagulated by various means, but after separation of the curd, produced either by excess of acetic or lactic acid or by rennin, the whey no longer reduces nitrate to nitrite. Saturation with ammonium sulphate or the addition of basic lead acetate (6 cc. to 100 cc. of milk[1]) likewise completely removes the nitrate-reducing agent with the proteins, the clear whey then giving negative results. It is this fact which makes the separation of the active principle from milk a matter of considerable difficulty and attempts to do this have so far not met with success.

With regard to the measurement of the activity of the nitrate-reducing agent, the fact that milk contains much protein renders the estimation of small amounts of nitrite a matter of no little difficulty. Many methods were tried and the one finally adopted as giving the most reliable results was as follows. To the volume of milk an equal volume of a saturated solution of ammonium sulphate was added, the clot was filtered off and the whey saturated with ammonium sulphate by the addition of an adequate quantity of the crystalline salt. The mixture was then again filtered through a folded filter paper. The nitrite contained in the filtrate was estimated colorimetrically by means of metaphenylene diamine. On theoretical grounds this procedure

[1] It is well to centrifuge off the fats first.

may be open to question in view of the possible interaction between the ammonium sulphate and the sodium nitrite: it was found, however, that in the time spent in salting out the proteins at the room temperature at which this operation was carried out, the loss of nitrite from this cause was, if any, within the margin of error.

The following illustrates a typical experiment on the reduction of nitrate by milk.

Exp. 2. 450 cc. of milk, contained in a stoppered bottle, were mixed with 30 cc. of 4 % sodium nitrate and 6 cc. of 10 % acetaldehyde. After a thorough shaking, the mixture was left at rest in a thermostat at 45° and only shaken up regularly every 15 minutes when taking a sample for estimation. The experimental results obtained are shown in the form of a curve (Fig. 1).

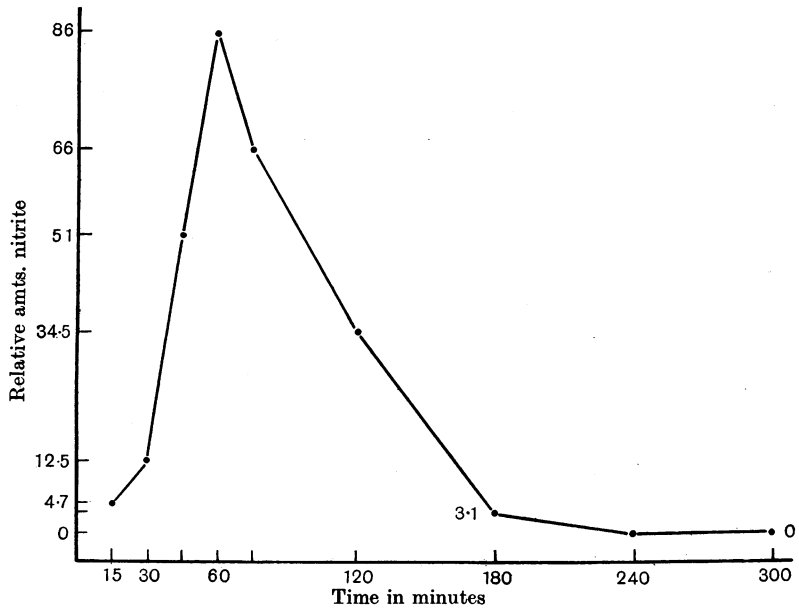

Fig. 1.

This curve is typical for the conditions named, of which the most important is the amount of shaking, since this not only preserves the homogeneity of the fluid but also ensures uniform aeration. The significance of aeration will later be apparent (p. 675).

The curve is peculiar and merits some consideration. If the reduction of the nitrate is a simple enzymic reaction, the curve should ascend until the whole of the nitrate is reduced to nitrite; if the agent is a reducing substance but not an enzyme, the curve should ascend until the whole of this substance is used up. In neither case should there be a fall to zero and it therefore becomes necessary to account for the disappearance of the nitrite. It is unreasonable to suppose that the same agent which reduces the nitrate,

should suddenly break off its action on the nitrate, when the latter is still in excess, and initiate an attack on the nitrite. Even if this were so (provided the agent is enzymic or, if non-enzymic, is in sufficient abundance) the curve should ascend again, since in the experiment there was nitrate sufficient to produce more nitrite. On the assumption that the system is a single agent capable of reducing both nitrate and nitrite, the ultimate result should be that some nitrite should remain just so long as there is an excess of nitrate present, but, the ultimate result should not be zero until all the nitrate has disappeared. The fact that the nitrite ultimately disappears, although excess of nitrate remains, strongly indicates that the mechanism responsible for the production of nitrite has ceased its activity, wherefore it probably is not an enzyme but rather a reducing substance present in limited quantity. Moreover, if the two reductions were really brought about by one and the same substance, whether enzymic or not, the final product of the combined actions alone should be detectable, since the nitrite produced by the first reduction would be in the most favourable condition for immediate further reduction and so would have no opportunity for accumulation to a maximum as is actually the case. These considerations indicate strongly the presence of two active substances; the one reducing nitrate to nitrite, for which we propose the term "atite[1]" the other converting the nitrite into something else. Considerations such as these led to much experimentation which in time yielded evidence which proved the existence of an agent, other than atite (p. 678). Before dealing with this it may be mentioned that on the assumption that the nitrite itself was further reduced, milk was quantitatively examined for ammonia before and after treatment. To do this, increasing quantities of sodium nitrite were added to a series of tubes containing milk and a little acetaldehyde (1 cc. of 10 % aldehyde to 75 cc. of milk) placed in a thermostat at 45°. A small quantity of the mixture from each tube was periodically tested and that tube from which most nitrite had disappeared was selected for estimation as having, presumably, most ammonia. The details of the procedure followed may be omitted since the result showed that the quantity of ammonia present after the destruction of nitrite was no greater than that found in the original milk.

In view of the presence in milk of a reductase capable of reducing methylene blue directly, and also an indirect reductase (Schardinger enzyme) only capable of bringing about this reduction in the presence of formaldehyde [see Lane-Claypon, 1913; Harden and Lane-Claypon, 1912], the relationship between the nitrate reducing agent and the Schardinger reducing enzyme must be inquired into.

That the nitrate reducing agent of the potato is not identical with the Schardinger enzyme is indicated by the following experiment.

Exp. 3. Four tubes are made up and treated as follows:—(1) 1 g. of potato tuber is ground in a mortar under 10 cc. of 4 % sodium nitrate; the

[1] A convenient term since it indicates the conversion of nitr*ate* to nitr*ite*.

mash is placed in a test tube and the temperature raised to 57° in a thermostat; three drops of 10 % acetaldehyde are then added. (2) The same preparation, but the sodium nitrate is replaced by three drops of a 1 % aqueous solution of methylene blue. (3) 5 cc. of milk are mixed with 5 cc. of 4 % sodium nitrate and then as for the first tube. (4) The same preparation as (3), but three drops of methylene blue in place of the sodium nitrate. The four tubes, together with two controls lacking sodium nitrate, are then placed in a thermostat at 57°; numbers (1) and (3) and the controls are removed after two or three minutes and tested for nitrite with Griess-Ilosvay reagent, whilst numbers (2) and (4) are allowed to remain for a time sufficient to give a result. Typical reactions are as follows:

Potato	1. Griess-Ilosvay	+	Control −
	2. Schardinger	−	
Cow's milk	3. Griess-Ilosvay	+	Control −
	4. Schardinger	+	

It may be argued that the negative result with methylene blue is due to the conditions of the experiment: this may be true; but without considering in any detail the botanical aspects of the question, which consideration is reserved for a future occasion, it may be stated that in no instance has success in reducing methylene blue with the sap of various plants been achieved under conditions which invariably gave positive reactions with milk.

II. Oxidation of nitrite to nitrate.

On p. 673 allusion has been made to the significance of shaking, and, in consequence, of aeration. This is well brought out in the following experiments:

Exp. 4. A mixture of 500 cc. milk, 6·6 cc. of 10 % acetaldehyde, and 33·3 cc. of sodium nitrate was placed in a glass container of 1 litre capacity which was throughout the experiment rotated in the thermostat at 45° to ensure continuous agitation and aeration. A precisely similar mixture contained in a vessel of the same capacity was placed in the thermostat but not rotated and only shaken every 15 minutes. Samples from each were periodically taken and the amount of nitrite estimated.

Table II.

Time in minutes	Relative amount of nitrite: Continuously rotated	Relative amount of nitrite: Periodically shaken	Time in minutes	Relative amount of nitrite: Continuously rotated	Relative amount of nitrite: Periodically shaken
15	0	0·04	90	0	0·14
30	0	0·11	120	0	0
45	0	0·22	180	0	0
60	0	0·52	240	0	0
75	0	0·55			

The results indicate that with thorough aeration no measurable amounts of nitrite are detectable, whereas, when the aeration is limited, quantities of

nitrite rising to a maximum and falling off again are produced as in the experiment represented by Fig. 1. A further test was applied by conducting two experiments side by side, the only difference being that the one lacked oxygen.

Exp. 5. 300 cc. of milk together with 20 cc. of 4 % sodium nitrate were placed in a bottle fitted with a cork through which passed a delivery tube reaching to the bottom and an exit tube; the air was exhausted from the bottle and a rapid stream of nitrogen was then bubbled through for about half-an-hour, to wash out the last traces of air. 4 cc. of acetaldehyde were thereupon rapidly added and nitrogen was again bubbled through. The vessel was then closed and placed in the rotating mechanism of the thermostat at 45°. A similar mixture of aerated milk, nitrate and aldehyde was placed in a bottle of suitable capacity and fixed in the rotating mechanism of the same thermostat. Both bottles were thus equally agitated throughout the experiment. Samples from each were periodically removed, without admitting air to the anaerobic tube, and the nitrite estimated, with the following results:

Table III.

Time in minutes	Relative amount of nitrite: Aerobic conditions	Relative amount of nitrite: Anaerobic conditions	Time in minutes	Relative amount of nitrite: Aerobic conditions	Relative amount of nitrite: Anaerobic conditions
30	0	0·024	120	0	1·06
45	0	0·125	180	0	1·18
60	0	0·461	240	0	1·30
75	0	0·80			

The figures in Table III representing the relative amounts of nitrite produced under anaerobic conditions show a gradual falling off, but the duration of the experiment was insufficient to show whether there was a limit to the amount of nitrite which could be produced. For this reason an experiment similar in all respects to the foregoing, with the exception that hydrogen was used instead of nitrogen, was carried out for a longer period. The results are set out in Table IV and Fig. 2.

Table IV.

Time in hours	Relative amount of nitrite	Time in hours	Relative amount of nitrite
1	0·74	5	2·67
2	1·47	7	2·90
3	2·03	8	2·89
4	2·30	9	2·89

From these and like experiments it is clear

(1) That the action of atite is best manifested in the absence of oxygen which element is not a requisite, in that the action of atite is a reductive process.

(2) That the estimations of nitrite obtained in an atite experiment, Fig. 1 for example, represent the balances between the formation and destruction

of nitrite in a given time under the experimental conditions, of which the degree of aeration is of the greatest importance.

(3) That atite is present in limited amount and can only reduce a certain quantity of nitrate.

(4) That the destruction of nitrite, since it is so rapidly effected in a free supply of oxygen, is an oxidative process rather than a reductive process, as was first supposed. This latter conclusion was proved to be correct by the following experiment in which the production of nitrate was established.

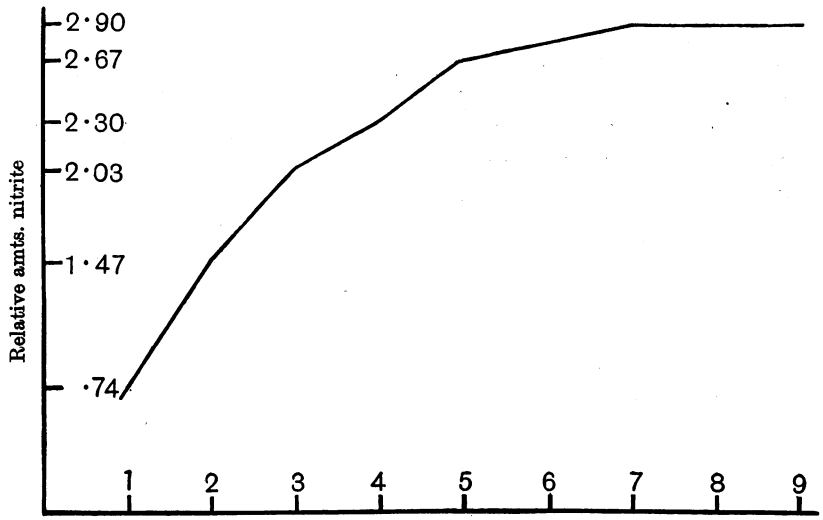

Fig. 2.

Exp. 6. A number of tubes were prepared each containing milk and aldehyde in the proportion of 75 cc. of milk to 1 cc. of 10 % aldehyde; to each tube was added a standard solution of sodium nitrite in serially increasing amounts. The tubes were then rotated in the thermostat at 45° and the contents tested with Griess-Ilosvay reagent at definite intervals of time for nitrite. The accompanying table, from which the lower additions of nitrite have been omitted, sets out the results.

Table V.

Time in minutes	Cc. of standard sodium nitrite added to each tube[1]													
	40	45	50	55	60	65	70	75	80	85	90	95	100	110
30	−	−	+	+	+	+	+	.	.	.	.	.	.	.
60	.	.	−	−	−	−	−	+	+	+	+	+	+	+
90	.	.	.	.	.	.	.	−	+	+	+	+	+	+
120	.	.	.	.	.	.	.	.	−	−	+	+	+	+
150	.	.	.	.	.	.	.	.	.	.	−	+	+	+
210	.	.	.	.	.	.	.	.	.	.	.	−	−	+
390	.	.	.	.	.	.	.	.	.	.	.	.	−	+

[1] In order to prevent undue dilution a stronger solution of nitrite was actually employed for these experiments so that the actual volumes of liquid added were only 1/10 of those given in these columns.

The tube to which 10 cc. of strong nitrite equivalent to 100 cc. of standard sodium nitrite had been added when tested after 6½ hours with Griess-Ilosvay reagent was found to give no reaction, indicating absence of nitrite. In order to ensure absence of traces of nitrite the solution was further warmed with pure nitrate-free urea and hydrochloric acid; it was then tested for nitrate with diphenylamine when a very strong positive reaction was given; this observation has been several times confirmed and the conclusion is therefore reached that the nitrite formed by the action of atite is reconverted back into nitrate. Furthermore this reconversion is effected by some agent contained in the milk, since controls to test the action of atmospheric oxygen and of acetaldehyde upon sodium nitrite in the absence of milk gave negative results. The facts set forth in Table V indicate that this agent, which is responsible for the oxidation of nitrite to nitrate and for which the name of *itate*[1] is proposed, is present in milk in limited amount, the quantity present in 75 cc. of milk being unable to effect the oxidation of 110 cc. of 0·182 % standard nitrite even after 6½ hours. As has been seen, it is only active in the presence of free oxygen and like atite it is dependent on some accelerator or activator such as acetaldehyde (see p. 679). Like atite it is precipitated with the proteins when milk is coagulated and is destroyed in five minutes at a temperature of 99°, in fact its thermolability is similar to that of atite, the thermal inactivation point being between 70 and 75° (Table VI).

Table VI.

Temp. °C.	Reaction with Griess-Ilosvay reagent after the stated intervals of time: 5 minutes	10 minutes	15 minutes
80	+	+	+ } all reactions
75	+	+	+ } very strong
70	+ weak	+ weak	+ weak
65	+ weak	+ weak	+ very weak

After these observations had been made, it was found, on referring to the literature, that Bach [1911] had also noticed in his experiments on milk a tendency for the nitrite to disappear during the course of the reaction; he was however unable to find a reason for this.

It is well known that peroxidase is described as occurring in milk [for literature see Lane-Claypon, 1913]. The question arises whether the oxidation of nitrite to nitrate is not due to the activity of this peroxidase, or, in other words, whether itate and peroxidase are identical. Experimental evidence goes to show that there is undoubtedly a close connection between the two: invariably has it been observed that the disappearance of the itate activity is coincident with the disappearance of the positive guaiacum reaction and *vice versa*, and conditions which destroy the one will destroy the other. If then itate is identical with milk peroxidase it would at any rate appear doubtful whether it is enzymic in nature, since it is used up in the course of its activity.

[1] Since it converts nitr*ite* into nitr*ate*.

ERRATA

Vol. 17 p. 678, lines 1–2, *for* "standard sodium nitrite"
read "0·0182 % sodium nitrite"

line 15, *for* "110 cc. of 0·182 % standard nitrite"
read "11 cc. of 0·182 % sodium nitrite"

Furthermore, since it was observed that milk which gives a positive reaction with guaiacum and hydrogen peroxide is unable to oxidise nitrite in the absence of acetaldehyde, even if oxygen be present, a comparative experiment was set up using the strong direct oxidase of the potato. But in this case also it was found that no oxidising action was exerted upon added nitrite.

Also it has been found that neither the oxidase of the potato tuber nor the peroxidase of horse-radish is destroyed in the presence of acetaldehyde or of nitrite, whilst the peroxidase reaction of milk is so destroyed under the same experimental conditions. From these facts it is concluded that the so-called peroxidase of milk at any rate differs materially in its properties both from a typical peroxidase and from a typical oxidase of plant origin.

III. The action of acetaldehyde.

Acetaldehyde, or some comparable substance, is either requisite, or is necessary as an accelerator, for the activity of atite, itate and Schardinger reductase. It is, however, peculiar in the fact that it will destroy atite, itate and Schardinger enzyme in the presence of oxygen, which leads to the paradox that the same substance which activates also destroys. The following experiment illustrates this.

Exp. 7. Tubes of milk with added acetaldehyde and sodium nitrite in the proportion of 1 cc. of 10 % aldehyde and 4 cc. of a standard nitrite solution (of ten times the usual strength) to 75 cc. milk were rotated in the thermostat at 45°. After 25 minutes they were removed and tested:

Table VII.

Mixture	Guaiacum	Griess	Methylene blue
Milk alone	+	.	+
„ and aldehyde	−	.	−
„ „ nitrite	+	+	+
„ „ nitrite and aldehyde	−	−	−

The results show that heat alone does not destroy the active principles, that aldehyde destroys both the oxidising itate and the reducing Schardinger, and that itate without aldehyde cannot oxidise the nitrite in the given time.

This dual action of acetaldehyde offers a possible explanation of an observation many times made that itate can in a given period oxidise a definite amount of sodium nitrite in the presence of acetaldehyde; but if the nitrite be presented in two portions, the sum of which is the same as the original amount, oxidation is never completed.

Exp. 8. Four tubes were half filled with 75 cc. milk, 1 cc. acetaldehyde, and 6, 7, 8 and 9 cc. standard sodium nitrite respectively. Rotated in thermostat at 45° and tested after one hour:

Table VIII.

	Amount of nitrite added			
Reagent	6	7	8	9
Griess-Ilosvay	−	−	−	−
Guaiacum	−	−	−	−
Diphenylamine	+	+	+	+

To the first three tubes there were then added 3, 2 and 1 cc. of sodium nitrite, *i.e.* sufficient to bring the total amount of nitrite to 9 cc. which amount was completely oxidised in one hour. Even after the lapse of 2½ hours the tubes gave strong positive reactions for nitrite showing that the preliminary heating with aldehyde had destroyed the itate which should have been capable of oxidising the further added quantities of nitrite.

Similarly atite is destroyed by its accelerator acetaldehyde, a phenomenon illustrated in the following experiment.

Exp. 9. 150 cc. of milk with 2 cc. of 10 % aldehyde were rotated in the thermostat at 45° and periodically tested. After 15 minutes, the milk no longer gave a positive reaction with Guaiacum tincture and therefore presumably the itate was destroyed. The milk, which still contained acetaldehyde, was then divided into two equal portions, to one (A) were added 3 cc. of standard nitrite (strong) and to the other (B) 5 cc. of 4 % nitrate. A control (C) containing 75 cc. of milk, 1 cc. aldehyde and 3 cc. nitrite was also put up. The three tubes were rotated in the thermostat at 45° and periodically examined:

Table IX.

Time in minutes	Test	(A) Added nitrite	(B) Added nitrate	(C) Control
15	Griess	+	–	–
	Guaiacum	–	–	–
45	Griess	+	–	.
	Guaiacum	–	–	.
120	Griess	+	–	.
	Guaiacum	–	–	.

Since the control (C) by its negative reaction with Griess showed that the itate was active, the failure to destroy the added nitrite in (A) must be attributed to the disappearance of the itate, it having been inactivated by the preliminary treatment with aldehyde. Similarly the failure to produce nitrite in (B) must be due to the destruction of atite since, as is shown by (A), the preliminary heating with aldehyde had destroyed the itate. Otherwise expressed there are only two ways in which the absence of nitrite in (B) can be accounted for; one of these is the inactivation of atite and the other the excessive activity of itate in oxidising nitrite to nitrate; this latter alternative is however excluded by (A), wherefore it must be concluded that the atite had been destroyed.

It has been stated above that oxygen is essential for this destruction to take place; to show this the following experiment may be quoted.

Exp. 10. The oxygen was replaced by nitrogen in a mixture of 225 cc. milk and 3 cc. of acetaldehyde and rotated, under anaerobic conditions, in the thermostat at 45°. After 2¾ hours, 15 cc. of 4 % sodium nitrate were added, precautions being taken to exclude air, and the rotation continued. After the lapse of one hour the mixture was titrated for nitrite and gave the value 0·677; at the end of the second hour, the titration value was 1·42.

From this it follows that neither prolonged heating, nor the presence of aldehyde in the absence of oxygen impairs the activity of atite. After the titration was made the remainder of the milk was well aerated, replaced in the thermostat and tested with Griess-Ilosvay reagent after the lapse of 15 minutes. A negative reaction was obtained showing likewise that neither prolonged heating nor the presence of aldehyde in the absence of oxygen impairs the activity of itate.

By similar experimentation, details of which it is unnecessary to give, it was found that Schardinger reductase is destroyed by acetaldehyde in the presence of oxygen.

Summarising the evidence relating to the action of acetaldehyde; whilst acetaldehyde is an accelerator, it also in presence of oxygen destroys atite, Schardinger reductase, and itate, that is both reducing and oxidising systems. It is difficult to understand the function of the oxygen in the destruction of substances of so diametrically opposed properties.

IV. The nature of atite and itate.

With respect to atite, Bach considers it to be an enzyme; Kastle and Elvove suggest it might be an oxidisable substance; and Harris and Creighton consider it to be an enzyme and term it reductase.

Any such views as may have been held with regard to atite by earlier authors do not apply to itate since this substance has not been previously described by other workers, although the fact of the disappearance of nitrite is mentioned by Bach. In deciding whether these substances are enzymes or oxidisable and reducible substances respectively, much depends on the relative importance attached to the characters of thermolability and of indestructibility in the normal reaction. For us the latter would appear the more important, and from the experimental evidence brought forward it seems that the most reasonable explanation of the nature of atite and itate is that they are respectively oxygen accepting and donating substances present in milk in limited amount; limited, in view of the fact that they are only capable of reducing or oxidising definite amounts of nitrate or nitrite respectively. At the same time we have not overlooked the possibility of the toxic effect of the aldehyde in gradually depressing the activity of both atite and itate in such a manner as ultimately to mask what to us appears the most characteristic feature of enzyme action, namely indestructibility in a normal reaction. The possibility of atite and itate being the oxidised and reduced forms respectively of one and the same substance, comparable with glutathione, is excluded for the reason that the amount of nitrite which can be oxidised by itate is very much greater than the amount of nitrite which the atite can produce in the course of a normal experiment.

SUMMARY.

1. Milk contains an oxidisable substance which has the power under certain conditions of reducing nitrate to nitrite. Although possessing one of the characteristics of enzymes, namely thermolability, the evidence indicates that it is not a true enzyme, in that it is destroyed in the process of reduction. It is precipitated by the methods commonly employed in coagulating milk. The name of *atite* is proposed for this substance pending more information regarding its nature.

2. Milk also contains a substance capable under certain conditions of effecting the oxidation of nitrite to nitrate. It is active only in the presence of oxygen and under the most favourable conditions will oxidise the nitrite formed by atite as quickly as it is formed, so that under the requisite conditions of the experiment the presence of neither of these two active bodies is discernible. This substance is termed *itate*, and the evidence points to the fact that it is not a true enzyme, in that it is destroyed in the course of its oxidative activity. Its characters resemble those of atite as regards thermolability and precipitation.

3. For both atite and itate an accelerator is required, aldehydes, especially acetaldehyde, being the most potent. The action of acetaldehyde is peculiar in that in the presence of oxygen it destroys atite, itate, and also Schardinger enzyme.

4. The reducing substance known as atite is distinct from the reducing enzyme of Schardinger.

5. Since the disappearance of the guaiacum-peroxide reaction of milk is coincident with the destruction of itate the latter is possibly identical with "peroxidase"; since moreover the conditions which bring about the inactivation of milk "peroxidase" do not similarly affect either the peroxidase of horse-radish or the oxidase of the potato it appears that milk peroxidase differs fundamentally in properties from plant peroxidases.

6. No direct physiological significance is attached to the presence of these two active principles, atite and itate; indeed it is difficult to rationalise such a circulation, nitrate→nitrite→nitrate, in a secretion. To what extent they are present in the milk of mammals generally has not been ascertained.

REFERENCES.

Bach (1911). *Biochem. Z.* **33**, 282.
—— (1912). *Biochem. Z.* **38**, 154.
Harden and Lane-Claypon (1912). *J. Hyg.* **12**, 143.
Harris and Creighton (1912). *Proc. Roy. Soc.* Lond. B, **85**, 486.
Kastle and Elvove (1904). *Amer. Chem. J.* **31**, 606.
Lane-Claypon (1913). Reports to the Local Government Board on Public Health and Medical Subjects. New Series, No. 76.
Rullmann (1913). *Z. Unters. Nahr. Genussm.* **6**, 603.
—— (1914). *Z. Unters. Nahr. Genussm.* **7**, 81.
Seligmann (1906). *Z. angew. Chem.* **19**, 1540.