given off consists of both hydrocarbons. Further, when carbon monoxide, diluted with nitrogen to make the action less violent, was acted upon by magnesium, the black residue gave very little silver precipitate, but when hydrochloric acid gas was added to the mixed gases then a good yield of unsaturated hydrocarbon was obtained and the analysis of the silver precipitate indicated nearly pure allylene. We conclude, therefore, that it is magnesium allylide that gives the allylene and not a carbide.

CHEMICAL LABORATORY, WASHINGTON UNIVERSITY,
ST. LOUIS, MO.

STUDIES IN THE ACTION OF HEAT ON MILK.[1]

BY R. R. RENSHAW AND F. C. WARE.

Received January 11, 1910.

A number of experimenters[2] have noticed that when milk is heated, the lactose apparently diminishes in quantity. The subject, however, has not been systematically investigated.

Richmond[3] first called attention to the fact that the specific rotatory power of lactose in milk materially diminished on heating, but that the reducing power decreased only slightly. Later he and Boseley[4] determined lactose in samples heated for $1^1/_2$ to 3 hours at 100° and found that there was a decrease of from 8.8 per cent. to 23 per cent. of the sugar as measured by the optical method. In another series where sugar was determined gravimetrically, no change was noticed. They offered the suggestion that this may be due to the formation of caramel, since the rotatory power decreased roughly in proportion to the increase of yellow color in the milk.

It did not seem to the present authors that caramelization could be the primary cause of the difference in the optical and gravimetric determinations, since the rotatory and reducing power should decrease nearly proportionally, due to the relatively large molecule of the caramel compared to that of lactose. It was for the purpose of investigating this that the following experiments were undertaken. We were particularly interested to find out the character and extent of the change taking place at the temperatures which might be used in pasteurization.

A number of possible causes for the change in rotatory power of the milk serum presented themselves among which may be mentioned changes

[1] Submitted by F. C. Ware, in partial fulfilment of the requirement for the degree of Master of Arts at Wesleyan University, June, 1908. The experimental work was carried on in the Chemical Laboratory, Stamford High School, Stamford, Conn.

[2] Wroblewski, *Oest. Chem. Ztg.*, 1, 5 (1898); Leeds and Coon, *Pharm. J.*, **23**, 86; Cazeneuve and Haddon, *Compt. rend.*, **120**, 1272; and others.

[3] *Analyst*, **17**, 222.

[4] *Ibid.*, **18**, 141 (1893).

producing soluble *l*-rotatory glycerophosphates,[1] or small amounts of a highly *l*-rotatory amino acid, not precipitated by the clarifying agents used. In order to test these, the sugar was determined by both methods, as well as total phosphorus and total nitrogen in the protein-free serum, on samples of milk heated different lengths of time up as high as 80°. A rapid decrease of sugar was noticed, but strange to say nearly identical results were obtained by each method, and in all subsequent work concordant results were obtained where great care was used in maintaining uniform conditions, particularly as to temperature of pipetting samples,[2] complete washing of the asbestos filter, etc. With such results from the preliminary experiments it was thought not necessary to consider phosphorus and nitrogen determinations.

We next took up a study of the behavior of lactose in a solution which contained approximately the same active mass of hydroxyl ions that occur in milk, in order to see if the alkaline salts were instrumental in the decomposition. Owing to the great complexity of milk as a solution, with its varying amounts of di- and triatomic acids and bases, each with a different temperature coefficient of ionization, and to the incalculable chemical changes caused by heat, it is impossible to compute ionic concentrations. Practically, however, an approximation could be obtained easily, by making a solution containing equivalent percentages of the principal electrolytes found in milk, with the understanding that precipitation of solids and evolution of carbon dioxide would occur. For this purpose 1 gram each of hydrochloric and citric acids,[3] 2.42 grams phosphoric acid, and calcium carbonate containing 1.9 grams of calcium oxide, were mixed with water and the solution neutralized to phenolphthalein with 0.1 *N* sodium hydroxide and 148 cc. (alkalinity × 10) in excess added. In this, 4.87 grams of lactose were dissolved and the whole made up to a liter, giving a solution containing one-tenth of the percentage of sugar that was found in the milk we were working with. No change in sugar content was noticed after heating this for two hours and a half at 85°. This solution, containing approximately the same amounts and similar kinds of positive and negative elements or radicles as occur in milk, except, of course, that all were in inorganic combination, should afford as high, if not a higher, hydroxyl ion concentration than the milk. It seemed then that the salts had no effect on the sugar at this temperature.

Series of tests were next run on milk heated to 60, 70, 80, and 85°. Three sets of samples were made up for each temperature from the same specimen. One of pure milk, the second containing formaldehyde, 1 : 2500, and the third the same, 1 : 20000. The aldehyde was used

[1] Bordas and Raczkowski (*Ann. chem. anal.*, **8**, 168 (1903)) have shown that when milk is heated the lecithins are decomposed.

[2] It was noticed that temperature differences materially affected results. The analytical work was done in a room fitted up with a constant temperature regulator.

in these two concentrations in the hope of distinguishing between bacterial and enzymatic action.[1]

For sugar, the Wiley and Erwell optical and the Defren gravimetric methods were used. The acidity was determined with phenolphthalein without the removal of carbon dioxide and is expressed below in cc. of 0.1 *N* sodium hydroxide per 100 cc. milk. Lacmoid was used for alkalinity. It also is expressed in cc. 0.1 *N* solution per 100 cc. milk.

The presence of a sediment containing calcium phosphate was confirmed in the pasteurized milk.

The sugar, acidity and alkalinity were determined immediately after cooling the sample. The milk used in the 70 and 80° tests was of the same lot, from a mixed herd, and was about five hours old at the beginning of the 70° test. That for the 80° test stood about four hours longer in a good refrigerator. The tests at 60 and 85° were run about a month later on milk from the same dairy and at about the same length of time after milking.

The results for 60 and 80° are given below in detail:

RESULTS ON MILK HEATED AT 60°.—LACTOSE.

Time heated.	0 hr.	¼ hr.[2]	½ hr.	¾ hr.	1 hr.	1½ hrs.	2 hrs.	2½ hrs.	Diff.
Milk alone, opt.	4.42	4.34	4.3	4.27	4.24	4.13	4.02	3.96	0.46
Milk alone, grav.	.....	4.32	.....	4.26	.....	4.11	.....	3.96	0.49
" and ald. 1 : 2500, opt.	.....	4.37	4.35	4.35	4.34	4.32	.....	4.33	0.09
" and ald. 1 : 20000, opt.	.....	4.39	.....	4.37	.....	4.25	.....	4.24	0.18
Acidity	21.4	22.0	22.5	22.8	23.4	24.5	25.6	26.0	4.6
Ald. 1 : 2500	.....	21.6	21.4	21.6	21.8	21.7	21.8	22.0	0.6
Ald. 1 : 20000	.....	21.8	.....	22.2	.....	22.6	.....	22.7	1.3
Alkalinity	31.4	30.4	29.9	29.6	29.2	28.6	28.2	27.6	3.8
Ald. 1 : 2500	.....	31.2	31.24	31.0	31.2	30.8	31.0	30.78	0.62
Ald. 1 : 20000	.....	30.9	.....	30.6	.....	30.2	.....	30.0	1.4

RESULTS ON MILK HEATED AT 80°.—LACTOSE.

Time heated.	0 hr.	¼ hr.	½ hr.	¾ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.	Diff.
Milk alone, opt.	4.52	4.37	4.21	4.08	4.05	4.01	4.0	4.0	0.52
Milk alone, grav.	4.48	4.33	4.17	4.07	3.98	3.96	3.97	3.96	0.53
Ald. 1 : 2500, opt.	.....	4.48	.....	4.46	.....	4.47	.....	4.44	0.08
Ald. 1 : 20000, opt.	.....	4.46	.....	4.44	.....	4.41	.....	4.41	0.09
Acidity	16.8	18.4	19.5	20.3	20.7	21.3	21.8	21.7	4.9
Ald. 1 : 2500	.....	16.9	.....	17.0	.....	17.1	.....	17.2	0.4
Ald. 1 : 20000	.....	17.1	.....	17.2	.....	17.6	.....	17.7	0.9
Alkalinity	33.4	32.1	31.4	30.9	30.5	30.1	29.5	29.2	4.2
Ald. 1 : 2500	.....	33.4	.....	33.2	.....	33.0	.....	32.9	0.5
Ald. 1 : 20000	.....	33.2	.....	33.4	.....	32.7	.....	32.5	0.9

[1] Price (*Abs. U. S. Hyg. Lab., Bull.* 41, 379) has shown that many enzymes can withstand formaldehyde 1 : 20000, while the growth of the more common bacteria is prevented. A strength of 1 : 2500 stops the action of most enzymes.

[2] This does not include the time of heating necessary to bring the milk up to the required temperature in the thermostat but means 1/4 hr. at the designated temperature. For the 85° test this required 15 minutes and for the lower temperatures a somewhat shorter time.

In the series at 60°, a fairly regular and continuous decrease was noticed up to $2^1/_2$ hours in the sugar and alkalinity, while there was a corresponding increase of acidity. Practically no changes occurred in the sugar and acidity when formaldehyde was present in the proportion of 1 : 2500, and only slight changes with a strength of 1 : 20000. At 80° the changes were more rapid at the beginning and had nearly ceased by the end of the first hour. Figures obtained at 70° showed that the changes here stood intermediate between the two given. At 85° the changes went more rapidly at the beginning and were practically over after the first $^1/_2$ hour. There was neither as much decrease in sugar or alkalinity, nor as much increase in acidity, however, as in the case of the lower temperatures.

It would seem then that the major cause of the change in the sugar was bacterial or enzymatic action, for, although no very strict relationship seems to exist between the amount of acid formed and the decrease of sugar, as the following table exhibits, still, the results are in accord with Haacke's[1] observation that the amount of lactic acid found at any one time is not strictly proportional to the amount of lactose decomposed, because (probably) the lactic acid is transformed into other substances. The acid never exceeds one-third of the amount of sugar decomposed.

	60°.	70°.	80°.	85°.
Per cent. sugar loss after $2^1/_2$ hours..	10.4	11.94	11.28	6.33
Increase of acid calc. as gs. lactic	0.0414	0.0513	0.0441	0.0333
Loss of lactose in gs..	0.46	0.54	0.52	0.28

It is difficult to interpret in the light of our results, the data found in the literature showing the differences in optical and gravimetric determinations for sugar in heated milk, because the temperatures used were higher (100°), and neither the history of the milk nor the details of analysis were given. We regret that time has not permitted investigating the sugar changes at 100°. It seems certain, however, that the data furnished by the difference between the optical and gravimetric methods for sugar in pasteurized milk cannot be used as confirmatory evidence of pasteurization, as has been recommended.

Theoretically, of course, very different results would be obtained from different samples of milk, due to varying amounts (as well as kinds) of inoculation, for it has been shown that the lactic acid organisms vary greatly in the extent, rapidity and persistency of their action. Tissier and Gasching[2] state that *B. acidi-paralactici* has a high order of resistance, that it works actively and vigorously, and produces mostly *d*-lactic acid. Heinemann[3] seems to have shown that ordinary *B. acidi-lactici* gives

[1] *Arch. Hyg.*, **42**, 16–47 (1902).
[2] *Ann. inst. Pasteur*, **17**, 540–563 (1903).
[3] *J. Biol. Chem.*, **2**, 603–612 (1907).

chiefly the *l*-acid, and this appears to be augmented by higher temperatures.[1]

Observations by Rullman,[2] that it requires an hour's heating at 68–69° to kill the major portion of the bacteria in milk, and by Mullen,[3] that oxidizing ferments increase in activity from 0 to 60°, and that they are still active at 80°, are noteworthy. The investigations of many others[4] support the statement that the milk ferments are not materially injured by heating at 60–65° for some time. They are weakened only at 65–70° and are finally destroyed at 70–80°.

These facts, along with the knowledge recently obtained concerning the action of Metchnikoff's *B. bulgaricus*, largely remove the cause for surprise at the large amount of sugar transformed. It has been shown that *B. bulgaricus* is universally found in milk. It works best at temperatures above 40° and under the anaerobic conditions which would prevail, at least in bottle pasteurization, and it propagates prodigiously, producing extraordinary amounts of lactic acid. It is hence not necessary to assume cytolysis, with an accompanying liberation of an intracellular enzyme having a higher thermal death-point than the organism. It is to be regretted that so little work has been done in determining the important relation between the thermal death-points of the organisms and their characteristic enzymes.

From the small differences between the results obtained by using the two strengths of formaldehyde, it would appear that one was dealing with bacterial rather than enzymatic action. This is further substantiated by the fact that it is an exceedingly difficult matter to procure lactic acid enzymes from lactic acid organisms.[5] Such small differences as are observed might be due to only partial killing of the bacteria at the lower concentration, or to a slight secretion of an extracellular enzyme.[6] The very rapid transformation of sugar into lactic acid (in one case 0.24

[1] These facts indicate that for very close work certainty of complete precipitation of the lactic acid by the clarifying agents is necessary if the fermentation has occurred at higher temperatures, for under these conditions equivalent amounts of *d*- and *l*-acid may not be formed.

[2] *Z. Nahr.-Genussm.*, **7**, 81–89 (1904).

[3] *Arch. Hyg.*, **44**, 132–3 (1902).

[4] For a comprehensive review of this subject and for some original contributions, see article by Kastle and Roberts, *U. S. Hyg. Lab., Bull.* 41, 309–408 (1908).

[5] The senior author has attempted, so far unsuccessfully, to get a lactic acid enzyme from B. *acidi-lactici* in sufficient quantity to experiment with. It is hoped that this may yet be done. Herzog (*Z. physiol. Chem.*, **381** (1903)) got a very slightly active solution from this organism which would transform lactose into lactic acid in the absence of organisms.

[6] Dox, in a paper on "The Development of Catalase in Lower Fungi," read at the Boston meeting of the American Chemical Society, Dec., 1909, stated that he found a rise of temperature favored the formation of extracellular enzymes.

gram giving 0.0252 gram acid in $^1/_2$ hr.) seemed extraordinary to the authors. It has been abundantly proven, however, that there are optimum temperatures for catalyzers in general, that is, temperatures allowing a maximum in the reaction along with a minimum of destruction to the catalyzer itself.

Many instances could be cited of the extraordinary influence of rise of temperature on the reaction velocity of catalytic actions, and particularly is this true of organic catalyzers (enzymes) within narrow margins of temperature. For instance, Tammann[1] has found that the temperature coefficient for emulsin on salicin between 60 and 70° was 12.3. What is true of enzymes is also true in a degree of cells, for broadly speaking these also are catalyzers, or rather systems of catalyzers. The organisms, then, may continue very rapidly to increase in activity until finally they die in hyper-activity. With this in mind, the increased velocity of sugar decomposition as higher temperatures were used becomes clear. This affords an explanation, too, of the occasional curdling of milk on pasteurizing, and gives further reasons for using a not too highly inoculated milk in such processes.

Conclusions.

1. The alkaline salts have no effect on the lactose in milk when it is heated at 85° for some time.

2. Perfectly concordant results can be obtained by the optical and gravimetric methods for lactose in pasteurized milk.

3. Concordance between the results by the optical and gravimetric methods can not be taken, as has been suggested, as evidence that the milk has not been pasteurized.

4. In bottle pasteurization it is impossible to heat the milk quickly enough to prevent appreciable amounts of lactose being decomposed, unless the milk has a very small bacteria count.

5. Some lactic acid organism or organisms work prodigiously at a temperature of 80–85°, though for a short time. They work more slowly but for a longer time at 60°.

6. The presence of a sediment containing calcium phosphate in pasteurized milk has been confirmed.

WESLEYAN UNIVERSITY, MIDDLETOWN, CONN.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MICHIGAN EXPERIMENT STATION.]

ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS.

BY S. L. JODIDI.

Received January 27, 1910.

When we take into consideration that not less than one-seventh of the total area of the state of Michigan consists of swamp lands largely covered

[1] *Z. physiol. Chem.*, 16, 323.