gives striking color reactions with H_2SO_4, HNO_3, KOH, CaOH and other reagents.

A solution to which a few drops of silver nitrate are added gives a pink color changing quickly to a dull green.

Iodine as Lugol's solution gives a pink color which quickly fades.

With ferric chloride a very dark blue or black changing to brown.

On the addition of Fehling's solution it turns green and on boiling a slight reduction occurs.

It does not reduce Fehling's solution in the cold on long standing even after it had been previously boiled with acid and again rendered neutral. This leads to doubt as to its being a glucoside.

With Millon's reagent it turns a port-wine color rapidly becoming darker which suggest the possibility of its belonging in the group of the phenols.

69 (1816)

The effect of heat on the calcium salts and rennet coagulability of cow's milk.*

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When milk is boiled a precipitation of a portion of the calcium phosphates occurs, the amount of fixation being proportional in general to the amount and duration of heat applied. Söldner[1] first called attention to this fact and his observations have been confirmed by numerous investigators, among whom may be mentioned Boekhout and de Vries,[2] Purvis, Brehaut and McHattie,[3] and Grosser.[4]

It has been commonly believed, also, that some fixation of the

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[1] Söldner, F., *Landw. Versuchs.*, 1888, xxxv, 351.

[2] Boekhout, F. W. J., and de Vries, J. J. O., *Landw. Versuchs.*, 1901, lv, 221.

[3] Purvis, J. E., Brehaut, A. H., and McHattie, A. C. N., *Roy. Sanit. Inst. Journ. Trans.*, 1912, xxxiii, 154.

[4] Grosser, Paul, *Biochem. Zeitschr.*, 1913, xlviii, 427.

calcium phosphates takes place during the holding process of pasteurization. The fact that pasteurization of milk retards the coagulability of the casein by rennet and the fact that this property can be restored by the addition of calcium chloride to the milk have been presented in support of the view that heat changes some of the soluble calcium salts to an insoluble form. The experimental evidence for such a change is, however, contradictory. Solomin[1] noted that a little phosphorus falls out of milk when the temperature is raised to 80° C. Diffloth[2] found a decrease of 26 per cent. in the soluble phosphates when milk was held at 60° C. for 30 minutes. Rupp,[3] however, approached the problem by filtering raw and pasteurized milk through a clay filter and analyzing the filtrate for calcium and phosphorus. He found no change in the calcium and phosphorus after holding the milk for 30 minutes at 68.3° C. Milroy[4] held the fresh milk at a temperature just below the boiling point for one hour and, after filtering through an ordinary filter, noted a decline in calcium. He explained this result on the basis of a transformation of dicalcium phosphate into basic calcium phosphate. Grosser made similar studies on samples of milk which had been boiled for 5, 10 and 15 minutes, respectively, and noted a negligible loss in phosphorus in the filtrate, but a slight loss in calcium.

Daniels and Loughlin[5] have recently obtained qualitative evidence that calcium phosphates are thrown down when milk is pasteurized by the holding process, in that they have noted a nutritional calcium and phosphorus deficiency of such milk which could be prevented by feeding the washings from the walls of the vessel in which the milk was pasteurized or by addition of calcium phosphate to the rations of the animals (rats) in the experiments.

The explanation commonly held for the effect of heat on the calcium phosphates of milk originated with Söldner who believed that the calcium of milk is present as mono- and dicalcium phos-

[1] Solomin, P., *Arch. f. Hyg.*, 1897, xxviii, 43.

[2] Diffloth, Paul, *Bull. d. Sci. Pharm.*, 1904, x, 273; *Zeit. Nahr. Genussm.*, 1906, xi, 455.

[3] Rupp, Philip, U. S. Dept. of Agr., Bureau of Animal Ind. Bull., 1913, clxvi, 1–16.

[4] Milroy, T. H., *Biochem. Jr.*, 1915, ix, 221.

[5] Daniels, A. L. and Loughlin, R. J., *Biol. Chem.*, 1920, xliv, 381.

phates which, on heating, pass to tricalcium phosphate and are thus precipitated. This explanation has apparently never been submitted to critical examination. Monocalcium phosphate is readily soluble in water and its solutions decompose on boiling giving rise to dicalcium phosphate whose solubility is so low that a heavy precipitation of phosphates occurs. The solubility of dicalcium phosphate is, however, only 0.135 to 0.561 part per 1000 of water, depending on the saturation of the water with CO_2. The more highly concentrated solution naturally gives up some of its calcium phosphate on heating, due to the loss of CO_2. The solution in pure water also clouds up on boiling.

The facts just cited seem to support, in general, Söldner's theory. However, the experimental results of Grosser[4] and Rupp,[7] cited above, show that there is actually little if any decrease in the calcium phosphates dissolved in milk when the milk is held at pasteurization temperatures or boiled for some minutes. At the same time there is abundant evidence, as indicated, that heat does precipitate calcium phosphates from milk. How are these divergencies in results to be explained?

It occurred to the writer that a simple explanation of these divergencies is afforded by the experimental evidence brought forth by Van Slyke and Bosworth[1] that the calcium phosphate of cow's milk is wholly in the form of dicalcium phosphate, amounting to about 1.75 parts per 1000, on the average. These figures are greatly in excess of the maximum solubility of dicalcium phosphate, even in water saturated with CO_2. These investigators found, moreover, that the dicalcium phosphate of cow's milk was retained on the Pasteur-Chamberland filter when milk is filtered through this medium. The natural conclusion to be drawn from these results is that the calcium phosphate of cow's milk, which appears to be wholly in the form of $CaHPO_4$, is present in colloidal solution, and that the aggregates of particles are sufficiently large that they do not pass through the Pasteur-Chamberland filter, or even through the Bechloidt filter used by Grosser, or the clay filter used by Rupp. This conclusion coincides with the results of Grosser and Rupp who obviously were

[1] Van Slyke, L. L., and Bosworth, A. W., *N. Y. Agr. Exp. Sta. Tech. Bull.*, 1914, xxxix, 1–17.

not dealing with the colloidal matter of milk in their analyses of filtrates from the clay filters which they used. The experiments which have shown a gross decline in calcium phosphates or in which the precipitated phosphates have been seen, when milk is heated, are to be explained, therefore, solely by the effects of heat on colloidal solutions of dicalcium phosphate. As a matter of fact the loss of calcium and phosphorus from milk on boiling observed by Söldner showed a ratio of one molecule of calcium to one of phosphorus such as exists in dicalcium phosphate.

EXPERIMENTAL.

In order to determine what the effect of heat is on colloidal $CaHPO_4$ solutions, such a solution was prepared by grinding $CaHPO_4$, which had been washed free from electrolytes, to an impalpable powder in a porcelain ball mill. This powder was then ground further in the mill in the presence of a 0.6 per cent. gelatin solution. After settling, the supernatant fluid presented a very satisfactory colloidal suspension of $CaHPO_4$. It was distinctly milky and showed the usual Tyndall effects in a striking manner. The concentration of $CaHPO_4$ was not, however, as high as had been expected, the solution being found to contain only 0.542 gram per 1000. Possibly a higher concentration would have been obtained if a stronger solution of gelatin had been employed or a better colloid stabilizer used. Gelatin was chosen, however, because it is not coagulated by heat.

The effect of heat on this colloidal solution of $CaHPO_4$ was determined qualitatively only by heating a portion of it in a water bath at 63° C. for 30 minutes. A heavy precipitation of $CaHPO_4$ resulted and the filtrate showed much less evidence of a colooidal suspension.

This simple experiment shows rather conclusively that it is not necessary to assume any transformation in the composition of the calcium phosphates of milk during heating to account for the partial fixation of these salts. The phenomenon is readily accounted for by the effect of heat on a colloidal solution of $CaHPO_4$ which renders such a solution much less stable and causes the aggregates to pass, in part at least, to the crystalloid form.

The results secured in this experiment have a bearing, also, on the alleged effect of heat on the calcium salts of milk as affecting the coagulability of milk by rennet. It seems evident that the only calcium salts affected by heat are colloidal calcium salts and the question is therefore raised as to the possibility of the colloidal $CaHPO_4$ of milk playing a part in the rennet coagulability.

In order to determine whether this is true or not, two 200 c.c. portions of fresh whole milk were dialyzed in collodion bags against running distilled water for 48 hours, using 1 per cent. toluene as preservative. When rennet was added to this milk there was no coagulation even after several hours. One drop of 4 molar $CaCl_2$ solution added to 100 c.c. of the rennet treated milk caused instant coagulation. The same result followed the addition of 2 or 3 drops of dilute HCl solution. The addition of 10 c.c. of the colloidal gelatin solution of $CaHPO_4$ to 100 c.c. of the rennet treatment milk was, however, without any effect.

It is apparent that the colloidal $CaHPO_4$ of the milk does not play any part in the rennet coagulation. The indications are, also, that the effects of heat on rennet coagulation which can apparently be overcome by the addition of soluble calcium salts are not to be explained on the grounds of an effect of the heat on the calcium salts of the milk but rather on the grounds of an effect of heat on the casein itself. Just what this may be is not definitely clear, as yet. The author has this problem under investigation and hopes to be able to present definite data on it at a later date. It will be sufficient to point out at this time that the explanation of this phenomenon involves the fact that rennet coagulation is unquestionably both a chemical and a colloidal reaction. The calcium caseinate of milk is in colloidal solution. Rennet appears to hydrolyze the calcium caseinate into two molecules of calcium paracaseinate. The clotting of the calcium paracaseinate is a secondary phenomenon which is a gellation, perhaps of the nature of a crystallization of colloid—in this case a hydrophyllic colloid in a state of hydration. The conditions which govern what is regarded as a normal clotting of the calcium paracaseinate are evidently disturbed by the application of heat to the colloidal calcium caseinate of the milk. Zoller[1] has recently shown how

[1] Zoller, H. F., *J. Ind. Eng. Chem.*, 1921, xiii, 510.

the properties of the casein of milk are affected by heat so as to have a marked influence on the precipitation of casein by acids and on the hydrophyllic properties of the casein thus precipitated. Apparently the addition of soluble calcium salts to milk helps to restore the conditions existing in raw milk which govern the normal rennet clot. All the interrelations of calcium and rennet coagulation have obviously not been determined. A study of these relations is at present in progress in this laboratory.

SUMMARY.

It is shown that the partial fixation of the calcium salts of milk by pasteurization or boiling is readily explained simply on the grounds of the effect of heat on colloidal solutions of $CaHPO_4$, the calcium phosphate natural to cow's milk.

It is shown further that the effect of heat in retarding the rennet coagulability of milk is not related directly to the loss of colloidal $CaHPO_4$ because the addition of colloidal $CaHPO_4$ to dialyzed milk does not restore its coagulation by rennet, while the addition of $CaCl_2$ or HCl does restore this property.

The phenomenon of rennet coagulation is discussed briefly from the standpoint of the chemical and physico-chemical reactions involved, and also from the standpoint of the possible bearing which the addition of calcium salts to heated milk has on this phenomenon.

70 (1817)

The velocity of development of the demarcation current in the frog's sartorius.

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Urano and Fahr have experimentally established the fact that the potassium ion is almost exclusively the only cation within the muscle cell of the frog's sartorius. Overton has shown that the demarcation current of the frog's sartorius may be inhibited or have its sign reversed by replacing the lymph fluid surrounding the muscle cell by a fluid containing K ions in place of the normally