THE PROTEIDS OF COW'S MILK.

By Albert R. Leeds.

I.

*Upon the three varieties of casein found in milk by E. Duclaux.**

According to the first communication of this author, there is not simply one variety of casein in milk. Besides solid casein, which is seen to fall to the bottom of the vessel on the milk standing (in one case representing 0.4 p. c. of all the casein), there is casein in a colloidal state, passing through all paper filters, but unable to pass through a filter of baked porcelain. After filtration through porcelain, the filtrate, containing according to Duclaux, no casein, gives a precipitate upon the application of heat (Albumin). After filtering off this precipitate, subacetate of lead and Millon's reagent throws down a third albuminoid substance which is the lactoprotein of Millon and Comaille.

He states that if the material arrested by the filter be removed and suspended in water, it will nearly all dissolve ($\frac{3}{4}$ of it in the course of three years) and that it dissolves as lactoprotein, or at least with all the characteristics of lactoprotein. Simply suspending the casein in water serves to bring about the appearance of all those materials which have been met with and sought for as affording characteristic reactions in milk. All these are presented, the casein passing from one to another by insensible transitions, but tending more and more towards those which are perfectly soluble. This is stated to be especially perceptible when the casein is suspended in water which is slightly acid or alkaline. He concludes by stating that casein seems to be a plastic substance modifying itself in an acid, alkaline or neutral medium and dissolving in variable quantities, varying with the time and also with the composition of the liquid. And hence, if the reaction of the liquid be

* Sur les Matieres Albuminoids du lait. E. Duclaux. Comptes Rendus de l'Academie des Sciences, 98, 373 (1884).

changed from an acid to an alkaline, or vice versa, or if any substance be added, a precipitate may occur, and thus be originated the numerous bodies which have been extracted from milk.

In a later communication (ib. **98**, 438), Duclaux claims for his procedure with a porcelain filter, the merit of a new method for the analysis of milk. He regards the determination of albumin and lactoprotein as vain and illusory, believing that the important matter is to separate and determine the casein only. The casein, according to this second communication, exists in three forms:

First. Dissolved casein.

Second. Casein in suspension.

Third. Casein in a colloidal condition.

He restricts himself to the determination of the dissolved casein only, because he regards this dissolved casein as "The final form towards which the two other forms tend, and because its variations exhibit all the transformations which milk undergoes whether in industrial use or in alimentation."

By the porcelain filter he arrests the elements "in suspension, viz.: the fat, the last two varieties of casein and also a part of the phosphate of lime," whilst the elements "in true solution" pass through the filter; viz.: the milk sugar, the remainder of the phosphate of lime and the other mineral salts.

Heat, Duclaux goes on to state, influences the casein but little, giving to the suspended casein cohesiveness and changing it from a mucous state to one more condensed, which is seen from the deposit on the tube of porcelain after filtration. In boiled milk this deposit is always more resistant and less voluminous than in raw milk. Slight acidity causes a small portion to pass from the colloidal into the solid state while a slight alkalinity sends a portion of the solid into the colloidal condition, but neither of these influences sensibly change the proportions of the dissolved casein. The same may be said to be true of pressure. Hence the quantity of dissolved casein in a sample of milk is very stable, and what is more, is seemingly independent of the nature of the milk. Duclaux states that he found nearly the same proportion in cow's milk from different sources, and in goat's, ass's and human milk. Also, that there are two influences which augment the quantity of casein in solution, viz.:

First.—The addition of water which, however, is but slightly active.

Second.—Intervention of a diastase ("casease"). The latter is the more powerful influence, and this casease is prepared by introducing the microbes which produce it, and which act in a manner similar to a pancreatic ferment.

I have repeated the experiments of Duclaux, the especial interest of which lies in the character and quantities of the substances separated out by the porcelain filter, and while my experiments accord with his in respect of the great changes in the composition of the filtered milk thus obtained, yet there are important, and as it appears to me, very significant discrepancies.

Duclaux obtained :

	"In Suspension."	"In Solution."
Fats	3.32 per cent.	....
Milk sugar	....	4.98 per cent.
Casein	3.31 per cent.	0.84 " "
Calcium phosphate	0.22 " "	0.14 " "
Soluble salts	....	0.39 " "
	6.85	6.35

In my own experiments a new Chamberland-Pasteur filter was employed, the milk being placed in a glass tube 8 feet in height and in a funnel reservoir at its top. The funnel was plugged with cotton wool to exclude atmospheric micro-organisms. After three hours not a drop had come through and aspiration was found requisite. The reaction of the filtrate was feebly acid, but not more so than the milk before filtration. Its specific gravity was 1.021 at 15°C, while that of the original milk was 1.033.

	Raw Milk.	Filtrate.	Residue.
Fats	2.18	none	2.18
Milk sugar	4.749	4.291	0.458
Casein	2.96	none	2.96
Proteids other than casein (lactalbumin?)	0.69	0.085	0.605
Ash	0.751	0.414	0.337
Total Solids	11.33	4.790	6.540

The first important difference is that a portion of the milk sugar is arrested, ten per cent. of the total amount present remaining in the residue.

The next is in regard to the casein. The substance termed casein in my analysis is the proteid precipitable by dilute acid. In the raw milk the total proteids were determined according to Rittthausen, the casein by dilute acid, and the other proteids as the difference. In the filtrate the total proteids were found by determining the nitrogen and multiplying by 6.45. Dilute acid produced no precipitate and hence casein is put down as entirely absent. It is noteworthy that while the proteids as found in the filtrate by the above method were 0.119 per cent., their amount as determined by precipitation with alcohol was 0.0848 per cent. These other proteids had the deportment of "lactalbumin" and are so reported.

According then to my experiment 2.96 per cent., or all the casein, was taken out by the procelain filter and also 0.571 per cent. (or 83 per cent. of its total quantity) of the lactalbumin.

It is still a matter of debate as to the form in which the casein exists in milk, many regarding it as in part suspended and in part dissolved, whilst Duclaux adds a third condition—the colloidal. Certain it is that the porcelain removed it all.

But there has never been any question as to the condition of the lactalbumin, all holding it to be in a state of solution. Nevertheless, the filter, assisted by the dense coating originated during the process of filtration, has removed 83 per cent. of this dissolved substance.

The phosphoric anhydride in the solution was 0.085, corresponding to the tribasic calcium phosphate 0.0185 if we grant that it is in this form the phosphoric acid is combined.

The preceding experiments left quite undecided some questions arising from the statements of Duclaux.

In the first place, the statement that in the course of time nearly all the casein redissolves, and that this redissolved casein is filterable through the porcelain, and that the filtrate presents all the characteristics of lactoprotein. "The mere placing of the casein in suspension in water is sufficient to provoke the appearance in

the liquid of all the series of materials which people have encountered in milk, and to which they have endeavored to ascribe distinctive characters. All the terms of this series are present in the beginning, the casein passes from one to the other by insensible transitions, but it tends more and more towards those which correspond to a state of perfect solubility."

Later on, where he speaks of casein as a plastic substance, Duclaux objects to giving different names to the various precipitates obtained from milk, regarding the determination of the percentage of dissolved casein as the only point necessary, all the other soluble proteids in milk being simply in a condition of transition towards this state of dissolved casein.

If these suppositions are correct, then on washing the residue left by filtration on the exterior of the filter by passing a sufficient volume of wash water through it, removing it from the filter, diffusing it in water, dissolving as far as possible the soluble from the fatty and other matters and again filtering, the filtrate should present the characters of casein, and not of lactalbumin or other soluble proteid.

The results of this treatment were as follows :

	Raw Milk.	First Residue.	First Filtrate.	Second Residue.	Second Filtrate.
Casein.....	2.47	3.19		3.1474	
Other Proteids	0.94		0.22		0.043
Fats.....	4.39	3.568	5.101		
Milk-sugar....	4.279				
Phosphoric Anhydride.....	0.223	0.084	0.139		
Calcium Phosphate (calculated)	0.487	0.183	0.303		
Ash	0.751	0.112	0.639		
Total Solids	12.83	6.87	5.96		1.04

It will be seen that the statements of Duclaux are not borne out by the analytical results. Nearly all the proteids were arrested

by the first filtration including all the casein and all the other proteids, except the small amount of the truly soluble proteid which carried the ferment (0.22 p.c.). And that on the second filtration, no casein was present in the filtrate, but, as in the first instance, was all arrested by the filter. The still smaller amount of the truly soluble proteid which passes through (0.043 p.c.) was in all probability the minute amount left in the first residue of total casein and lactalbumin and not removed from them by washing.

The conclusion which may fairly be drawn from these results is:

First. That whatsoever is the condition of the casein (and the reactions are most in accordance with the supposition that it is present as an alkaline caseinate), it is held in the milk in a colloidal state, and can therefore be filtered out on a porcelain filter. Furthermore, that the lactalbumin is likewise held in the milk in a colloidal state, and is filtered out along with the casein. But the starch liquefying ferment, the "galactozymase," is in a state of true solution and passes through the filter.

The milk sugar reported as being present in the residue is simply that part which is detained in the dense skin or coating of proteids and fats, and not removable by washing. A separate experiment was made with a milk sugar solution filtered through a new Pasteur filter. It was found that the filtrate contained precisely the same percentage of dissolved milk sugar as the original solution, showing that there was no stoppage of this truly dissolved substance owing to the magnitude of its molecules and the fineness of the pores of the filter.

Nearly as much of the phosphates present are retained on the filter as pass through, and they constitute nearly, if not all, the mineral residue in the former case. Indeed, if calculated as tribasic calcium phosphate, the amount would much exceed the total ash of the residue on the filter. That they do not exist in this form is evident, but in what condition of partial saturation has not been determined. In this connection the influence of lime upon the precipitation (or coagulation?) effected by rennet is noteworthy, as is also the relation which the phosphoric acid bears to the casein molecule.

II.

The casein obtained by the action of rennet, and the casein obtained by the action of dilute acid.

In a recent communication by Prof. W. D. Haliburton,* the proteid which is present in the milk is termed caseinogen, and that which composes the curd found under the ferment action of rennet is termed casein. And still further the casein-producing substance (or caseinogen) is not only called caseinogen while it stands for the proteid as it exists in milk, but after it has been precipitated out of solution by the action of a dilute acid, like acetic acid.

The nomenclature appears to be based on the interpretation given by Hammersten to the results which he obtained while studying the action of rennet, and from which he concluded that the rennet acting as a ferment produced two substances neither of which exist pre-formed in the milk. One of these is the insoluble part, the curd, called above casein, the other a soluble proteid, called by Haliburton whey-proteid.

In other words, caseinogen plus rennet produces casein plus whey-proteid, and caseinogen plus acetic acid produces caseinogen plus acetate of ——?

According to Hammersten the above casein (from rennet) differs from the casein (produced by acid) by its lesser solvent power upon calcium phosphate, and especially by its no longer having the property of coagulating under the influence of rennet. According to Haliburton in order to restore to casein (produced by acid, his caseinogen) the casein producing property, it must be redissolved in an alkali, preferably lime, with the addition of phosphoric acid, in order to form the necessary amount of calcium phosphate, and so finally rendered coagulable by rennet. Only after this manipulation does it give the insoluble curd and the soluble whey-proteid.

I find it very difficult to draw the inferences above stated from the observed facts. That both alkaline substances and phosphoric acid play an essential part in the phenomena is admitted. But no one, so far as I am aware of, has traced out the effect of these

* The Proteids of Milk, Journal of Physiology, **11**, 449.

mineral salts satisfactorily, nor shown what manner of combinations they form with a proteid (probably a complex organic acid) like that we are dealing with.

The hypothesis at present most generally entertained is, that the casein exists in combination with the alkali only, and that on forming with the aid of the dilute acetic acid an alkaline acetate, the casein is set free. But this reaction and the difficulty of identifying the casein such as it exists in milk with alkali-albuminate in all respects, are both reconcilable with the possibility of the existence in milk itself of a complex compound of the casein-producing substance and the mineral substances present.

When rennet is used, the casein-producing substance is in part precipitated along with some of the phosphoric acid and mineral constituents, and another part remains in solution associated with the remainder of the phosphoric acid and mineral salts. And until these two portions, the insoluble and the soluble, are obtained free from the mineral salts accompanying them it would appear to me to be premature to state that the proteid precipitated by rennet is in itself different from the proteid precipitated by dilute acid. And furthermore, until the influence of these mineral constituents is allowed for, that it is premature to state that rennet acts as a ferment in such wise as to form two new proteids, an insoluble and a soluble, neither of which existed pre-formed in the milk.

These difficulties were not lessened by my own experimental results. On coagulating some milk with rennet, and examining both curd and whey I found :

	Raw Milk.		Curd.	Whey.
Proteids precip. by acid	2.47	Total Proteids	2.41	....
Proteids non-precipitable by acid	0.94	Total Proteids	....	1.00
Phosphoric anhydride	0.223		0.102	0.121
Calculated as				
Tricalcic phosphate	0.486		0.222	0.264
Ash	0.751		0.247	0.504

From which result it would appear that the proteid precipitable by acid (casein), and that non-precipitated (lactalbumin) in the raw

milk are respectively the same as the total proteids in the curd or casein produced by rennet and in the whey. If a new soluble proteid (the whey proteid) is produced by the action of rennet, then I should have anticipated that the total soluble proteids in the whey would have been materially increased. But this is not the case.

Furthermore, both the curd and the whey contain about the same amount of phosphoric acid, and this and the other mineral bodies remain in each to modify profoundly their deportment with the reagents used as tests.

III.

*Upon the Three Varieties of Proteids Present in Cow's Milk according to A. Bechamp, and upon the Phenomena of Coagulation**.

This author discusses and objects to the many distinct phenomena which chemists describe under the one term coagulation. In this article he applies the term to the production of proteid precipitates insoluble in water, by means of alcohol and by heat. And on the supposition that the casein in cow's milk exists as an alkali-albuminate from which the coagulum is thrown down simply by displacing the alkali with the aid of an acid, he speaks of precipitating the casein, not of coagulating it. His experiments are directed to showing, moreover, that casein is a soluble substance and that it is not coagulable.

He finds that besides casein there are two other proteids present in milk, one of which is coagulable by alcohol (lactalbumin) while the other is not (galactozymase). Both are coagulable by heat, and are rendered by it insoluble in their natural solvents (by which he refers more especially to water).

The procedure followed by Bechamp is as follows: To prepare pure casein he adds to the fresh milk, in the cold, acetic acid, drop by drop, until the liquid plainly turns litmus paper the color of onion skin, at which point the dicaseinate first formed is completely decomposed. Soon after the milk is curdled. The whey filters clears whilst the casein in precipitating, brings down the

*Bull. Soc. Chim. (3), **4**, 181–186.

"milk globules" and "microzymes." The precipitate is then washed to free it from all the soluble matters, and after draining its fat is extracted with ether. Then, after being again washed with water, it is diffused through a volume of water equal to that of the milk originally taken, and which has been rendered distinctly alkaline with ammonium sesquicarbonate. The precipitate dissolves, but the solution becomes turbid from the debris of the globule envelopes and from the microzymes. The casein is then precipitated by adding just sufficient acetic acid, and washed with water. If it is pure, the wash waters are not rendered turbid either by ebullition, or by the addition of alcohol. If they are, it is necessary to redissolve and reprecipitate, etc. By prolonged washing the casein is obtained in a pure condition always presenting the same characters.

It dissolves slowly and in small amount at common temperatures, a litre of water dissolving during 52 hours 1.005 grm. It melts at higher temperatures and its solubility increases; 2.37 grms. going into solution in a litre of water raised to ebullition.

It deports itself as a feeble acid which acetic acid precipitates from its solutions, as in milk (so Bechamp states, although it would appear from his earlier statements in this article, that it is with alkali caseinate we have to do in milk and not pure casein), on account of its relative insolubility. It is not coagulated by acid or heat. Its solutions redden litmus paper in the same manner as carbonic acid; but it can form with potassa, soda, ammonia and lime, soluble acid caseinates which redden litmus and which carbonic acid does not precipitate. The caseinates of these bases form solutions which are not precipitated by alcohol and which are not coagulable by heat. At the boiling point, however, a solution of calcium caseinate appears to coagulate, but on cooling the apparent coagulum dissolves. All these reactions distinguish casein from albumen.

The whey from the casein is coagulable by heat and the coagulum is at the same time insoluble in water and in ammonium sesquicarbonate. To the clear whey alcohol of 95% is added as long as a precipitate forms, two volumes at least being required. The voluminous precipitate is freed from milk sugar by means of alco-

hol of 80%, then drained and before drying it is stirred into water and after an interval thrown upon a filter. Something dissolves which is precipitable by alcohol, and the washing is therefore continued until the wash water gives no precipitate with alcohol.

The undissolved portion is dissolved by dilute solution of ammonium sesquicarbonate, leaving an insoluble residue of mineral matters. The ammoniacal solution, treated by acetic acid, forms a precipitate which when collected and washed with water represents *lactalbumin*.

The part precipitated by alcohol but soluble in water is *galactozymase*. When it has been freed from lactalbumin by repeated solutions and reprecipitations, it is completely soluble in water, and it can come to pass that alcohol will no longer precipitate it except on the addition of a trace of sodium or ammonium acetate. The galactozymase of cow's milk liquefies starch paste but without saccharification.

It is evident that lactalbumin exists in the whey in a soluble form. But on precipitation with alcohol it loses its solubility in water, while remaining soluble in ammonium sesquicarbonate. If, however, it is diffused through water and heated to 100 degrees, it contracts without softening and becomes insoluble not only in ammonium sesquicarbonate but also in dilute ammonia.

Galactozymase is in no wise coagulable by alcohol, but yields solutions which heat coagulates whilst depriving them at the same time of their zymasic function.

The specific rotatory power of the aqueous solution of casein for sodium light is (a)j=—117° ; the rotatory and other definite properties of lactalbumin and galactozymase distinguish them absolutely from casein and from albumin, and prove them to be sharply defined, distinct chemical species.

Inasmuch as I have been attempting to discriminate between raw and heated milk by whatever processes appeared available, I have repeated the valuable work of Bechamp in this connection, and have obtained certain interesting results. The method was employed as a basis for analysis, the milk analyzed (A) being in part raised to boiling (B) and in part heated in a sterilizer for one hour at 100° (C).

	A	B	C
Casein,	3.370	3.559	3.653
Lactalbumin,	0.428	0.534	0.366
Galactozymase,	0.314	0.055	0.056
	4.112	4.148	4.075

The conclusions arrived at from these analyses are :

1st. Raising the temperature of milk to the boiling point, and still more the retaining of it in that condition for a lengthened period, as in sterilization, converts a considerable portion of the soluble into insoluble proteids.

2d. The effect of heat is greatest upon the galactozymase, which is as much thrown out of solution by raising the milk to boiling, as it is by keeping it at 100° for an hour.

3d. By prolonged heat the lactalbumin is also partly thrown out of solution in the milk. I say milk, because I am speaking of what takes place in presence of the saline and other constituents of milk, and not what may be true of the coagulability and insolubility of these proteids under other conditions.

4th. If these analyses are correct, raw milk should possess the power of liquefying starch. Milk from which the casein is removed should have that power. Both whole milk and milk deprived of casein should lose the power of liquefying starch merely on its temperature being raised to the boiling point. These suppositions were confirmed by experiments narrated under a separate head.

5th. By heating, the percentage of what is put down in analyses as casein is increased, the increase and the error being greater as the boiling is continued.

6th. I have been unable to satisfy myself that the portion of the casein insoluble in ammonium carbonate represents the debris of the envelopes of the fat globules and the "microzymes." If so, and these envelopes exist, they must be of marvelous tenuity, since the weight of this portion amounted to only 0.0256°.*

* On repeatedly shaking milk with bisulphide of carbon, the latter separates out, carrying with it a white substance which imparts to the

"The theory of an envelope to the fat globules in milk is persistent, but difficult to maintain. Babcock's experiments on butter making showed that churning cream above 85° F. merely reduced the size of the globules. The globules cannot renew their envelopes in proportion to their size as they break up; yet they are not changed in properties, as they would be if the envelope had been destroyed by rupture.

"Apart from the action of fat solvents on milk fat when in its normal condition, there is little to suggest the existence of an envelope. May not the apparent properties of an enveloped globule be equally well explained by the theory of a globule of fat suspended in a liquid of different constitution from pure water, somewhat ropy or mucilaginous, as milk apparently is, and therefore exhibiting different relations of surface tension with reference to the fat globule, as compared with the relations held to the latter by pure water?"

IV.

Upon the Starch Liquefying Ferment in Cow's Milk and Human Milk.

If it be true, as the conclusion set forth under the fourth head in the preceding article would intimate, that there is naturally present in untreated milk a starch-liquefying ferment, the result would be of much interest from its bearing upon infant nutrition.

Inasmuch as changes might be produced in the milk by the processes and reagents employed, I experimented in the first place upon the limpid sterilized liquid obtained with the Pasteur filter,

---

bisulphide layer at the bottom almost the appearance of barium sulphate precipitate. After many washings with water, this yielded after evaporation a percentage of nitrogen corresponding to 0.51 p. c. of the milk treated. It was thought that by osmose the bisulphide might extract the fat, leaving the envelopes of the fat globules intact. I do not consider the above as adding anything additional to the inadequate experimental evidence on which the existence of these hypothetical envelopes rests, and quote the following comments by Prof. Breneman upon this point. His description of the character of the fluid part of the milk, as "somewhat ropy or mucilaginous," I have quoted as aptly expressing a similar conception on my own part.

and which contained in all but 0.119 per cent of proteid matter. The amount and energy of the ferment cannot be otherwise but small, for on treating a paste of one grm. of starch in 200 c. c. water with 5 c. c. of this liquid, it required from 3 to 6 hrs. digestion at 34.5° C. to render the starch entirely fluid. As such it ran readily through a filter, whereas before digestion with ferment it was unfilterable.

On heating to 75° C. the ferment was destroyed, a white coagulum being formed, and the filtrate from the Pasteur filter being entirely without action on starch.

The same results were obtained with 5 c. c. of the original cow's milk. It liquefied the starch paste, but after heating to 75°, or still more readily on boiling, entirely lost this property.

The whey obtained from the milk by the action of rennet was also destitute of a ferment capable of liquefying starch. As a control experiment, the rennet itself was tested and likewise found to be without such action.

On throwing down the casein with dilute hydrochloric acid, the filtrate appeared to have a slight action on the starch.

Human milk behaved in the same manner as cow's milk, but appeared to act with somewhat greater energy. On heating to 75° C., it entirely lost its power of liquefying the starch.

The percentage of galactozymase it will be noted is quite comparable to that of the lactalbumin in the analysis previously given, being as 0.314 to 0.428 per cent. But the result of the repeated washings and the solution would all tend in this direction: that is, to raise the percentage of what is put down as galactozymase. In the same milk, after heating to boiling, and at 100° C. for an hour. the same body is obtained by analysis though in much smaller amount, being only 0.055 per cent. It is doubtful if it is present at all since these heated specimens had entirely lost the starch liquefying property.

It is more probable that the correct amount of the galactozymase is more nearly represented by the small percentage of coagulable proteid obtained in the filtrate from the Pasteur filter 0.119, and which possessed more strikingly the property of starch liquefaction than any other condition or derivative of the cow's milk experimented upon.

It is, therefore, to be inferred that there is in cow's milk, besides the casein and albumin existing in a colloidal condition, and which are removed by filtration through a porcelain filter, a third proteid which is soluble and dissolved in such a condition that is passes through the filter. This proteid appears to be the galactozymase, which already has been differentiated from lactalbumin on the ground of greater solubility and other properties.

V.

The Behavior of Raw and Sterilized Cow's Milk with Acid and Rennet at 34.5° C.

In the preceding comparisons of raw and heated cow's milk, the tests and analyses were made upon the samples when cold. But it is evident that the natural physiological processes of digestion take place at a higher temperature, and that deductions based upon tests made in the cold might lead one quite astray in relation to the phenomena of nutrition. The milk was diluted with eight times its volume of water.

	Raw.	Sterilized one hour at 100° C.
0.2 p. c. hydrochloric acid.	No precipitation. Merely a turbidity like dilute milk.	Similar to the raw. But more opaque (clots of albumen).
Rennet.	Imperfect coagulation. Opaque liquid and filtrate.	Similar to raw. No curd separating, but slimy clots.

The experiments were then repeated on the raw and sterilized milk without dilution. After digestion with dilute acid and rennet for fifteen minutes they were cooled to 15° C., then diluted with eight times their volume of water and the precipitate, if possible, separated from the filtrate.

	Raw.	Sterilized one hour.
0.2 p. c. hydrochloric acid.	Imperfect precipitation. Turbid liquid and filtrate.	Similar to raw, but nonfilterable. Clots.

Rennet.		Curdled at once.	No curd separating. Clots (separated albumen) as found in milk long heated to 100° C.
		Curd, Whey } See Analyses.	
Rennet + 0.3 p. c. hydrochloric acid.	15 mins.	Same appearance as with HCl alone.	Same as raw.
	1 hr.	As above.	" "
	30 hrs.	Some clots in clearing liquid.	Some clots. Liquid opaque.

Preceding experiments repeated, but with 0.3 p. c. pepsin added. The curdling with rennet was done before dilution, the raw milk setting at once to a stiff jelly, the sterilized forming no jelly.

		Raw.	Sterilized.
0.3 p. c. HCl + 0.3 p. c. pepsin.	15 mins.	Very similar to expt. with HCl alone.	Same as raw.
	30 mins.	Granular, from an incipient separation.	No change.
	1 hr.	Large flocks in turbid menstruum.	Beginning to granulate.
	1½ hrs.	As preceding, but menstruum clearing.	Some separation, flocks beginning to form.
	30 hrs.	Fine coagula at surface, liquid perfectly clear.	Similar to raw, but liquid not perfectly clear.
Rennet + 0.3 p. c. HCl + 0.3 p. c. pepsin.	15 mins.	Curd rapidly dissolved. Same as with gastric juice alone.	Coagula attacked and finally like raw.

30 mins.	Granular.	Unchanged.
1 hr.	Flocks in turbid menstruum.	Unchanged, no flocks.
1½ hrs.	Menstruum clearing.	Unchanged.
30 hrs.	Fine coagula on surface. Liquid clear.	Coarse coagula on top. Liquid turbid.

VI.

Behavior of Raw and Sterilized Milk with Acid, Rennet and Artificial Gastric Juice at 43° C.

Having noted the results obtained with acid alone and rennet alone, it was desirable to repeat them in connection with the peptic ferment, to determine the relative action of the reagents employed, and the relative digestibility in these media of the milk before and after sterilization. 25 c.c. of the milk was used and made up to 200 c.c., the solution containing 0.3 p.c. real hydrochloric acid. The experiments were first repeated with these materials alone and then afterwards with rennet, no pepsin being employed.

	Time.	Raw.	Sterilized.
0.3 p. c. Hydrochloric Acid.	15 mins.	Translucent, the solids somewhat dissolving.	Same as raw.
	1 hour.	Same as above. No Precipitation.	" " "
	30 hours.	No further change.	" " "
Rennet alone.	15 mins.	Imperfect coagulation. Opaque and like the mixture of milk and water alone.	Similar to raw.
	1 hour.	Similar to above.	" " "
	30 hours.	Coagulated.	Slimy clots, not curd.

The milk employed in these experiments was carefully analyzed with the hope of determining the amount and nature of the changes. But inasmuch as the hydrochloric acid appeared not only to combine with and carry some of the casein into solution, but also to form with the casein imperfectly or not at all precipitated, a turbid non-filterable liquid, the analyses could not be proceeded with. The only quantitative result was that yielded by rennet. But even the curd formed by it could not be filtered from the dilute and turbid whey, by means of ordinary filters. It was necessary to use a Pasteur filter. This separated all the proteids except that minute amount which appears to be persistently soluble, and did not give a true result as to those which were present in the curd and those which remained behind in the whey.

	Original Milk.	Filter Residue.	Filtrate.
Casein	2.317	3.01	0.123
Other proteids	0.813		
Fats	4.12	4.65	3.07
Milk-sugar	3.578		
Ash	0.772	0.254	0.518

VII.

Artificial Digestion of Raw and Sterilized Cow's Milk.

An attempt was then made to determine quantitatively the amount of change effected in raw and sterilized milk, by operating upon portions of the same sample of milk before and after heating, with artificial gastric juice and with pancreatic extract.

25 c.c. of milk was used in each experiment, the peptic digestion being as previously stated. In the pancreatic the 25 c.c. was made up to 200 c.c. with water containing in solution 195 mgms. (3 grains) sodium bicarbonate and 65 mgms. (1 grain) pancreatin. After digestion for six hours at 43°, the liquids were allowed to stand over night and then filtered. The peptic products could be filtered in the ordinary manner, since the liquid portions were clear and limpid. But those from the pancreatic digestion were not clear and were only filterable through porcelain.

The raw milk which was employed in the experiments was completely analyzed with a view of determining the condition of all the constituents in the product of digestion, but the research was beset with so many difficulties, that at present the only figures reported are those for the residues:

	Original Milk.	Peptic Digestion. Residue from		Pancreatic Digestion. Residue from	
		Raw	Sterilized.	Raw	Sterilized.
Casein	2.58				
Other proteids	0.84				
Total proteids	3.42	0.153	0.449	1.26	2.596
Fats	2.26				
Milk-sugar	4.69				
Ash	0.71				

The fat globules appeared to remain quite unaltered during the process of digestion, and this was true not only of the peptic, but of the pancreatic action, although in the latter the reaction of the menstruum was alkaline. Under the microscope in the latter case, no change could be discovered in the appearance of the fat globules, either as to size, form or probable number. This remark is more especially true of the products from the raw milk which exhibited but little else than the fat globules, the shreds of residual nitrogenous matter, being relatively inconspicuous. The residue from the sterilized milk exhibited more undigested nitrogenous matter, and this adhered in many places to the fat globules, somewhat distorting their outlines with sharp angular indentations. But the phenomena were not in accordance with the supposition that the fat globules were originally encased in a membranous envelope. In one instance at least, the nitrogenous residue was submitted for many hours to further digestion (the residue from the peptic action on raw milk) and no further solution occurred. Furthermore, this particular residue, amounting to only 0.153 p.c., in all probability was suspended as insoluble matter in the original raw milk. It gave the reactions of Nuclein. In the ordinary course of analysis it would be thrown down along with the precipitate produced by dilute acid, and would be included in the casein.

The residue from the sterilized milk was much greater in both the peptic and pancreatic digestion than that from the raw. With both varieties of milk the latter digestion gave greater residues than the peptic, but the amount of pancreatin employed was relatively less also. That the effect of sterilizing was greatly to retard the rate, and diminish the amount of digestion was evident. The clinging of the undigested residues to the fat globules of the sterilized milk was also a prominent adverse factor.

CONCLUSIONS.

From the preceding discussion it would appear that there are three classes of substances which are present in normal cow's milk.

1st. Substances in suspension, including:

Nuclein, an organic compound containing phosphorus, the composition of which has not as yet been satisfactorily determined.

Fat globules, destitute of envelopes, and swimming in a colloidal ("somewhat ropy or mucilaginous") fluid, the physical characters of which are favorable to their persistance as separate globules under ordinary conditions of temperature and reaction.

2d. Substances present in a colloidal condition, including:

Casein and lactalbumin, the former of which appears to be in combination with alkali, and probably also with lime and phosphoric acid, and is precipitable by dilute acid.

3d. Substances present in solution, including:

A nitrogenous starch liquefying ferment "galactozymase" milk-sugar.

Common salt and certain soluble compounds of phosphoric acid.

The noted effects of sterilization are:

The starch liquefying ferment is destroyed and coagulated. After coagulation the "galactozymase" is insoluble and is carried down with and included in the precipitate obtained on addition of dilute acid.

A portion of the lactalbumin as it exists along with the other substances present in milk, is coagulated. But this coagulation is

only partial, even after long continued heating. Its effect, however, is to thicken the milk and intensify its colloidal (ropy or mucilaginous) character.

The casein is not coagulated by the heat, but is less readily coagulated by rennet, and yields slowly and imperfectly to the action of pepsin and pancreatin.

The fat globules themselves are somewhat affected by the heat, and after standing for some time lumps of butter-fat have sometimes been observed on the surface of the milk. But the coagulated proteid matters attach themselves to the fat globules and probably have an influence in bringing about the less perfect assimilation of fat, which has been noted by various observers as true of infants nourished upon sterilized milk.

The milk-sugar by long continued heating is completely destroyed, and is probably affected to a certain extent during the interval ordinarily allowed for sterilization.

Sterilized milk is less readily and less perfectly digestible than raw milk, and if sterile milk is sought for, the present desideratum is to obtain it either directly from the animal, or by a process not accompanied by such serious drawbacks. In the performance of the many analyses required in the course of this investigation, I desire to acknowledge the aid of my assistant, Mr. William G. Johnston.