ON THE PRESENCE OF SO-CALLED "COMPLEMENT" IN MILK.

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Attention was first directed to the presence of a substance in milk acting as a complement by Pfaundler and Moro (1) and from that time onwards there has been much conflicting evidence published on this subject. Kopf (2) stated that complement could not be demonstrated in milk, using the method of Pfaundler and Moro, and the work of Sassenhagen and Bauer (3) would seem to show that complement is not present in milk unless the milk of cows suffering from mastitis be present. Sassenhagen (4) in a later paper states that complement is present in the colostrum of cows and goats and that it disappears as lactation progresses. Lane-Claypon (5) has followed Pfaundler and Moro and states that complement can be found in almost any milk.

The mere presence or absence of complement in milk is of somewhat academic interest, except from the point of view of the pediatrician, but the stress laid on the fact that its presence is almost diagnostic of mastitis, necessitates a clear understanding of this question.

We have carefully re-investigated the matter using milk from single healthy cows, mixed milk of healthy cows, colostrum, and milk from cows suffering from very slight inflammatory or catarrhal affections of the udder, or possessing abnormal characteristics in one or more quarters. On account of the ease of procedure, we have used the haemolytic

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system proposed by Pfaundler and Moro, but employing (at first) the quantities given by Bauer and Sassenhagen, viz. :

1·0 c.c. milk,
0·5 c.c. 5 % suspension of guinea-pig corpuscles.
0·2 c.c. inactivated ox-serum.

From our experiments it is quite clear that much of the conflicting evidence on this subject has been due to the very variable content of amboceptor present in different samples of inactivated ox-serum.

Some preliminary experiments showed that, in general, 0·2 c.c. of the heated serum might be sufficient, but in many cases it was necessary to employ 0·4 or 0·5 c.c. to produce haemolysis in a control using 0·1 c.c. guinea-pig complement.

In any case, it is essential that the ox-blood should be defibrinated and the serum obtained by centrifugalisation as rapidly as possible. After inactivation at 55°, the serum keeps fairly well in the ice chest.

Some preliminary experiments showed that the order of addition of the various members of the haemolytic system was immaterial as was also (within limits) the quantity of guinea-pig corpuscles. The haemolytic effect is entirely dependent on the quantity of amboceptor added. This is seen in the following table:

5 % suspension of g.-p. corps.	Saline	Heated Ox-serum	Milk	Result*
0·1 c.c.	0·7 c.c.	0·1 c.c.	1·0 c.c.	+ + + +
0·2 „	0·6 „			+ + + +
0·3 „	0·5 „			+ + + +
0·4 „	0·4 „			+ + + +
0·5 „	0·3 „			+ + + +
0·1 „	0·4 „	0·4 c.c.		+ + + +
0·2 „	0·3 „			+ + + +
0·3 „	0·2 „			+ + +
0·4 „	0·1 „			+ + +
0·5 „	0·0 „			+ + +

* The following signs are used to designate the haemolytic effect:

+ + + + = complete haemolysis
+ + + = slight deposit of unlaked cells
+ + , + = increasing amount of unlaked cells, but pink colour distinct
— = negative result, no haemolysis.

The milk used in the above case always gave a strong haemolysis with guinea-pig corpuscles and heated ox-serum. It was used, after passing through a separator, as the peculiar action of the fat (as noted

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also by Lane-Claypon (*loc. cit.*)) is obviated. That the complementary substance in milk is not attached to the fat globules is seen by the following result obtained with the same milk as used above:

		Result		
Milk or Cream	Saline	Whole Milk	Skim Milk	Cream
1·0 c.c.	0·0 c.c.	+ + + +	+ + + +	v. sl. +
0·5 ,,	0·5 ,,	+ + +	+ + +	—
0·25 ,,	0·75 ,,	+ +	+ +	—
0·15 ,,	0·85 ,,	—	—	—

In all the following experiments recorded the quantities of milk used alone are given. It is to be understood that the quantity was always made up to 1·0 c.c. with saline, that 0·5 c.c. of the 5 % suspension of guinea-pig corpuscles was always employed, and the quantity of heated serum varied from 0·2 c.c.—0·5 c.c. as was required. The tubes used were 75×6 mm. and the order of filling was: (1) milk, (2) saline, if necessary, (3) heated ox-serum, and, after shaking, (4) guinea-pig corpuscles. The contents of the tube were then mixed by inversion two or three times. The tubes were kept in water at 37° C. for 2 hours, the contents being mixed after 30 minutes and 60 minutes, and were finally centrifuged for 10 minutes at 2000 revolutions.

In all cases controls were carried out and as no experiments are recorded in which there was any failure in these, they are not further alluded to.

Cellular elements as a source of complement.

In the greater number of the milks examined, a count was made of the number of cellular elements present, in order to ascertain if there were any connection between these and the presence of complement. The method employed was that described by ourselves and Villar (6).

It will be seen that there is no general connection to be traced, though, as a rule, any cause which leads to a considerable cell count usually leads to the presence of a "complementary substance." That this latter, however, is not directly due to the increased number of cells is seen from the following experiments:

A large quantity of these cellular elements was obtained from the milk of a cow, whose milk always gave a reaction for "complement" (see cow 24 *infra*), by centrifugalisation, and the cells, after washing twice with saline, were diluted 1 : 20 with saline. The emulsion was

kept for 24 hours at about 5° C. and then tested, but the presence of "complementary substance" could not be detected in 1 c.c. of the emulsion.

COLOSTRUM.

Specimen I.

Heifer, 1st calving. (*a*) *Sample taken 24 hours after calving.*

Appearance.	As ordinary milk.
Blood.	Present in fair quantity.
Cells per c.c.	432,000, many typical large cells present.
Haemolysis.	1·0 c.c. ++++, 0·5 c.c. ++, 0·25 c.c. ?, v. sl. +.

(*b*) *Sample taken 72 hours after calving.*

Appearance.	Normal milk.
Blood.	Trace.
Cells per c.c.	224,000.
Haemolysis.	Negative.

Specimen II.

Shorthorn cow, 6 years old. (*a*) *Sample taken 12 hours after calving.*

Appearance.	Very yellow (due to colour of fat), long-chain streptococci present.
Blood.	Trace.
Cells per c.c.	6,912,000.
Haemolysis.	1·0 c.c., 0·5 c.c., and 0·25 c.c. all ++++, 0·15 c.c. ++.

Mixtures of this colostrum with ordinary milk were made and the presence of 5 % could be easily detected by haemolysis in 1·0 c.c. of the dilution.

This sample of colostrum was kept 24 hours at about 15° C. and again tested with practically similar results.

(*b*) *Sample taken 60 hours after calving.*

Appearance.	As the 12 hour sample, but not quite so yellow.
Blood.	Trace.
Cells per c.c.	11,840,000.
Haemolysis.	As in 12 hour sample.

Mixtures of this sample with ordinary milk could be detected down to 10 %

(*c*) *Sample taken 5 days after calving.*

Appearance. As last.
Blood. Not present.
Cells per c.c. 7,072,000.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. ++, 0·25 c.c. and 0·15 c.c. negative.

Mixtures with milk which had been heated to 55° for 30 minutes gave slight haemolysis in 10 % dilution.

Specimen III.

Heifer, 3 years old. Cross bred Jersey and Shorthorn.

(*a*) *Sample taken 24 hours after calving.*

Appearance. As specimen (II), 12 hour sample.
Blood. Slight trace.
Cells per c.c. 720,000.
Haemolysis. 1·0 c.c., 0·5 c.c., 0·25 c.c., 0·15 c.c. all ++++.

Mixtures with an ordinary milk allowed of the detection of 2 % of the colostrum.

(*b*) *Sample taken 3rd day after calving.*

Appearance. Somewhat yellow, but otherwise normal milk.
Blood. Not present.
Cells per c.c. 176,000, but many very small cells not included.
Haemolysis. 1·0 c.c. and 0·5 c.c. +++, 0·25 c.c. ++, 0·15 c.c. +.

Mixtures with milk heated at 55° for 30 minutes all gave negative results, there being no haemolysis in any case.

(*c*) *Sample taken 5th day after calving.*

Appearance. As last.
Blood. Not present.
Cells per c.c. 160,000, not including many very small cells.
Haemolysis. As last sample.

Mixtures with heated (55°) milk gave a very slight haemolysis in 10 % dilution.

Specimen IV.

From a cow whose previous history was unknown. Quite healthy. The first sample was taken 24 hours after calving. Six hours later the

cow was suffering from an acute attack of milk fever. By suitable treatment recovery rapidly ensued, and 24 hours later she seemed quite normal again.

(*a*) *Sample taken* 24 *hours after calving.*

Appearance. Yellow, but otherwise normal milk.
Blood. Not present.
Cells per c.c. Not counted.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. +++, 0·25 c.c. +, 0·15 c.c. v. sl. +.
Haemolysis, with g.-p. corpuscles only. All tests negative.
Haemolysis, with sheeps' corpuscles and special amboceptor[1]. All tests negative.

Mixtures of the above with ordinary milk showed a + result in 10 % admixture and a slight haemolysis in 5 % and 2 % admixtures.

(*b*) *Sample taken* 4 *days after calving and about* 48 *hours after recovery from milk fever.*

Appearance, blood, etc. as in sample (*a*).
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. +++, 0·25 c.c. ++, 0·15 c.c. sl. +.
Haemolysis, with g.-p. corpuscles only. 1·0 c.c. ++. Rest negative.
Haemolysis, with sheeps' corpuscles and special amboceptor. Result doubtful; there appeared to be a very slight haemolysis, but the control, without complement, also showed a distinct though slight haemolysis. It is probable that no real action took place.

Mixtures with ordinary milk showed a + result in 10 % admixture, sl. + in 5 %, and negative in 2 % admixture.

(*c*) *Sample taken* 8 *days after calving.*

Appearance. Almost normal milk, only slightly yellowish.
Blood. Not present.
Haemolysis. 1·0 c.c. sl. +. Rest negative.
Haemolysis, with g.-p. corpuscles only. 1·0 c.c. sl. +, rest negative.
Haemolysis, with sheeps' corpuscles and special serum. All tests negative.

[1] This was a very active haemolytic serum obtained by injecting a horse with sheeps' red-blood corpuscles, for a supply of which we are indebted to Messrs Burroughs, Wellcome & Co., per Dr Dale (see also below, p. 495).

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Mixtures with ordinary milk were all negative. The cells in this sample were not counted, but there was only a small number present. An analysis of this sample gave the following results: fat 4·10 %, T. solids 13·74 %, N.F.S. 9·64 %, lactose 4·75 %.

From the above results, it is quite evident that colostrum contains a " complementary substance " in considerable quantity and that this persists over a period within which it is quite possible that the milk would be brought into general supply. It is quite useless to lay down a definite period within which such admixture should not be made as the persistence of abnormal milk after parturition varies very considerably in duration, and it is certain that a farmer will use such milk as soon as it is normal in appearance, without any regard to regulations to the contrary. From the above results it is also evident that such admixture may be detectable in other milk, at any rate when present in a dilution of 1 : 10, which might quite easily occur in practice.

MILKS FROM SINGLE COWS IN HEALTHY CONDITION.

The following experiments were made with milk from single cows in perfectly healthy condition, the examinations being made at intervals in some cases. All these cows were in about the 7th month of lactation.

Cow 3. Shorthorn, 5 years old. 10 quarts per day.

(1) 20th Oct. 1913. Cells per c.c. 160,000.
Haemolysis. All tests negative.

(2) 25th Nov. 1913. Cells per c.c. 864,000.
Haemolysis. 1·0 c.c. +, others negative.

(3) 20th Jan. 1914. Cells per c.c. 480,000.
Haemolysis. 1·0 c.c. +++, others negative.

This sample had an extraordinary high acidity when milked, viz. 25·7 degrees Thörner, but was quite normal in taste and appearance.

(4) 3rd March 1914. Cow nearly dry.
Haemolysis. 1·0 c.c. ++. Rest negative. Using as a control a very sensitive system of sheeps' blood corpuscles and horse serum amboceptor, *no* haemolysis resulted in 1·0 c.c.

The acidity of the milk was still rather high, 21·3 degrees Thörner. The analysis of this last sample gave, fat 4·65 %, T. solids 14·28 %, N.F.S. 9·63 %, lactose 4·72 %.

Cow 5. Shorthorn, 6 years. 13 quarts per day.

(1) 20th Oct. 1913. Cells per c.c. 368,000.
Haemolysis. All tests negative.

(2) 25th Nov. 1913. Cells per c.c. 192,000.
Haemolysis. All tests negative.

(3) 2nd Dec. 1913. Cells per c.c. 208,000.
Haemolysis. 1·0 c.c. ++, 0·5 c.c. v. sl. +. Rest negative.

(4) 20th Jan. 1914. Cells per c.c. 128,000.
Haemolysis. 1·0 c.c. v. sl. +. Rest negative.

The acidity in this case also was singularly high, viz. 23·6 degrees Thörner.

(5) 3rd March 1914. Cow nearly dry.
Haemolysis. All tests negative.
Analysis. Fat 3·50 %, T. solids 12·82 %, N.F.S. 9·32 %, lactose 4·75 %, acidity 21·3 degrees Thörner.

Cow 9. Shorthorn, 9 years.

(1) 20th Oct. 1913. Cells per c.c. 160,000.
Haemolysis. All tests negative.

(2) 28th Nov. 1913. Cells per c.c. 80,000.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. +++, 0·25 c.c. ++, 0·15 c.c. ++.

The deposit showed many " capsular " bodies and many large cells.

(3) 3rd March 1914. Cow nearly dry.
Haemolysis. All tests negative.
Analysis. Fat 3·45 %, T. solids 12·96 %, N.F.S. 9·51 %, lactose 4·58 %.

Cow 12. Shorthorn, 7 years.

(1) 20th Oct. 1913. Cells per c.c. 672,000.
Haemolysis. All tests negative.

(2) 28th Nov. 1913. Cells per c.c. 304,000.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. +++, 0·25 c.c. +++, 0·15 c.c. ++.

Cow 15. Shorthorn, 6 years.

(1) 20th Oct. 1913. Cells per c.c. 608,000.
Haemolysis. All tests negative.

(2) 28th Nov. 1913. Cells per c.c. 368,000.
Haemolysis. 1·0 c.c. and 0·5 c.c. ++++, 0·25 and 0·15 c.c. +++.

(3) 3rd March 1914. Cow nearly dry.

Haemolysis. 1·0 c.c. ++, rest negative. Control with sheeps' corpuscles and horse serum amboceptor. All tests negative.

Analysis. Fat 3·30 %, T. solids 12·02 %, N.F.S. 8·72 %, lactose 4·26 %.

Cow 10. Shorthorn, 7 years. Nearly dry.

7th November 1913. Cells per c.c. 656,000.

Haemolysis. 1·0 c.c., 0·5 c.c. and 0·25 c.c. all +.

Cow 2. Shorthorn, 6 years. Nearly dry.

(1) 18th November 1913. Cells per c.c. 960,000.

Haemolysis. 1·0 c.c. +++. Rest negative.

(2) 2nd Dec. 1913. Cells per c.c. 1,440,000.

Haemolysis. 1·0 c.c. ++, 0·5 c.c. v. sl. +. Rest negative.

Cow 11. Shorthorn. Nearly dry.

18th Nov. 1913. Cells per c.c. 2,960,000.

Haemolysis. 1·0 c.c. +++, 0·5 c.c. +. Rest negative.

Cow 8. Shorthorn. Nearly dry.

5th Dec. 1913. Cells per c.c. 176,000.

Haemolysis. 1·0 c.c. ++, 0·5 c.c. +. Rest negative.

In the centrifugalised deposit of this sample " capsular " bodies were present and many large cells.

Analysis. Fat 3·95 %, T. solids 12·62 %, N.F.S. 8·67 %, lactose 4·08 %, acidity 15·8 degrees Thörner.

Cow 18. Shorthorn. Nearly dry.

5th Dec. 1913. Cells per c.c. 3,280,000.

Haemolysis. 1·0 c.c. +++, 0·5 c.c. ++, 0·25 c.c. +, 0·15 c.c. −.

Analysis. Fat 3·70 %, T. solids 12·38 %, N.F.S. 8·68 %, lactose 3·61 %, acidity 15·8 degrees Thörner.

Cow 20. Nearly dry.

19th Dec. 1913. Cells per c.c. 256,000.

Haemolysis. 1·0 c.c. ++, 0·5 c.c. +, 0·25 c.c. v. sl. +, 0·15 c.c. −.

Analysis. Fat 4·45 %, T. solids 13·54 %, N.F.S. 9·09 %, lactose 4·32 %.

Two other cows near the end of lactation were also examined but gave negative results as regards haemolysis. The cell content and analyses of these were:

(1) Cells per c.c. 2,480,000.
Fat 3·50 %, T. solids 12·46 %, N.F.S. 8·96 %, lactose 3·96 %.

(2) Cells per c.c. 160,000, many being very large and of indefinite character.
Fat 4·85 %, T. solids 14·34 %, N.F.S. 9·49 %, lactose 4·47 %.

MIXED MILK FROM SEVERAL COWS (8–10).

The samples of these were in all cases examined within 12–20 hours of milking.

(*a*) *Milks taken during principal calving period.*

(A) 24th Oct. 1913. Cells per c.c. 432,000.
Haemolysis. All tests negative.

(B) 6th Nov. 1913. Cells per c.c. 240,000.
Haemolysis. 1·0 c.c. slightly +. Rest negative.

(C) 8th Nov. 1913. Cells per c.c. 544,000.
Haemolysis. 1·0 c.c. ++. Rest negative.

(D) 11th Nov. 1913. Cells per c.c. 432,000 containing many large "colostral" bodies.
Haemolysis. 1·0 c.c. ++, 0·5 c.c. +. Rest negative.

(E) 12th Nov. 1913. Cells per c.c. 464,000.
Haemolysis. 1·0 c.c. +++, 0·5 c.c. ++, 0·25 c.c. v. sl. +, 0·15 c.c. −.

(F) Same source as (B).
14th Nov. 1913. Cells per c.c. 336,000 containing many "colostral" bodies.
Haemolysis. 1·0 c.c. ++. Rest negative.

(G) 14th Nov. 1913. Cells per c.c. 304,000.
Haemolysis. 1·0 c.c. ++, 0·5 c.c. sl. +. Rest negative.

(H) 9th Dec. 1913. Cells per c.c. 1,280,000.
Haemolysis. 1·0 c.c. v. sl. +. Rest negative.

(I) 20th Jan. 1914. Cells not counted.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. ++. Rest negative.

Three more samples done during this period all gave negative results. The cell content per c.c. was respectively: (1) 240,000, (2) 768,000,

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(3) 770,000. There was no reason to suspect mastitis in any of the above cases; in fact, in the case of (I), in which the strongest haemolytic effect was found, the herd was of a particularly good and healthy character.

Eleven samples of mixed milk were also examined during March 1914. Nine of these gave negative results, and two a very slight positive reaction with 1·0 c.c. of the milk.

MILK FROM COWS HAVING SOME ABNORMALITY OF THE UDDER OR SUFFERING FROM SLIGHT FORMS OF MASTITIS.

The samples of milk examined under this heading were all of such character that the milk would have been used in ordinary dairy work. There was not, in any case, such outward alteration of the milk as would have been noticed during milking. In one or two cases it was noticed that the first stream or two drawn was somewhat thick.

The veterinary notes are appended in brief to each case.

Sample A. Mixed milk of all four quarters of a young half-bred cow apparently quite healthy. Three quarters were normal, but the fourth had an elongated indurated nodule, probably of long standing. Tubercle not found.

Haemolysis. 1·0 c.c. ++, 0·5 c.c. +, 0·25 c.c. v. sl. +, 0·15 c.c. negative.

Sample B. Mixed milk of an old Shorthorn cow, apparently healthy. Three quarters normal, but a slight induration without hypertrophy in the fourth quarter.

Haemolysis. 1·0 c.c. ++, 0·5 c.c. +. Rest negative.

Sample C. Shorthorn cow, having had a fourth calf about two months previously. Milk fell suddenly from 15 quarts to 9 quarts (day previous to sampling). Both hind quarters were slightly swollen. A case of slight catarrhal inflammation, such as would be called a "chill."

The sample was of the whole mixed milk.

Cells per c.c. 30,000,000.

Haemolysis. 1·0 c.c. and 0·5 c.c. ++++, 0·25 c.c. ++, 0·15 c.c. −.

A test was made with the addition of guinea-pig corpuscles alone.

Haemolysis. 1·0 c.c. ++. Rest negative.

Sample D. Shorthorn cow having had fifth calf about two months previously. The left hind quarter was indurated at the upper part, but there was no loss of functional activity. About 16 quarts of milk per day were being given and the cow was in good health. During previous lactation the L.H. quarter was slightly bigger than the R.H. quarter, and there was then probably slight interstitial mastitis, which had become chronic. Sample taken from the L.H. quarter:

Cells per c.c. 608,000.

Haemolysis. 1·0 c.c. ++++, 0·5 c.c. ++. Rest negative.

The test, with g.-p. corpuscles only, gave negative results.

Sample E. Mixed milk of a cow with slight induration of one quarter probably due to a previous congestion arising from a chill; it was not due to any recent inflammatory condition. Milk quite normal.

Haemolysis. All tests negative.

Sample F. A "three quarter" cow. One quarter completely atrophied by mastitis during present lactation (probably about three months previous to sampling). Milk normal.

Haemolysis. All tests negative.

Sample G. From a cow with three quarters quite normal. The L.H. quarter had a slight chronic diffused induration probably due to congestion arising from a chill. Not due to any recent inflammatory condition.

(*a*) *Milk from three normal quarters.*

Haemolysis. 1·0 c.c. +++, 0·5 c.c. +. Rest negative.

Haemolysis, with only g.-p. corpuscles added. All tests negative.

Haemolysis, with sheeps' corpuscles and special amboceptor. All tests negative.

(*b*) *Milk from L.H. quarter.*

Haemolysis. 1·0 c.c. ++. Rest negative. With g.-p. corpuscles only and also with special haemolytic system, all tests were negative.

Sample H. In this case the two right quarters of the cow were quite normal, and the milk was also normal. In the two left quarters there was slight mastitis (probably interstitial) of three days' standing. The milk of these quarters was somewhat watery and the fat slightly coagulated.

(*a*) *Milk of two sound quarters.*

Haemolysis. 1·0 c.c. sl. +. Rest negative.

Tests with g.-p. corpuscles only and also with special system were all negative.

(*b*) *Milk of two affected quarters.*

Haemolysis. 1·0 c.c. and 0·5 c.c. ++++, 0·25 c.c. +++, 0·15 c.c. −.

Haemolysis, with g.-p. corpuscles only. 1·0 c.c. ++++ (other tests not done).

Haemolysis, using special system. All tests negative.

It was particularly noticed in this case that the control of the special system using 1 : 10 guinea-pig complement, gave complete haemolysis in ten minutes. Milk has no inhibitory effect on this system, as a parallel control, using inactivated milk to dilute the guinea-pig complement, gave a rapid and complete haemolysis.

Sample I. This was a particularly interesting case, as it was possible to follow it through up to the end of lactation. The cow in question received a kick in the right fore quarter which caused a bruise and some swelling. The milk was outwardly unchanged.

28th Oct. 1913. *Cells per c.c.* 7,040,000.

Haemolysis. 1·0 c.c. +++, 0·5 c.c. ++, 0·25 c.c. sl. + 0·15 c.c. −.

4th Nov. 1913. The milk of the separate quarters was examined :

R.H. *Cells per c.c.* 1,360,000.
Haemolysis. 1·0 c.c. ++, 0·5 c.c. +, 0·25 c.c. v. sl. +.
Analysis. Fat 2·25 %, T. solids 11·30 %, N.F.S. 9·05 %, lactose 4·30 %.

R.F. (the bruised quarter). *Cells per c.c.* 9,680,000.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. and 0·25 c.c. +++.
Analysis. Fat 2·10 %, T. solids 10·08 %, N.F.S. 7·98 %, lactose 3·57 %.

L.H. *Cells per c.c.* 4,912,000.
Haemolysis. 1·0 c.c. ++++, 0·5 c.c. and 0·25 c.c. +++.
Analysis. Fat 2·05 %, T. solids 10·12 %, N.F.S. 8·07 %, lactose 3·77 %.

L.F. *Cells per c.c.* 4,992,000.
Haemolysis. As L.H. quarter.
Analysis. Fat 2·45 %, T. solids 10·40 %, N.F.S. 7·95 %, lactose 3·67 %.

Tests were made on the following dates:

Date	Haemolysis 1·0 c.c.	0·5 c.c.	0·25 c.c.	0·15 c.c.	Cells per c.c.
7th Nov. 1913	+ + + +	+ + + +	+ + + +	+ + +	7,912,000
11th Nov. ,,	+ + + +	+ + + +	+ + + +	+ + +	5,920,000
18th Nov. ,,	+ + + +	+ + +	+ +	—	2,992,000
25th Nov. ,,	+ + +	+ +	+	v. sl. +	4,960,000
2nd Dec. ,,	+ + + +	+ +	sl. +	—	5,760,000
5th Dec. ,,	+ + +	+ +	sl. +	—	1,344,000
16th Dec. ,,	+ +	+	—	—	4,000,000
16th Jan. 1914	+ + + +	+ + +	sl. +	—	not done
3rd Feb. ,,	—	—	—	—	not done
3rd Mar. ,, (practically dry)	+ + + +	+ + + +	+ + +	—	uncountable

The milk was also tested on Dec. 16th 1913 and March 3rd 1914 with g.-p. corpuscles only, with a negative result in each case.

It was also tested on one occasion with the special haemolytic system with a negative result, and a negative result was obtained with the last sample of March 3rd 1914.

The corresponding analyses of the milk were:

Date	Fat	T. Solids	N.F. Solids	Lactose
28 Oct. 1913	3·17	11·66	8·49	4·05
7 Nov. ,,	3·10	9·43	6·33	3·23
12 Nov. ,,	3·10	11·52	8·42	3·96
18 Nov. ,,	3·30	11·89	8·59	4·26
25 Nov. ,,	3·10	11·58	8·48	4·15
5 Jan. 1914	3·50	12·00	8·50	4·28
16 Jan. ,,	3·35	11·96	8·61	4·36
3 Mar. ,,	3·30	11·99	8·69	1·64

The milk was quite normal in appearance throughout the whole period, except on March 3rd 1914, when the cow was within a few weeks of calving. The milk was then brownish, and had an excess of protein as seen by the analysis. The injury to the udder healed quite readily, and all swelling had disappeared before the end of December. The change in the quality of the milk during and after the injury is easily seen from the above table, the sugar being practically the only constituent affected[1].

From the results recorded above, it is evident that a substance acting as "complement" in a haemolytic system, of which the other members

[1] A sample taken from this cow after her calving, and after colostrum had quite ceased, still showed a powerful haemolytic activity.

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are inactivated ox-serum and guinea-pig corpuscles, is present in (*a*) *colostrum*, both immediately, and for at least six days after parturition, and that this can be detected in 10 % admixture with other milk; (*b*) *in ordinary milk*, both of single cows, and of several cows, in healthy condition, and that its presence is often very marked in the case of cows which are nearing the end of their lactation period; (*c*) *in the milk of cows which are suffering from the slighter forms of mastitis or "chill"* not of sufficient severity to cause the milk to be rejected, and also in the milk of cows which have suffered in this manner, and have quite recovered.

Further that the presence of this "complement" is not limited to the quarters which are actually affected but that a "sympathetic" influence is exerted on the sound quarters.

There seems to be no relation between the presence of "complement" and the chemical composition of the milk.

There also appears to be no direct relation between the content of "cellular elements" and the presence of "complement," and in fact the examination made of these, as already stated, would negative any such relation. There appears, however, to be so far a correlation that whatever cause may bring about a rise in the number of these cells will also probably cause the exhibition of, or increase in, the "complementary substance." The general results obtained from the "cell" counts made during this investigation are entirely in accordance with the results obtained previously by ourselves and Villar (6).

Both persistence of high cell count and of "complementary substance" after injury or slight mastitis are remarkable and not easily accounted for. It must be noticed that it was only in some of the cases of mastitis that two members of the haemolytic system were apparently present, and that haemolysis resulted on the addition of guinea-pig corpuscles alone.

The most remarkable feature of these experiments, however, is that, on no occasion could haemolysis be brought about by the use of a specific haemolytic system of great delicacy. This system consisted of sheeps' corpuscles (0·5 c.c. of a 5 % suspension) + horse serum amboceptor (0·1 c.c. of 1 : 30 dilution) of which the minimum haemolytic dose was 0·00066 c.c. With 1 c.c. of a 1 : 10 dilution of guinea-pig complement, haemolysis was complete in five minutes.

This immediately opens up the question as to whether the substance which acts as complement in the natural system (inactivated ox-serum and guinea-pig corpuscles) is *true* complement, as theoretically the

natural system could scarcely be looked upon as being as delicate as the former (specific) system, yet the natural system is capable of producing complete haemolysis when the specific system fails. The questions that this has opened up are being investigated and will form the subject of a further communication. The results obtained so far are somewhat remarkable and point to the fact that, in the case of milk, the natural system (inactivated ox-serum and guinea-pig corpuscles) is specific in character and of great delicacy.

Conclusions.

When using the haemolytic system consisting of inactivated ox-serum and guinea-pig corpuscles in the quantities stated above, the following conclusions have been arrived at:

1. Milk, from the colostral stage to the end of lactation, may contain a "complementary substance."
2. Colostral and mastitis milks contain larger quantities of this substance than ordinary milk.
3. In the case of mastitis milk, amboceptor may be present as well as complementary substance, but this would not be detectable in admixture with other milk.
4. Colostrum gives evidence of its presence in 5 % dilution with other milk.
5. Even in the slighter forms of mastitis, "complementary substance" is present in the milk long after subsidence of the inflammatory condition.
6. The presence of "complementary substance" is not specifically diagnostic of mastitis milk.
7. There is no connection to be traced between the number of cellular elements and the presence of "complementary substance," though the appearance of the latter is often accompanied by a rise in the number of the former.
8. The above-mentioned haemolytic system is of extraordinary delicacy in the case of milk.
9. There is some doubt as to the true nature of the "complementary substance" in milk.

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