

ON THE NATURE OF THE CELLULAR ELEMENTS PRESENT
IN MILK. PART II. QUANTITATIVE AND QUALITA-
TIVE RESULTS.

(FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.)

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One Text Figure.

In a preliminary report (Hewlett, Villar and Revis, 1909, p. 278) published last year a summary of conclusions at which we had arrived with regard to the nature of the cellular elements present in milk was set forth. These conclusions were formulated on results obtained during a prolonged quantitative and qualitative examination of these cellular elements in milks obtained from known sources under veterinary supervision, and possess, we believe, a chronological significance which, with the exception of results published by Russell and Hoffmann (1907), has been somewhat lacking in many investigations. For this purpose we selected four dairy farms at which milk was being produced for sale on ordinary commercial lines, but under various conditions of environment, both architectural and sanitary. Together with these we were also enabled, through the kindness of Professor Percival, to make use of cows in the experimental herd of the University College of Reading.

Our method was to select six cows, generally a month or so after calving, and after careful veterinary inspection of the animals, to have samples of the carefully mixed milk once a week until the cows were so near the end of lactation, that in the ordinary way their milk would

be no longer sold for consumption. As the animals dropped out at varying times we were enabled concurrently to investigate the effect on the cell count, of the termination of lactation. We were thus also in a position to note what warning might be given of the advent of mastitis when it occurred, and what effect on the mixed milk the inclusion of milk from cows suffering from mastitis might have. The farms being run on commercial lines we also knew to what extent the milk of cows suffering from mastitis would be included in a general supply, it being the common practice of farmers to include milk so long as it is unchanged in appearance and derived from quarters of the udder not apparently affected by any diseased condition. This practice, though often condemned, is in our opinion probably without danger to the community, except in certain special cases to which we shall refer.

The term "mastitis" includes all forms of inflammatory disease of the mammary gland. It may be interstitial or catarrhal, acute, sub-acute, or chronic, localised to a small portion of one quarter of the gland or involving a quarter, quarters, or the whole of the gland. It varies in intensity from a slight, transitory and hardly perceptible condition to one in which there is a considerable swelling and thickening, tenderness, local heat and general constitutional disturbance and fever. Definite suppuration with abscess formation seems to be rare. In a definite catarrhal mastitis the normal secretion is replaced by a yellowish serous fluid.

The slighter forms of so-called mastitis are probably very common and may arise from slight injuries, or even careless milking. The effect on the cell count, as we believe we show, seems to be as marked in the slight and transitory cases, in which the condition is revealed only by a careful examination, as in the more severe and obvious cases.

We also regard the character of the secretion as indicative of the severity of the condition. It must be clearly understood that when the term "mastitis" is used in this paper there was not the slightest indication of even a trace of suppuration in any case.

It is to be noted that it is quite common in the early period of lactation for the breasts of the suckling woman to become unequally swollen, knotty and painful. This is ascribed to obstruction in the lacteal ducts preventing a free outflow of the secretion. In the severer cases there may be general constitutional disturbance, thickened lymphatics and enlargement of the axillary glands. The condition almost always ends in resolution. It seems very probable that a similar condition may obtain in the cow and constitute these mild forms of so-called mastitis, especially that termed "interstitial mastitis."

We have also investigated in a similar manner the effect of the commencement of lactation, but have been forced to leave the consideration of the effects of feeding, and also of the microscopical structure of the udder tissue itself, to a further report.

The number of cows selected, viz. six, may at first sight seem too small to represent practical conditions, but in our opinion milk is constantly sold from such a small number, and further, if the number be large, the work becomes unwieldy, and the supervision not sufficiently rigid; and moreover, in such a small herd, the inclusion of one abnormal animal would produce a more marked and noticeable effect than if the number of animals were large.

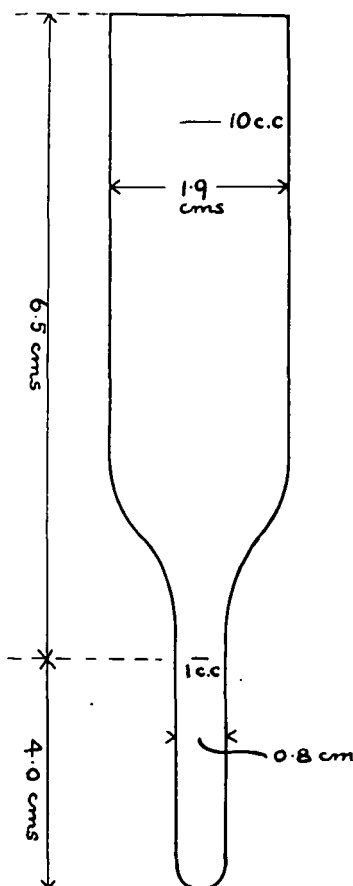
We have attempted to approach our results in a frame of mind unbiassed by prevailing ideas, so that the conclusions at which we have arrived regarding the nature of these cellular elements seem to compel a reconsideration of the causal relationship of streptococci to mastitis, and we hope shortly to make this the subject of experimental investigation.

I. *The quantitative examination of milk for cellular elements.*

For quantitative examination we have adopted a uniform method which is here described:

Early in the investigation our attention was directed to the work of Russell and Hoffmann (1908), who have shown that by heating milk to 60°—70° C. for 10 minutes before centrifugalisation, a very great increase in the number of cells takes place, and they attribute this to an effect on the fat globules, which being broken down from their cluster formation by the heat cease to have an entangling effect which normally results in the carrying of cells to the surface, and a much larger deposit is therefore obtained. This work has been amplified by Campbell (1909, p. 15), who not only confirms the work of his predecessors, but concurs in their conclusion as to the action of heat. We also concur in the action of heat as an agent increasing the number of cells, but differ as to the explanation.

Before Russell and Hoffmann's work had come into our hands we had found that the addition of about 6 drops of formalin (40% formaldehyde) to 60—70 c.c. of milk had the effect of greatly increasing the number of cells if the milk be allowed to stand 24 hours. We have compared this method with the "heating" method and find that within the limits of error of counting, the two methods give very similar results. There is a further advantage in the use of formalin, viz. that this liquid can be introduced into milk the moment it is obtained from the cow, and the



In a tube of the shape and dimensions shown in the figure are placed 5 c.c. of the well mixed milk, diluted to the 10 c.c. mark with 0.8% salt solution. After inserting a rubber stopper the contents are well mixed. The tube is then centrifuged at about 2000 revs. per min. for two minutes, the cream is broken up by violently shaking the upper part of the tube and the rotation continued for four minutes longer. A glass rod, fitting roughly the narrow neck of the tube, is inserted and the major part of the milk poured off and the upper part of the tube well rinsed with water to remove cream, etc.; the contents of the narrow end down to within $\frac{1}{4}$ inch of the deposit are sucked out with a fine glass pipette, the upper part of the tube wiped clean, and the tube then filled to the 10 c.c. mark with salt solution. The tube, having been violently shaken till all the deposit is distributed through the liquid, is then rotated for four minutes and the liquid down to within $\frac{1}{4}$ of an inch of the deposit again removed. In the case of small deposits 2 to 3 drops of saturated aqueous solution of methylene blue are added, and the deposit stirred up by blowing through a fine glass capillary pipette (which is afterwards used for filling the counting chamber). After 15 minutes, water is added to the 1 c.c. mark and counting done in the usual way with a Thoma-Zeiss blood-counter. Counting should not be restricted to the ruled spaces, but the field so arranged that a definite number of squares is included and fields counted all over the chamber. At least two different preparations should be made of the same deposit for counting.

In the following examinations, the field was so arranged that its diameter was 8 small squares of the counter (Leitz-Thoma-Zeiss); then if n be the number of cells per field:

$$n \times 16,000 = \text{number of cells per c.c.}$$

or the method proposed by Savage (1906, p. 127) may be adopted.

sample used after 24 hours without further manipulation, and at the same time the cells themselves are preserved without alteration for microscopical examination.

Now it is evident that there can be no action on the fat globule clusters in this case, such as does occur to a certain extent when milk is heated to 60°—70° C., and we must look for another explanation. One of the most striking effects of formalin or heat is the disruptive action on clusters of the cells themselves, and it would seem more probable that these agents break down aggregations of cells, perhaps by destroying some agglutinative property present, and by thus producing a more even distribution of these cells cause an apparent numerical increase. As a temperature of 70° C. and formalin both have a destructive effect on enzymes and substances of similar nature, this explanation seems reasonable.

The carrying power of the fat globules not only for cells, but also for blood, bacteria, etc. is undoubted, and it may well be that some attractive force between the fat globules and the cells is also broken down by heat or by formalin.

The formalin method has been used throughout this investigation (with one exception), and this must be taken into account in considering the figures given.

Should the deposit for counting be large, a correspondingly larger amount of methylene blue must be added and the whole diluted to 5, 10, or more cubic centimetres, as may be required.

A preliminary experiment to determine the distribution of cells in the milk stream.

A number of counts were made of milk drawn from one quarter of a cow in successive portions in order to determine whether these cells appeared uniformly during the process of milking or not. The results obtained are tabulated on the opposite page.

From these results it is fairly evident that the cells appear practically uniformly throughout the milking, the slight increase in the strippings being probably caused by increased manipulation of the udder by the milker in stripping. It seems safe to assume that these cells pass into the milk regularly during its elaboration in the alveoli and are not a response to any stimulus given to the tissues by the process of milking. They do not therefore appear to be connected with any particular constituent of the milk, but to be the result of the general activity of the gland tissue.

		No. of cells per c.c.	Quantity of milk in c.c.	Fat	T. solids
Exp. I.	Fore milk	17,000	170	3.95	13.64
		40,000	340	4.60	14.56
		31,000	284	5.60	15.20
	Strippings	32,000	284	6.30	16.01
Exp. II.	Fore milk	155,000	260	1.80	11.12
		155,000	300	2.52	11.80
		165,000	300	3.20	12.32
	Strippings	230,000	170	4.00	13.02
Exp. III.	Fore milk	24,000	150	0.80	10.24
		20,000	150	0.70	10.18
		30,000	170	0.70	10.16
	Strippings	20,000	240	1.42	10.72
Exp. IV.	Fore milk	30,000	200	2.22	11.86
		32,500	400	2.60	12.24
		30,000	450	2.40	12.03
	Strippings	70,000	460	4.37	13.76
Exp. V.	Fore milk	147,000	160	1.90	10.48
		137,000	430	3.00	12.12
		145,000	240	3.05	12.00
	Strippings	290,000	130	4.45	13.16
Exp. VI.	Fore milk	5,300	330	0.68	10.44
		4,700	990	0.55	10.31
		2,200	415	0.60	10.33
	Strippings	1,500	460	0.62	10.32

In general, in the case of any particular cow, apart from changes produced by some special causes, the number of cells per c.c. is fairly constant over the lactation period, from which we may infer that these cells are closely connected with milk production, though no light is thus thrown upon their character. As however we shall see that in general in the case of cows which are in calf there is a large increase of these cells at the end of lactation, while in the case of cows which are barren, no such increase usually takes place, there seems a certain amount of support to the view that these cells are tissue cells, as in the former case we should expect a considerable regenerative action to be taking place in the gland tissue, but not in the latter.

DAIRY FARM A.

To determine the effect of the introduction of newly calved cows on the cell count of the mixed milk.

Six cows were selected and at intervals two were dropped out and two newly calved cows introduced in their place. The results were as follows:

(The counts in this experiment were done without the use of heat or formalin, the numbers are therefore lower than would otherwise have been obtained.)

No. of cows	Date	No. of cells per c.c.	Remarks
Six	17. 11. 08	670,000	—
„	25. 11. 08	287,000	—
„	1. 12. 08	278,000	—
„	9. 12. 08	384,000	—
„	17. 12. 08	261,000	One dropped out and one newly calved in
„	18. 12. 08	287,000	—
„	23. 12. 08	705,000	—
„	31. 12. 08	230,000	—
„	6. 1. 09	—	Two out and two newly calved in.
„	12. 1. 09	250,000	—
„	19. 1. 09	587,000	—
„	26. 1. 09	205,000	—
„	3. 2. 09	11,000	—
„	9. 2. 09	220,000	—
„	19. 2. 09	511,000	Two out and two newly calved in.
„	24. 2. 09	272,000	—
„	30. 2. 09	220,000	—
„	8. 4. 09	572,000	Two out and two newly calved in (one three days only since calving).
„	13. 4. 09	237,000	—
„	20. 4. 09	697,000	—
„	28. 4. 09	412,000	(The two new cows were examined separately, see below.)
„	5. 5. 09	798,000	—
„	11. 5. 09	1,528,000	—

On 28. iv. 09 the milks of the two last cows were separately examined, (1) because the number of cells had increased suddenly the week before, and (2) because the milker considered that the milk of one of the cows had become slightly ropy, though there was no evidence of this in the samples received by us.

The results of the examination were as follows :

Cow I.	28. iv. 09	1,390,000 cells per c.c.
Cow II.	„	90,000 „ „

Analyses.

Cow I.	Total solids	12·30%	Fat	3·50%
Cow II.	„ „	13·14%	„	4·20%

The milk of Cow I was undiminished in quantity and was not in the least abnormal in appearance.

On May 13 this cow (No. I) was examined by Mr Villar, who gave the following report :

“ I examined the cow in Kent yesterday. She is a good sort of Shorthorn and had her second calf about six weeks ago.

I am told that at her first calving she had mastitis affecting the right front quarter of her udder—that quarter is now atrophied and has lost its functional activity, so that we have not been receiving milk from that particular quarter.

There is a slight sub-acute interstitial mastitis of the right hind quarter, following, I should think, a more acute process.

The milk from the three quarters had the appearance of normal milk:—her afternoon yield was 12 lbs.”

The cowman had however noticed no signs of inflammation, or abnormality of the udder, which would point to a more acute process.

The milk of this cow was examined several times during the next two months. Except in the case of the first two and the fifth samples the milk samples of the two sound quarters were mixed, and that from the suspected (R. H.) quarter examined separately. The milk was also analysed more or less completely, in order to ascertain the effect of the high cell output on the quality.

The results were as follows :

Date		No. of cells per c.c.	Analyses		
			Fat	T. solids	
6. 5. 09	Mixed milk	5,110,000	3·60	12·18	
11. 5. 09	„	5,850,000	3·50	12·06	
18. 5. 09	(2 sound quarters)	2,905,000	3·20	11·87	Lactose 4·62
„	(R. H. quarter)	3,632,000	3·30	12·18	Lactose 4·48
21. 5. 09	Mixed milk	4,485,000	3·75	12·52	
27. 5. 09	(2 quarters)	3,700,000	3·45	12·25	
„	(R. H. quarter)	10,000,000	3·20	12·10	
4. 6. 09	(2 quarters)	3,710,000	2·40	11·40	
„	(R. H. quarter)	5,125,000	3·25	12·08	Lactose 4·45
9. 6. 09	(2 quarters)	1,656,000	3·30	12·06	
„	(R. H. quarter)	3,242,000	3·65	12·60	
15. 6. 09	(R. H. quarter)	2,995,000	2·95	12·04	
17. 6. 09	(2 quarters)	2,280,000	3·95	12·87	

The cell count which was at first very much higher in the suspected quarter, gradually fell till the quarters were eliminating cells fairly

evenly, though the total count remained very high. There was not, at any time, any abnormal appearance of the udder to the ordinary observer.

Two months later (23. VIII. 09) the milk from this cow was again examined with the following results :

(2 quarters)	5,280,000 cells per c.c.
(R. H. quarter)	3,120,000 " "

Analyses of above:

	Fat	T. S.	N. F. S.	Lactose	Protein & Ash
(2 quarters)	4.20	13.08	8.88	4.26	4.62
(R. H. quarter)	3.75	12.26	8.51	4.26	4.25

The cell content of the milk of the two sound quarters now exceeded that of the so-called affected quarter. The milk was quite normal chemically, and the high count of cells was not in the case of this cow accompanied with an abnormally depressed percentage of lactose, which, as we shall see, is often to be observed in such cases. This case is very interesting, as we have here a cow which has a slight and transitory affection of one quarter of the udder, producing for a long time a large number of cells from the udder, but without any change in the milk secretion either in quantity or quality, showing that the vital activity of the gland tissue was in no way affected. There is no evidence to show that such milk is injurious; yet if a cell count be relied upon, such milk might at any time have been supposed to be the product of a cow or cows suffering from severe mastitis. Reference will be made to this cow again in our general conclusions.

DAIRY FARM B.

Six cows were selected here and carefully examined. The individual cell counts of these cows at the start, and their dates of calving, were as follows :

Ref. No. of Cow	Age	Last calving	Cells per c.c.
25	5 years	Feb. 12, 1909	14,000
26	6 "	Mar. 1, "	533,000
27	5 "	Jan. 6, "	264,000
28	5 "	Mar. 7, "	75,000
29	4 "	Feb. 14 "	12,500
30	6 "	Mar. 4 "	32,500

Their usual feed consisted of a mixture of chaff, grains, flaked maize, bran, bean meal, and half a bushel of mangels a day, and as much hay

as they could eat with about 4 lbs. of linseed and Waterloo cake mixed. The first milk was received on April 7th, 1909, and the samples continued till the cows were dry.

The weekly results were as follows:

No. of Cows	Date	No. of cells per c.c.	Remarks
Six	7. 4. 09	82,000	—
„	13. 4. 09	157,000	—
„	20. 4. 09	120,000	—
„	27. 4. 09	367,000	—
„	4. 5. 09	456,000	Cows turned out to grass after this sample.
„	11. 5. 09	115,000	—
„	18. 5. 09	256,000	—
„	25. 5. 09	142,000	—
„	1. 6. 09	315,000	—
„	8. 6. 09	161,000	—
„	15. 6. 09	667,000	—
„	22. 6. 09	329,000	—
„	29. 6. 09	726,000	—
„	6. 7. 09	467,000	—
„	14. 7. 09	725,000	—
„	27. 7. 09	421,000	The cows were examined and found healthy
„	4. 8. 09	458,000	by Mr Villar, one (27) considered to be far in calf.
„	7. 8. 09	—	Individual counts made again with following results:
		Ref. No. of Cow	No. of cells per c.c.
		25	35,000
		26	1,410,000
		27	3,545,000
		28	128,000
		29	32,000
		30	100,000
		The cows were examined and 27 reported quite healthy, but one quarter (L. F.) of 26 seeming slightly abnormal she was dropped out after the next sample and her milk was examined separately (see below).	
Six	11. 8. 09	770,000	—
Five (Nos. 25, 27, 28, 29, 30)	18. 8. 09	1,075,000	—
„	20. 8. 09	—	Cow 28 reported to have a bad quarter (L. H.). She was dropped out.
Four (Nos. 25, 27, 29, 30)	25. 8. 09	829,000	—
„	31. 8. 09	240,000	—
„	7. 9. 09	207,000	—
„	15. 9. 09	557,000	—

Cellular Elements in Milk

No. of Cows	Date	No. of cells per c.c.	Remarks
Four			
(Nos. 25, 27, 29, 30)	23. 9. 09	1,210,000	} Nothing to account for these fluctuations.
"	6. 10. 09	295,000	
"	12. 10. 09	1,735,000	
"	19. 10. 09	250,000	
"	20. 10. 09	—	
Three	27. 10. 09	445,000	Higher than last week with cow 27 in.
(Nos. 25, 29, 30)	3. 11. 09	44,000	—
"	9. 11. 09	46,000	—
"	16. 11. 09	42,000	Cow 30 dried off.
Two	23. 11. 09	22,000	—
(Nos. 25, 29)	30. 11. 09	62,000	—
"	7. 12. 09	17,000	—
"	17. 12. 09	26,000	—
"	21. 12. 09	133,000	—
"	28. 12. 09	22,000	—
"	4. 1. 10	22,000	Cows dried off.

These cows gave some very remarkable results. In general it must be noted that the end of lactation with the three cows—Nos. 25, 29 and 30—had no effect at all in raising the cell count and they were all barren. Cow 27 was a very disturbing factor, and while at no time showing any signs of disease gave very large cell counts towards the end of lactation, and it is evident from the figures obtained between 15. IX. 09 and 20. X. 09, that this cell count was of a very fluctuating nature. Below are given some further details of this cow.

On 7. VIII. 09 there was apparent evidence that Cow 26 might be developing mastitis, which however did not occur; the only disturbance being a diminished quantity of milk from her L. F. quarter. On the other hand Cow 28, which on 7. VIII. 09 gave no evidence at all of anything wrong, by 20. VIII. 09 had developed a severe mastitis in her L. H. quarter, so that the cell count was of no premonitory value and the onset very sudden. A similar case is referred to later.

Further Examination of Cow 26.

This cow was reported on 7. VIII. 09 as having a very slight abnormal appearance of the L. F. quarter. It was not sufficient to diagnose any definite disease, but the milk from this quarter was diminished in quantity. Cell counts from each quarter were made as follows:

Date		Cells per c.c.	Remarks
19. 8. 09	R. H.	200,000	All samples quite normal in appearance.
	L. H.	356,000	
	L. F.	6,960,000	
	R. F.	424,000	
25. 8. 09	R. H.	736,000	The quantity of milk from L. F. quarter was still small in quantity.
	L. H.	252,000	
	L. F.	8,840,000	
	R. F.	2,292,000	

Analysis of milk of L. F. quarter :

Fat 1.27%, T. Solids 8.18%, N. F. S. 6.91%, Lactose 2.22%, Protein & Ash 4.69%.

The milk is abnormal only as regards lactose, a very common occurrence when low solids are found, and generally with a high cell count.

Date		Cells per c.c.
7. 9. 09	R. H.	64,000
	L. H.	128,000
	L. F.	10,640,000
	R. F.	372,000

This cow is a very good example of one giving a persistent high cell count with but slight and transitory cause, and the milk was never changed in appearance.

Further Examination of Cow 27.

This cow on 7. VIII. 09 gave a very high count in her mixed milk. She was reported by the veterinary surgeon as drying off rapidly being far in calf, but she did not become dry till 20. X. 09, when she dried off with great suddenness, and calved on 28. XII. 09 in a normal manner.

Cell counts and analyses of the milk from each quarter were made with the following results :

Date		No. of cells	Remarks
10. 9. 09		3,920,000	Mixed milk.
14. 9. 09	R. H.	7,200,000	
	L. H.	940,000	
	L. F.	6,800,000	
	R. F.	2,240,000	

<i>Analysis :</i>	Fat	T. solids	N. F. S.	Lactose	Protein & Ash
R. H.	3.15	11.36	8.21	3.64	4.57
L. H.	2.80	11.98	9.18	4.50	4.68
L. F.	3.40	12.20	8.80	3.98	4.82
R. F.	2.40	11.78	9.38	4.58	4.80

The diminished sugars correspond to the high cell counts.

This cow on 20. x. 09 gave abnormal fluid from one quarter (R. H.), but not caused by disease. The R. F. and L. F. quarters gave very large deposits, but were not counted. The milk from these three quarters (R. F., L. F and L. H.) was quite normal in appearance and would have been put in with other milk and undoubtedly have caused a very large cell count in the mixed milk. Each of the fore quarters would have contributed at least 80,000,000 cells per c.c.

The milk of this cow was again examined on 6. I. 10, nine days after calving, when the number of cells per c.c. was 3,340,000, so that the high cell count continued. The milk of each quarter was examined on 22. I. 10 with the following results:

	No. of cells per c.c.
R. H.	36,000
L. H.	360,000
L. F.	6,200,000
R. F.	5,200

It will be noted that the high cell count is continued after calving in the L. F. quarter, but in the R. H. quarter has fallen to a very small number. This is a remarkably good instance of the continuity of cell proliferation in quarters which have already given high counts, in spite of the regenerative tissue changes which presumably have taken place.

Cow 28.

This cow developed about 18. VIII. 09 a severe catarrhal mastitis of the L. H. quarter. The milk of each quarter was examined on 23. VIII. 09.

L. H.	Yellow watery liquid.
L. F.	452,000 cells per c.c.
R. F.	282,000 ,,
R. H.	1,470,000 ,,

It will be observed that the addition of the milk of the unaffected quarters to other milk would not materially affect the total cell count, and would therefore give no evidence that milk from a diseased cow had been added.

It is true that the week before this cow developed mastitis the total cell count of the six cows (q.v.) rose to over 1,000,000 per c.c., but as this number was exceeded on 23. IX. 09 and 12. x. 09 when the mixed milk was from cows not diseased, the indicative value of the cell count

is doubtful. From other observations it seems probable that there is no rise in the number of cells in the milk of a cow about to develop mastitis until a day or two before the affection is visible to the eye.

DAIRY FARM C.

(These cows were not examined by Mr Villar, but by the Veterinary Surgeon attached to the Farm.)

This farm is used for the production of milk for nursery use and only high-class tuberculin-tested animals are stalled. The feed is of rather a rich character including locust and bean meals, etc. The cows are kept in large, first class sheds, fitted with every up-to-date requirement, and they are under constant veterinary supervision.

The results are extremely interesting, as there was not the least trace of disease at any time in the shed; while the cell counts obtained were often extremely high.

Six cows were selected as usual and carefully examined. They averaged about five years old and were all Shorthorns.

An individual count at the commencement gave the following results:

Ref. no. of Cow	Date	No. of cells per c.c.
1	21. 4. 09	75,000
2	"	151,000
3	"	14,000
4	"	78,000
5	"	9,500
6	"	70,000

Weekly samples were then taken as usual:

Number of cows	Date	Cells per c.c.	Remarks
Six	5. 5. 09	322,000	—
"	12. 5. 09	136,000	—
"	18. 5. 09	295,000	—
"	26. 5. 09	635,000	—
"	2. 6. 09	478,000	—
"	9. 6. 09	303,000	—
"	16. 6. 09	563,000	—
"	23. 6. 09	343,000	—
"	30. 6. 09	357,000	—
"	7. 7. 09	385,000	—
"	14. 7. 09	740,000	—
"	28. 7. 09	671,000	—
"	4. 8. 09	1,186,000	Cows reported quite healthy by veterinary surgeon.
"	11. 8. 09	471,000	

Cellular Elements in Milk

Number of cows	Date	Cells per c.c.	Remarks
Six	14. 8. 09	—	Individual counts were made again :
			Ref. no. of cow Cells per c.c.
			1 1,745,000
			2 2,300,000
			3 68,000
			4 198,000
			5 462,000
			6 643,000
			Cow 3 was drying off and was dropped out here. All were reported quite healthy by the veterinary surgeon.
Five (No. 3 dropped out.)	18. 8. 09	817,000	Cow 6 stated to be giving very slightly less milk from one quarter (L. H.). The milk of Cow 6 was examined (see below). The veterinary surgeon reported as follows:—"Has a slight thickening in the near hind quarter of udder. Temp. 102.2°, pulse normal. She is quite healthy." N.B.—This thickening referred to disappeared practically entirely before the end of lactation. The lessened quantity of milk was only ephemeral, as eight days later each quarter was giving practically the same quantity when actually measured. Cows 1 and 2 were reported quite healthy.
"	25. 8. 09	640,000	Including Cow 6 which had recovered her quantity in L. H. quarter.
"	31. 8. 09	1,678,000	
"	8. 9. 09	1,450,000	—
"	15. 9. 09	1,070,000	—
"	23. 9. 09	660,000	—
"	29. 9. 09	815,000	—
"	7. 10. 09	1,580,000	—
"	13. 10. 09	1,635,000	—
"	20. 10. 09	650,000	—
"	27. 10. 09	669,000	Cow 1 dropped out here.
Four	4. 11. 09	1,680,000	—
"	10. 11. 09	330,000	—
"	16. 11. 09	786,000	—
"	24. 11. 09	1,020,000	—
"	1. 12. 09	4,255,000	Cows reported quite healthy.
"	3. 12. 09	—	Individual counts were again made with the following results :
			Ref. no. of Cow Cells per c.c.
			2 3,900,000
			4 644,000
			6 1,000,000
			7 460,000
			Cow 2 was very carefully examined and was found perfectly healthy in every way. Cow 6 is still contributing a high cell count. The cows are of course nearing the end of lactation, but still milking well.

Number of cows	Date	Cells per c.c.	Remarks
Four	8. 12. 09	778,000	Note the drop in the cell count.
„	15. 12. 09	981,000	—
„	21. 12. 09	519,000	—
„	29. 12. 09	985,000	—
„	5. 1. 10	1,982,000	—

Further Examination of Milk of Cow 6.

The milk of this cow was carefully examined on several occasions and also analysed with the following results:

	Date	No. of cells per c.c.				
	19. 8. 09	R. H.	7,210,000			
		L. H.	540,000			
		L. F.	112,000			
		R. F.	84,000			
<i>Analysis:</i>						
	Fat	T. Solids	N. F. S.	Sugar	Protein & Ash	
	R. H.	2·10	10·80	8·70	4·3	4·4
	L. H.	1·50	9·90	8·40	4·1	4·3
	L. F.	2·25	10·66	8·41	4·2	4·2
	R. F.	2·85	11·44	8·59	4·2	4·4

None of this milk was at all abnormal in appearance.

Date		No. of cells per c.c.	Date		No. of cells per c.c.
26. 8. 09	R. H.	5,360,000	29. 10. 09	R. H.	1,320,000
	L. H.	372,000		L. H.	256,000
	L. F.	120,000		L. F.	600,000
	R. F.	136,000		R. F.	300,000
10. 9. 09	R. H.	1,940,000	18. 1. 10	R. H.	920,000
	L. H.	1,040,000	(3 months	L. H.	1,496,000
	L. F.	318,000	later)	L. F.	1,800,000
	R. F.	152,000		R. F.	2,680,000

The results here are very curious as the R. F. quarter now gives the high count. The cow is nearly dry.

The milk of this cow is most interesting, as it is a good example of very high cell counts without satisfactory cause. The veterinary surgeon could give no explanation of the slight thickening of the one quarter, and there was no evidence at all of any diseased condition. There is no doubt that this cow gave fluctuating, and often very high, counts from the R. H. quarter, and was responsible for the high count experienced at times in the mixed milk, though there is no doubt that Cow 2 also contributed heavily at times. This cow was slaughtered on February 14th, 1910, and portions of the udder used for microscopical examination, the results of which will appear in a subsequent report.

Further Examination of Milk of Cow 2.

Date		No. of cells per c.c.
6. 12. 09	R. H.	1,112,000
	L. H.	21,200,000
	L. F.	776,000
	R. F.	2,800,000
10. 12. 09	L. H.	3,880,000

Analysis of milk from L. H. quarter :

Fat 3·35, T. Solids 12·22, N. F. S. 8·87, Lactose 3·82, Protein & Ash 5·05.

Note high protein and low sugar as usual with the high cell count.

Date		No. of cells per c.c.
31. 12. 09	R. H.	212,000
	L. H.	352,000
	L. F.	616,000
	R. F.	500,000

The milk of this cow was not diminished at any time, nor did the udder at any time present any abnormal appearance, nor could the least hardening be detected. There was no doubt that she was in perfect health. It will be noted that on 31. XII. 09 the milk showed a fairly normal cell count from each quarter.

Milk of Cow 3 analysed 23. 8. 09, when nearly dry.

Fat 4·00, T. Solids 13·83, N. F. S. 9·83, Lactose 4·08, Protein & Ash 5·75.

The protein and ash are abnormally high, but the milk was quite normal in appearance and taste.

There was no particular reason for examining this milk, as the cow was on 14. VIII. 09 giving only a small cell count, but it is interesting from the fact that the composition is practically the same as that from udders giving high cell counts.

DAIRY FARM D.

To determine the effect of calving on the cell content of the milk.

For this purpose cows were selected which had calved from one week to a fortnight before the samples were taken. We were not concerned with the actual immediate effect of parturition, but only with any effect that might be produced in milk sold for consumption,

for which purpose an interval of about a week is usually allowed. The following method was adopted:

Starting with one cow, samples were taken weekly from her, till another newly-calved cow was available when the mixed milk of these two was examined, and so on, till six newly-calved cows had been brought into use.

Number of cows	Date	No. of cells per c.c.	Remarks
1. (No. 33)	26. 10. 09	339,000	—
1. (No. 34)	3. 11. 09	110,000	Contained a trace of blood.
2. (Nos. 33 & 34)	4. 11. 09	279,000	„ „ „
1. (No. 35)	10. 11. 09	36,000	—
3. (Nos. 33, 34 & 35)	„	138,000	—
1. (No. 36)	17. 11. 09	415,000	—
4 (Nos. 33, 34, 35 & 36)	„	3,290,000	—
	22. 11. 09	—	The cows were examined separately with the following results:
			Ref. no. of cow
			Cells per c.c.
			33
			352,000
			34
			56,000
			35
			136,000
			36
			1,760,000
			(See below.)
1. (No. 38)	24. 11. 09	99,000	—
5. (Nos. 33, 34, 35, 36, 38)	„	483,000	—
	26. 11. 09	—	34 suffering from severe catarrhal mastitis in L. H. quarter. She was dropped out. 36 had a circumscribed swelling on R. H. quarter, but milk not affected.
1. (No. 37)	1. 12. 09	4,360,000	—
5. (Nos. 33, 35, 36, 38, 37)	„	1,295,000	—
	3. 12. 09	—	37 was carefully examined. Mr Villar reported:

“ She is a shorthorn of good class and had calved 11 days, was giving 16 quarts of milk per day and appears in very good health, but her temperature was one degree above normal.

She has what is known as a ‘fleshy’ udder—this is quite a normal condition, but in this cow there is an abnormality of right hind quarter, viz., it is slightly larger and the least bit more firm to the feel than the corresponding quarter—there is no pain or increased local temperature. Milk from it appears normal in quality and quantity. The condition is not observable, except on very careful examination.”

The cowman stated that ‘she ran her milk’ in the morning from the L. H. quarter. She was giving full normal milk from *all* quarters.

Number of cows	Date	No. of cells per c.c.	Remarks
1. (No. 34 A, instead of old 34)	8. 12. 09	730,000	—
6. (Nos. 33, 34 A, 35, 36, 38, 37)	„	885,000	—
	15. 12. 09	1,073,000	—
	22. 12. 09	1,174,000	—
	24. 12. 09	—	Mr Villar examined 36 and 37 and reported as follows:
			“Cow No. 37. The right hind is now quite normal, but the left hind quarter is obviously swollen throughout, the milk appears normal and the quantity from this quarter does not differ from that given from the other quarters, but there was at this afternoon’s meal (24th inst.) a considerable <i>total</i> falling off from that at my previous visit. The cow’s temperature was 102·6, and she has fallen away in condition and her appetite is not very good. I should regard it as a non-specific interstitial mastitis.
			<i>Cow No. 36</i> had also a marked <i>local</i> mastitis at the upper posterior part of the right hind quarter—there is no external sign of injury, although the symptoms rather suggested that cause. It was semi-acute and may go on to suppuration. The milk was not altered in appearance, and did not appear to be in quantity either from this quarter—cow in herself quite well—no sign of tuberculosis. I do not think that the actual secreting tissue is affected by the swelling.”
6. (Nos. 33, 34 A, 35, 36, 38, 37)	29. 12. 09	558,000	—
Ditto, (but not including milk from L. H. of 37.)	5. 1. 09	1,364,000	—

As sufficient samples had been examined, this supply was not continued further.

The interpretation of the above results is very difficult, as it is much complicated by the appearance of mastitis, or other udder affection, in the cows. Some points however are very noteworthy.

The original Cow 34 is the only one which developed a typical catarrhal mastitis and it is to be noted that she gave *no* premonitory symptoms as regards alteration in the count. On 17. XI. 09 the very large count rather pointed to some such trouble, but on 22. XI. 09 the cell count of this cow was only 56,000. Two days later, she had a severe mastitis of the L. H. quarter, from which milk ceased and was replaced by the usual yellowish watery fluid. The veterinary surgeon reported that it was of some days’ standing probably, but two days previously there was no evidence of the disease as indicated by the cell count.

On 30. XI. 09 each quarter was examined with the following results :

R. H.	64,000 cells per c.c.
L. H.	Yellow watery fluid.
L. F.	100,000 cells per c.c.
R. F.	20,000 „ „

The noteworthy point here is the low cell counts in the unaffected quarters. This is of very great importance, as *in practice the farmer would milk the L.H. quarter on the ground and mix the milk of the other quarters with other milk*, and there would not be the least indication that the milk was not from a healthy cow. The inadequacy of cell counts to detect or foreshadow mastitis in certain cases is well exemplified in the case of this cow.

She did not develop any disease of these other three quarters within the time of our experiment.

Cow 37.

The milk of all four quarters of this cow was examined on 13. XII. 09 with the following results :

R. H.	73,000,000 cells per c.c.
L. H.	(Sample broken.)
L. F.	11,240,000 cells per c.c.
R. F.	1,800,000 „ „

The milk was quite normal in appearance from all the quarters examined, but the deposit from the R. H. quarter contained quantities of long chain streptococci. Some of this deposit was injected into a young rabbit, but no ill effects at all followed.

On 17. XII. 09 the milk from these four quarters was analysed :

	Fat	T. Solids	N. F. S.	Lactose	Protein & Ash
R. H.	3.55	11.64	8.09	2.98	5.11
L. H.	3.90	12.62	8.72	4.74	3.98
L. F.	3.90	12.76	8.86	4.95	3.91
R. F.	3.25	12.14	8.89	5.01	3.88

All these milks were quite normal in appearance, and the low sugar in the case of milk of the R. H. quarter, which gave such a heavy cell count, is again to be noted. Here it is accompanied by a rise in protein.

Seven days later this R. H. quarter was quite normal (in fact, it had only been slightly abnormal in appearance from the first), but the L. H.

quarter was now swollen as noted above, and on 30. XII. 09 a sample of milk from each quarter was again examined :

R. H.	84,000,000 cells per c.c.
L. H.	79,000,000 " "
L. F.	684,000 " "
R. F.	1,148,000 " "

The milks were all quite normal in appearance, but the deposits both from the milk of the L. H. and R. H. quarters contained numbers of long chain streptococci.

The milk was again examined on 20. I. 10 :

R. H.	Uncountable (100,000,000 approx.) cells per c.c.
L. H.	" (" ") " "
L. F.	6,440,000 cells per c.c.
R. F.	2,820,000 " "

The deposits from the R. H. and L. H. quarters were full of streptococci in thick masses. The same organisms were also present in the two fore quarters. A large amount of deposit from the milk of each of the two hind quarters was injected into two young rabbits. In both cases no ill effects resulted. It is to be particularly noted that she ran her milk from both hind quarters, because if the sphincter muscle was not sufficiently strong to stop the egress of milk, it could not stop the ingress of streptococci.

The milk of the four quarters was analysed on 27. I. 10 with the following results :

	T. Solids	Fat	N. F. S.	Lactose	Protein & Ash
R. H.	9.76	2.40	7.36	2.18	5.18
L. H.	10.41	2.80	7.61	2.82	4.79
L. F.	13.40	4.30	9.10	4.42	4.68
R. F.	11.72	2.55	9.17	5.00	4.17

In the milk of the R. H. and L. H. quarters the lactose is much depressed and protein high. The milk of the hind quarters was very slightly brownish, but only noticeable in comparison with other milk. Mr Villar on 19. I. 10 reported as follows :

"Cow No. 37. Right hind quarter normal. Left hind quarter, mastitis slightly more marked than at my previous visit, quarter somewhat harder, but not any larger, first milk drawn flaky, and I thought slightly more yellow than normal, but the milker made use of it in the ordinary way. Cow's temperature two degrees above normal ; she coughed, and obviously not a healthy cow."

On 19. 2. 10. Mr Villar reported that this cow was much better in every way.

She was killed on March 11, 1910, and the results of the examination will appear in a subsequent report.

Cow 36.

The veterinary reports on this cow are given above. Counts of the cells from all four quarters were made on 31. I. 10 with the following results :

R. H.	12,400,000 cells per c.c.
L. H.	1,942,000 " "
L. F.	172,000 " "
R. F.	660,000 " "

No streptococci were apparently present and these milks were quite normal in appearance.

The chemical analyses of the milk on 1. I. 10 were as follows :

	Fat	T. Solids	N. F. S.	Lactose	Protein & Ash
R. H.	3.35	12.08	8.73	4.74	3.99
L. H.	3.50	11.74	8.24	4.15	4.09
L. F.	3.50	12.81	9.31	4.78	4.53
R. F.	3.15	11.91	8.76	4.82	3.94

All were quite normal in appearance and the milk of the quarter (R. H.) which shows the high cell count in this case does not show a depressed lactose figure, which curiously enough is found in the milk of the L. H. quarter which quarter is not in any way affected.

The milk was again examined on 20. I. 10 with the following results :

R. H.	336,000 cells per c.c.
L. H.	1,560,000 " "
L. F.	68,000 " "
R. F.	168,000 " "

Mr Villar reported as follows :

"The swelling is more diffused, assuming a chronic character and is extending into the mammary tissue."

This is most interesting as the lesion is becoming worse, and yet the cell count has fallen off considerably, and the *quarter giving the highest count is one not affected.*

The milk of each quarter was analysed on 26. I. 10 with the following results :

	T. Solids	Fat	N. F. S.	Lactose	Protein & Ash
R. H.	13.62	4.80	8.82	4.60	4.22
L. H.	12.18	3.75	8.43	4.23	4.20
L. F.	12.80	3.70	9.10	4.97	4.13
R. F.	12.34	3.70	8.64	—	—

All were quite normal in appearance. Here in the milk of the L. H. quarter, which now gives the highest cell count, the sugar is still depressed, but protein is normal.

Experimental Herd.

Six cows of the experimental herd of the Reading University College Farm at Shinfield were selected and samples received weekly from these cows as in the other cases.

These cows had calved as follows :

Ref. No. 19	March 19th, 1909.
„ „ 17	„ 1st, „
„ „ 16	Feb. 1st, 1909.
„ „ 11	Dec. 20th, 1908.
„ „ 9	Nov. 3rd, „
„ „ 14	May 24th, 1909.

The first sample of milk was received on June 30th, 1909.

The following are the results obtained :

Number of cows	Date	Cells per c.c.	Remarks
Six	30. 6. 09	67,000	—
„	7. 7. 09	88,000	—
„	14. 7. 09	126,000	—
„	28. 7. 09	70,000	—
„	4. 8. 09	67,000	—
„	11. 8. 09	200,000	—
„	18. 8. 09	184,000	—
„	25. 8. 09	304,000	—
„	31. 8. 09	161,000	—
„	7. 9. 09	212,000	—
„	15. 9. 09	830,000	Sudden heavy feed of green
„	23. 9. 09	195,000	maize—no illness.
„	29. 9. 09	189,000	—
„	6. 10. 09	359,000	—
„	13. 10. 09	165,000	—
„	19. 10. 09	228,000	—
Five	26. 10. 09	188,000	—
„	4. 11. 09	180,000	—
„	9. 11. 09	200,000	—
„	16. 11. 09	248,000	—
„	23. 11. 09	310,000	—
Three	30. 11. 09	126,000	—
„	7. 12. 09	137,000	—
„	14. 12. 09	139,000	—
Two	28. 12. 09	173,000	—
„	4. 1. 10	287,000	—
„	11. 1. 10	183,000	—
„	18. 1. 10	1,258,000	No disease.
„	25. 1. 10	1,015,000	—

Except for the incident of the heavy feed with maize, the cell count of these cows pursued a remarkably steady course. No illness beyond indigestion appeared at any time, and even this latter had no effect on the cell count.

The milk of this herd is hardly comparable with ordinary dairy farming, but it is very interesting in that it shows that rest and regular habits may have a good deal to do with keeping a steady low cell count.

The two last cows which at the end showed an increased cell count were nearly dry and both were in calf; the increased count is therefore to be expected (cf. the last cows of Dairy Farm B, where the cows were barren).

General Summary of the foregoing Quantitative Examinations.

In the following table are given some of the counts obtained from mixed milks of cows either all healthy, or some healthy and some abnormal, in order that they may be easily compared :

Healthy Cows.		Healthy and Abnormal.	
No. of cows	Cells per c.c.	No. of cows	Cells per c.c.
6	726,000	5	483,000
5	817,000	6*	885,000
4	829,000	6*	1,073,000
5	1,070,000	5	1,075,000
6	1,186,000	6*	1,174,000
4	1,210,000	5*	1,295,000
5	1,450,000	6*	1,364,000
5	1,580,000	4*	3,290,000
5	1,678,000		
5	1,638,000		
4	1,735,000		
4	4,255,009		

* These contained the milk of two abnormal cows, the other samples only one.

The above figures show conclusively how little reliance can be placed on a count of the cellular elements as an indication of the presence of udder disease, even in such small numbers of cows as were here employed. Attention has already been directed to the fact that in cases of catarrhal mastitis, there is often no warning given in the way of increased cell count until the actual onset of the disease.

A comparative study of the cows, whose milk was examined separately on several occasions because a high cell count had occurred, leads to some interesting results :

Of the two cows which actually developed a typical catarrhal mastitis, viz. Cow 34 Farm D, and Cow 28 Farm B, it is to be noted

that in both cases, in the unattacked quarters, low cell counts were found on the occasions recorded, viz. soon after the onset. It is true that the cell count usually increases in the other quarters gradually, and particularly if they also succumb to the disease, but in neither of these cases was there any further obvious spread of the lesion. As we have already pointed out the cell count gives no indication of the state of affairs.

Cow 37 Farm D provides a very interesting study. There was undoubtedly considerable disturbance in the udder of the cow progressively involving nearly all four quarters, but particularly the two hind quarters.

This disturbance could not be described as acute or as suppurative mastitis. Chemical analyses constantly showed the milk to be but slightly altered, and then only in such a way as we have always found when the cell content is increased from any cause. The milk was certainly slightly slimy due undoubtedly to the large cell content, and on some occasions it was stated to be slightly discoloured and abnormal in odour, but this did not come under our own observation. The whole condition of the udder indeed suggests that there was a want of tone and lowered vitality of that organ, and that the abundance of streptococci might have been a sequel rather than a cause of condition. It must not be forgotten that she "ran her milk."

The milk of this cow though laden with streptococci was sold, and used locally. We had sufficient faith in our view of the case not to hinder the sale of this milk. The presence of her milk might have been detected in a mixed milk as the cell counts were often very high, but the diagnosis of any danger to the consumer is, in our opinion, doubtful.

This opinion is further supported by some recent experiments made by Savage (1910) for the Local Government Board in connection with his "goat-test" for the differentiation of streptococci. The experiments seem to show very conclusively that sore throat in human beings is not caused by the streptococci found in bovine mastitis.

Savage went so far as to test the truth of this conclusion by personal inoculations with such streptococci, from which no harm whatever resulted.

Of the effect of what may be taken as external agencies, Cow 36, Farm D, and Cow 6, Farm C, provide good instances. In the case of the former the stimulus took the form of a circumscribed superficial semi-acute inflammatory process, which during the course of our observation slowly spread. It is doubtful whether the actual secreting

tissue was involved, but an increased cell count followed. The same result was observed in the case of the other cow, but here the nature of the lesion is exceedingly doubtful. It was probably caused in the first instance by a slight blow, though no diminution of milk followed. When the cow was killed on 14. II. 10 all trace of the thickening had gone. A similar case is reported by Hastings and Hoffmann (1909, p. 469). The persistent effect of this hardening, even though it was rapidly disappearing in the case of our own cow, is remarkable.

The case of Cow 1, Farm A, also shows in a very clear manner the long persistence of cell proliferation after a slight and transitory udder trouble. In this case too the lesion was scarcely such as to give any reason to suppose that the milk would be unwholesome; yet, to the end of the lactation period the response to the early stimulus was maintained almost unimpaired. It must of course be remarked that cows which have already lost a quarter through mastitis are well known to be liable to a recurrence of the trouble, and it may be suggested that the activity produced in the germinal and epithelial layers of the udder by the first attack lessens their resistance to other attacks, perhaps on account of the continuity of the epithelial layer being constantly disturbed by the elimination and replacement of its units.

Such a lessened resistance and liability to recurrence is of course well known in connexion with various inflammatory lesions.

In Cow 2, Farm C, and Cow 27, Farm B, and possibly also Cow 26, of the same farm, we have instances of high cell counts, often prolonged without any reason that it was possible to discover. To say that the cell count presupposes the disease is simply a "circulus in probando." Cow "Dorine," mentioned by Hastings and Hoffmann (*loc. cit.*) is a similar case.

The general impression that is forced on the investigator into this question after prolonged study, is the hopelessness of arriving at any really satisfactory explanation of the phenomena such as we have detected. The udder is evidently an organ so open to stimuli of a most varied nature, and yet showing practically only one form of response to such stimuli, that the cause is not to be diagnosed from the effect produced.

The idea so often held, that the cow is a stolid unimpressionable animal is quite erroneous. She shows all the response to outside influences that the human subject does. Cows are extremely nervous and often show profound changes in their milk when the usual surroundings or methods are changed, or if they are harassed or excited

in any way. The effect may be transitory, but it is none the less real. The wonderful power which a cow can exercise over the udder, such as retention of the milk when a new milker or unusual method of milking is employed, is quite well known to any who have had to deal with cows.

We are of the opinion that the cytological examination of milk does not admit of any inference of the existence of a diseased condition of the cows supplying the milk. It may point to the desirability of veterinary inspection, but gives no "a priori" grounds for condemnation of the milk.

The effect of Tuberculin injections on Cell Count.

In two cases milk was received from cows which were tested with tuberculin. The samples were taken 24 hours before the injection and three days after the following results:

	Time	No. of cells
I.	24 hours previous	95,000
	3 days after	87,000
II.	24 hours previous	251,000
	3 days after	241,000

It is evident that injections of tuberculin do not cause an increase in the number of cells.

II. *The nature of the cellular elements present in milk.*

The question of the nature of the cells has been investigated in the course of the work done on the number of these elements present in the samples of milk examined. From the deposit obtained by centrifugalising as detailed below, stained films have been prepared by the following method, and a large number of preparations have been studied.

The method of preparation of the stained films is as follows :

5 c.c. of the milk (which has been exposed to the action of formalin (see above)) are diluted with 5 c.c. of physiological (0.8 %) salt solution, rotated sufficiently and all the supernatant liquid then removed. (The cream is entirely washed away as in the case of obtaining deposits for counting.) The deposit is diluted to 10 c.c. with distilled water shaken up and again rotated, the supernatant liquid removed and the deposit mixed up with sufficient distilled water by blowing through a fine glass pipette. 1 c.c. of water is usually sufficient dilution, but if the deposit be large a proportionately larger quantity of water is required. The diluted deposit is distributed over two perfectly clean cover slips set on a level table, a cover being suspended over them to prevent the ingress of dust; they are allowed to dry by

evaporation. They are then placed in alcohol-ether (1 : 1) for 30 minutes, allowed to dry and stained as under :

Stain. Modified Geimsa.

Azur II Eosin	3.0 grams
Azur II	1.6 "
Glycerin (Kahlbaum)	250 "
Methyl Alcohol (Kahlbaum)	250 "

The dry stains must be left in a desiccator for 48 hours before use. For use, place three to four drops of this stain in a large weighing bottle (2 inches high by $1\frac{1}{2}$ inches in diameter) and add 1 c.c. of water. Mix well and add 1 c.c. pure methyl alcohol. The films prepared as above are placed face upwards at the bottom (one to each bottle) and left for 48–72 hours. At the end of this time 2 c.c. of a very dilute solution of acetic acid are added, consisting of acetic acid (Kahlbaum's absolute) 1.5 pts. per 100,000, mixing well. After four minutes pour off the stain and wash the film well for 30–40 secs. with distilled water by irrigation.

Dry and mount in Canada balsam. The time of action of the acetic acid may require modification in the case of individual observers. It must be allowed to act sufficiently long to cause the cytoplasm to stain pink and yet leave the nuclei a deep blue.

This method gives films in which the various cells on the whole are well stained and well differentiated. It is much superior to specimens prepared with methylene blue, borax methylene blue and eosin thionine blue, or with the Leishman stain. A few films have also been stained with haematoxylin and eosin after fixing: this also gives satisfactory specimens. From a study of a large number of films prepared in this way cells having the following characters may be distinguished.

(1) Cells having a large single nucleus (*large uni-nucleated cells*)¹.

These are roundish cells 9–12 μ in diameter, each containing a single nucleus, which occupies about half the cell. The nucleus is roundish, or sometimes elongated or semi-lunar in form, generally excentric, often quite on one side of the cell and then frequently semi-lunar in shape. The border of the nucleus is slightly ragged, the nucleoplasm stains deeply of a haematoxylin-blue colour, but not uniformly, showing lighter and darker irregularly-shaped portions.

The cytoplasm is structureless and stains well with the eosin. (In a few of these cells a double adjacent nucleus is present as though division of the nucleus had just occurred.) In many specimens this type is the predominant cell present.

Frequently naked nuclei, resembling the nucleus above described, are present. Cells apparently of this type are also met with, but in which the cytoplasm and nucleus are vacuolated and striated, so that no clear picture of the cell can be obtained. These are probably degenerate forms, or may have contained fat-droplets.

This large uni-nucleated cell is regarded as an epithelial cell derived from the secreting layer of the gland tissue. In the figures attached to Winkler's paper (*loc. cit.*) cells with semi-lunar marginal nuclei are here and there depicted. Winkler also mentions the occurrence of naked nuclei (VIII).

¹ The prefixes 'uni' and 'multi' have been adopted in describing the cells, to avoid any possibility of confusing them with the 'mono-' and 'poly-' nuclears of the blood.

(2) Cells having two or more small nuclei (*multi-nucleated cells*)¹.

These are roundish cells, smaller than the preceding, being about 8—10 μ in diameter. Each has 2—4, occasionally 5, small, generally roundish, occasionally irregular, nuclei, staining deeply and uniformly of a deep haematoxylin-blue colour. The nuclei may be separate, scattered or clustered, and are sometimes arranged in a crescent or horse-shoe. The cytoplasm is structureless and stains well with the eosin.

Similar cells are also present, but the nuclei of which stain a pale Cambridge blue. The two kinds are probably identical, the latter being perhaps degenerated.

If the nuclei are arranged crescentrically, or in a horse-shoe, these cells bear some resemblance to polymorphonuclear leucocytes, particularly if badly stained. When properly stained however, there can be no question that they are *not* polymorphonuclear leucocytes.

These cells are sometimes almost absent, sometimes they are numerous, sometimes abundant.

There can be little doubt that these are the "germinal cells" described by Winkler.

(3) Cells with a small single nucleus (*small uni-nucleated cells*).

These are roundish cells 7—9 μ in diameter. The nucleus is small with sharp edges, roundish, and stains uniformly and deeply of a haematoxylin-blue colour. The cytoplasm is structureless and stains well with the eosin, sometimes deeply, in which case the cell resembles a normoblast ('normoblastic' type). In some of these cells which stain deeply with eosin, the nucleus stains a Cambridge blue. These cells are generally scanty in numbers.

(4) Cells with eosinophilic granules (*eosinophile cells*).

These are roundish cells 7—9 μ in diameter. The nucleus is lobed or horse-shoe shaped and stains a Cambridge blue. The cytoplasm is filled with fine eosinophilic granules.

These cells are always scanty in numbers, or frequently absent.

(5) Vacuolated cells.

Cells of some size (10—15 μ) without, or with a faint staining, large, single nucleus. The cytoplasm stains feebly and appears to be vacuolated. These cells are probably fat-bearing cells, cells which have undergone fatty degeneration, or cells allied to colostrum corpuscles.

They are generally present in small numbers in all specimens.

(6) *Cells of indeterminate nature.*

A certain proportion of cells is generally present in all specimens which are indefinite in character, owing to feeble staining of cytoplasm and nucleus, giving them a hazy appearance, and cannot be classified under the above headings. An occasional lymphocyte-like cell is seen, also squamous cells.

¹ The prefixes 'uni' and 'multi' have been adopted in describing the cells, to avoid any possibility of confusing them with the 'mono-' and 'poly-' nuclears of the blood.

Cells also occur which cannot be definitely classified under the above heads and may have characters intermediate between the cells of one and another class. It would serve no useful purpose, though it might be done, to make groups to include these cells, and in the descriptions of the specimens which follow, a general survey of each film has been made and the general nature of the cells present is summarised.

The outstanding feature in the examination of some hundred films, prepared both from normal cows at different periods of lactation and from cows presenting slight signs of mastitis and representing many thousands of cells, is that *no cell having any decided resemblance to a polymorphonuclear leucocyte has been detected*, and phagocytosis of bacteria present is conspicuous by its absence.

We are not prepared at the present time to refer the cells other than those included under the headings 1 and 2 to any particular tissues of the udder until our investigation on the histology of the udder is completed, when reference will be again made to them.

General description of cells found on microscopical examination of stained films of milk deposits.

Mixed milk of six healthy cows.

FARM B.

No.	Date	Cells present
I.	7. 4. 09	Mostly of the large uni-nuclear type with rounded nuclei; some with divided nucleus. An occasional multi-nuclear and small uni-nuclear.
II.	14. 4. 09	Mostly of the large uni-nuclear type. The staining in this specimen is somewhat hazy and indefinite.
III.	21. 4. 09	Almost entirely of the large uni-nuclear type, mostly with rounded, a few with semi-lunar or horse-shoe nucleus. A few small uni-nuclears of the normoblastic type also present.
IV.	28. 4. 09	Almost entirely of the large uni-nuclear type, mostly with rounded, a few with horse-shoe, nuclei.
V.	5. 5. 09	Almost entirely of the large uni-nuclear type with rounded nuclei.
VI.	11. 5. 09	Mostly of the large uni-nuclear type with rounded nuclei. A few small uni-nuclears.
VII.	8. 6. 09	Mixture of cells of large uni-nuclear with some of the multi-nuclear types. (Haematoxylin and eosin staining.)
VIII.	15. 6. 09	Mostly of the large uni-nuclear type, both with rounded and with semi-lunar nuclei. Some small uni-nuclear cells.
IX.	24. 6. 09	Same as VIII.
X.	7. 7. 09	Majority of cells are large uni-nuclears with some multi-nuclears and a few small uni-nuclears.

Cellular Elements in Milk

EXPERIMENTAL HERD.

No.	Date	Cells present
XI.	7. 7. 09	Mostly multi-nuclears with relatively numerous small uni-nuclears. Some vacuolated cells. Hardly any large uni-nuclears.
XII.	18. 8. 09	Much the same as XI.

FARM C.

XIII.	28. 4. 09	Mostly large uni-nuclear cells with rounded nuclei, some with divided nuclei, with a few multi-nuclear cells.
XIV.	11. 5. 09	Same as XIII.
XV.	8. 6. 09	Large uni-nuclears with some multi-nuclears and a few small uni-nuclears.
XVI.	24. 6. 09	Mostly large uni-nuclears with a few multi-nuclears, an occasional small uni-nuclear and here and there an eosinophile.

Milk of newly calved cows.

FARM D.

No.	Date	Nature of slide	Cells present
I.	26.10.09	Cow 33 (Cow remained healthy)	Almost entirely of large uni-nuclear type with rounded nuclei. Some vacuolated cells.
II.	3. 11. 09	Cow 34 (Developed severe mastitis about 24. 11. 09)	Large uni-nuclear cells with some multi-nuclear and vacuolated cells. Large number of red-blood corpuscles.
IIa.	4. 11. 09	Cows 33 and 34 Mixed milk	Considerable number of multi-nuclear cells with some large uni-nuclear and vacuolated cells.
III.	10. 11. 09	Cow 35 (Remained healthy)	Mixture of large uni-nuclear, multi-nuclear and vacuolated cells (not well stained).
IV.	17. 11. 09	Cow 36 Developed a slow external inflammation of the udder R. H. quarter	Mostly multi-nuclear cells with a few large uni-nuclear, small uni-nuclear and vacuolated cells.
V.	24. 11. 09	Cow 38 (Remained healthy)	Large uni-nuclear and vacuolated cells with a few multi-nuclears.

Milk of single cows.

Cow 1. FARM A.

}	I.	5. 5. 09	Mixed milk of all quarters	A mixture of large uni-nuclears with multi-nuclears. Small uni-nuclear cells very scanty. A few vacuolated cells. No eosinophiles. Many of the large uni-nuclears have a semi-lunar nucleus.
	II.	5. 5. 09	Milk of Cow I. as above mixed with milk of 5 healthy cows.	Much the same as I. but multi-nuclear cells scanty. Many of the large uni-nuclears show an apparently dividing or divided nucleus.

No.	Date	Nature of slide	Cells present
III.	12. 5. 09	Mixed milk of all 3 quarters.	Much the same as II.
IV.	12. 5. 09	Milk of Cow I. and the milk of 5 healthy cows.	Much as II.
V.	19. 5. 09	Mixed milk of two sound quarters	Mostly of the large uni-nuclear type: not so well stained as usual. Many show the semi-lunar or divided nucleus. Hardly any other type of cell present; practically no multi-nuclears.
VI.	19. 5. 09	Milk of affected quarter	Much the same as I. but a few small uni-nuclears and vacuolated cells present in addition.
VII.	26. 5. 09	Two sound quarters	Practically all of the large uni-nuclear type.
VIII.	26. 5. 09	Affected quarter	Much the same as VII. Cells nearly all of the large uni-nuclear type, with a few small uni-nuclears mostly of the normo-blastic type.
IX.	1. 6. 09	Two sound quarters	Preponderating cells are of the multi-nuclear and small uni-nuclear types with a small admixture of large uni-nuclears.
X.	1. 6. 09	Affected quarter	Apparently mostly of the large uni-nuclear type, but the nuclear staining very poor and the cells therefore indefinite.
XI.	8. 6. 09	Sound quarters	Admixture of cells of the large uni-nuclear and multi-nuclear types.
XII.	8. 6. 09	Affected quarter	The same as the sound quarters with some small uni-nuclear cells.
XIII.	11. 6. 09	Sound quarters	Almost entirely of the large uni-nuclear type.
XIV.	15. 6. 09	Affected quarter	An admixture of large uni-nuclears and multi-nuclears with a few small uni-nuclears.
XV.	26. 6. 09	Sound quarters	Mostly of the large uni-nuclear type with semi-lunar nuclei.
The milk was again examined two months later.			
XVI.	30. 8. 09	Affected quarter	Mostly of the large uni-nuclear type with semi-lunar nuclei together with a few multi-nuclear cells, and an occasional small uni-nuclear.
XVII.	30. 8. 09	Sound quarters	Same as last with in addition a few vacuolated cells.

Cellular Elements in Milk

Cow 26. FARM B.

No.	Date	Nature of slide	Cells present
I.	7. 8. 09	Mixed milk. High cell count. Diminished milk. No disease developed	Mostly multi-nuclears with some vacuolated cells. Large and small uni-nuclears scanty.
II.	18. 8. 09	Milk of two quarters with low count	Some multi-nuclears, relatively large number of small uni-nuclears, and some vacuolated cells. Large uni-nuclears almost absent. A few red-blood corpuscles. (Staining indifferent.)
III.	18. 8. 09	L. F. quarter High count	Mostly multi-nuclears. Relatively large number of vacuolated cells. A few small uni-nuclears. Large uni-nuclears almost absent. No red-blood corpuscles.
IV.	7. 9. 09	L. F. quarter	Mostly multi-nuclears. A few large and small uni-nuclears. Relatively large number of vacuolated cells. No red-blood corpuscles.

Cow 27. FARM B.

I.	7. 8. 09	Mixed milk. High count. No disease developed	Mostly multi-nuclears with a few large uni-nuclears and vacuolated cells.
II.	8. 11. 09 (Cow nearly dry)	L. H. quarter	Much the same as I.
III.	8. 11. 09	L. F. quarter	Ditto, but preponderance of multi-nuclears.
IV.	8. 11. 09	R. F. quarter R. H. (No milk)	Ditto, with relatively numerous eosinophiles.
V.	10. 1. 10 (After calving)	Mixed milk High count	Some large uni-nuclear and vacuolated cells, many multi-nuclears and small uni-nuclears.

Cow 2. FARM C.

I.	10. 12. 09	L. H. quarter, very high count (21,000,000) from this quarter	Multi-nuclears with a smaller number of large uni-nuclears. Some vacuolated and eosinophile cells.
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Cow 6. FARM C.

I.	18. 8. 09	R. H. quarter, high count. No apparent disease	Mostly multi-nuclears with some large and small uni-nuclears, many of the latter of the normoblastic type.
II.	18. 8. 09	Two other quarters Low counts	Number of small uni-nuclears. Some multi-nuclears. Large uni-nuclears scanty (staining indifferent).

No.	Date	Nature of slide	Cells present
III.	15. 1. 10 (Nearly dry)	R. H. quarter, high	Multi-nuclears with a smaller number of large uni-nuclears. Some small uni-nuclear and vacuolated cells.
		count from this quarter	
IV.	15. 1. 10	L. H. quarter	Same as R. H. quarter.
Cow 37. FARM D.			
I.	8. 12. 09	Mixed milk	Considerable number of multi-nuclear cells with some large and small uni-nuclear and vacuolated cells.
II.	13. 12. 09	R. H. quarter, giving heavy deposit	Large uni-nuclears with some multi-nuclears and a few small uni-nuclears. Some streptococci.
III.	2. 1. 10	R. H. normal in appearance but heavy deposit	Same as I. with some vacuolated cells. Many of the large uni-nuclears with semi-lunar and horse-shoe nuclei. Many long chain streptococci.
IV.	2. 1. 10	R. F. normal	Mostly multi-nuclears with some large and small uni-nuclears. Long chain streptococci abundant.
V.	2. 1. 10	L. F. normal	Same as R. F.
VI.	2. 1. 10	L. H. swollen, heavy deposit	Many multi-nuclears with large and small uni-nuclears and some vacuolated cells. Long chain streptococci abundant.
VII.	3. 2. 10	Milk of two fore-quarters mixed. Normal in appearance	Large uni-nuclears and multi-nuclears. Some of the large uni-nuclears with semi-lunar nuclei and vacuolated. A few small uni-nuclear and vacuolated cells. Long chain streptococci abundant.

Cow A.

This cow was not one used in the preceding investigations, but was a healthy cow which received a blow, probably a kick, near the base of the R. H. quarter. The milk of the quarter was much diminished in quantity and streptococci (pathogenic to rabbits) appeared in this quarter. There was no outward sign of disease beyond the bruise, and on slaughtering the animal later, only a hard mass in the neighbourhood of the bruise was found, there being a very slight area of inflammation round this. Very heavy deposits were given by the milk from the bruised quarter.

I.	21. 4. 09 (Immediately after accident)	Mixed milk	Mostly large uni-nuclears, many of which show divided nucleus. Some multi-nuclears and a few small uni-nuclears. Some masses of long chain streptococci.
II.	28. 4. 09	Mixed milk of three good quarters	Mostly large uni-nuclears, some with semi-lunar or horse-shoe nucleus. Multi-nuclears comparatively scanty. A few red-blood corpuscles.

No.	Date	Nature of slide	Cells present
III.	5. 5. 09	Bruised quarter	Mostly large uni-nuclears, some with semi-lunar or horse-shoe nucleus. A few multi-nuclears.
IV.	12. 5. 09	Bruised quarter	Apparently mostly large uni-nuclears, which however are not well stained. Abundant long chain streptococci.
VI.	19. 5. 09	Bruised quarter	Almost entirely large uni-nuclears, which however are not well stained.
VII.	19. 5. 09	Three sound quarters	Almost entirely large uni-nuclears.

SPECIAL EXAMINATIONS.

I.	3. 2. 10	Milk of two cows (healthy and in calf) but nearly dry. High count	Large numbers of multi-nuclear cells present, both those with deep- and those with pale-staining nuclei. The large uni-nuclear cells are less numerous.				
II.	3. 11. 09	Milk of three cows, nearly dry. (Barren)	Large uni-nuclears with a good many vacuolated cells and a few multi-nuclears.				
III.	19. 1. 10	Milk of four cows, nearly dry. (Some in calf)	Mixture of all kinds of cells—large and small uni-nuclears, multi-nuclears, vacuolated and few eosinophiles.				
IV.	17. 5. 09	Milk of cow before tuberculin injection	Abundance of large uni-nuclear and multi-nuclear cells. Fair number of small uni-nuclear (ordinary and non-moblastic type) and vacuolated cells. No eosinophiles.				
IV a.		Milk of cow after injection. (Cow reacted but tuberculosis slight)	Practically the same as before injection.				
V.	10. 6. 09	Milk from cow suffering from cowpox	Mostly multi-nuclears with a few large uni-nuclears and an occasional small uni-nuclear.				
VI.	24. 8. 09	Cow 28, Farm B R. H. moderate count	Mostly large uni-nuclears with a few multi-nuclear and vacuolated cells.				
				VII.	24. 8. 09	R. F. and L. F. mixed milk. Low counts	Much the same as R. H. quarter.
				VIII.	24. 8. 09	Mastitis deposit from serous liquid	Almost entirely large uni-nuclears, many with semi-lunar or horse-shoe nuclei.
IX.		Mastitis deposit from serous liquid. Cow 34	Almost entirely multi-nuclear cells, with an occasional large uni-nuclear and vacuolated cell. No streptococci. Nothing like a polymorphonuclear leucocyte present.				

CONCLUSIONS.

It is difficult to formulate any general conclusions from this survey of the kinds of cells present in different conditions. All that can be said is, that in the milk of healthy cows in full milk and which do not give a high cell count, the majority of cells tend to be of the type termed "large uni-nuclears," with a small admixture of other cells. At the beginning and end of lactation, or when the cell count is high, the multi-nuclears tend to be the predominant cell, and this is the case whether the high cell count is without discernible cause, or whether a definite mastitis is present. That is to say, a high cell count seems to be due to an increase of the multi-nuclears, and may or may not be associated with mastitis. These conclusions are in accordance with the hypotheses we have put forward as to the effect of various stimuli on the gland tissue of the udder. Substituting the word "polymorphonuclear leucocyte" for "multi-nuclear cell," our results are in general in accord with Savage (1908, p. 33), *but we differ entirely as to the nature and origin of the actual cellular elements*. Even in the deposits from the serous fluid in catarrhal mastitis we do not find the presence of polymorphonuclear leucocytes, and must conclude that the cells of the deposit are not "pus cells" in the ordinary acceptation. *It is not in our opinion possible to recognise diseased conditions by means of a microscopical examination of the cells present*.

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