ON THE NATURE OF THE CELLULAR ELEMENTS PRESENT IN MILK. PART III. THE MILK OF ANIMALS OTHER THAN THE COW.

FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.

By R. TANNER HEWLETT, M.D., F.R.C.P., D.P.H.,
Professor of General Pathology and Bacteriology, King's College, London,

SIDNEY VILLAR, F.R.C.V.S.,
Veterinary Inspector for the County of Middlesex,

and CECIL REVIS,
Chief Chemist, Messrs Welford and Sons, Ltd.

Having arrived at the conclusions stated in our last report (Hewlett, Villar and Revis, 1910, p. 91), it seemed advisable to investigate the milk of animals other than the cow, to which hitherto we had confined our attention.

For this purpose we obtained samples of milk from the ass and goat, and also from the human subject. Naturally we were not able to carry out these investigations in the systematic manner we had hitherto employed, but such systematic treatment was at the same time scarcely necessary. These selected animals provide a field of enquiry very different from that which the cow presents. The ass may be taken as the type of an animal from which it is possible to obtain milk in but limited quantity and only so long as the foal is present. The lactation is in no way "artificial," if such a term may be used, and the animal itself is of a quiet, docile and stolid nature.

In the case of the goat we have an animal much more of the type of the cow, in that she will give milk over a fairly extended period

without the necessity of keeping the kid by her. The milk-producing power has not however been developed to such a degree as in the cow, at least in goats usually found in England, and certainly not in the case of those used in our experiments.

Milk production in the human subject is in type similar to that in the ass, but we have here to deal with a highly sensitive and nervous organization, which often affects lactation in a most profound manner.

It is most necessary to lay great stress on the fact that the milk-producing powers of the cow are the result of careful selection and breeding, so that both the udder itself and the process of lactation have become to a large extent artificial. The result is easily seen in the extremely sensitive nature of the cow's udder, making it react quickly and profoundly to external stimuli and rendering it very liable to damage and disease. It is for this reason that the cow's udder, in which the tendency to cell proliferation has been artificially encouraged by attempts to increase milk production, is liable to respond in a hyper-sensitive manner to minute causes, exactly in the way in which our experiments have indicated.

The relative position of the three specified animals is seen in a comparison of their body weight and average milk production, though it is of course extremely difficult to give either average weights or average milk yields in the case of any one of them.

	Body weight	Milk yield per day
Ass	450 lbs.	4 pints
Goat	100 ,,	2 ,,
Cow	1,100 ,,	24 ,,

Experiments were carried out exactly as in the case of cows; counts were made at frequent intervals and stained specimens were also prepared.

The Ass.

Counts of the cellular elements in the milk of this animal are much hindered by the constant presence of a white semi-crystalline deposit, which comes down on centrifugalisation. This deposit was examined and appeared to be of a protein nature, but no reason for its presence can be given, nor was the precise nature of it elucidated

Ass I. First sample about 3 weeks after foaling.

Date	Cells per c.c.	Date	Cells per c.c.
23. II. 10	32,000	14. IV. 10	168,000
2. III. 10	38,000	21. IV. 10	55,000
17. III. 10	198,000	27. IV. 10	436,000
24. III. 10	910,000	5. V. 10	21,000
31. III. 10	94,000	11. V. 10	66,000
6. IV. 10	444,000	Milk ceased.	

This ass showed greater variations and at times bigger cell counts than were observed in any of the other cases examined.

Ass II. First sample 4 weeks after foaling.

Date	Cells per c.c.	Date	Cells per c.c.
7. VI. 10	50,000	6. VII. 10	12,000
14. VI. 10	20,000	13. VII. 10	25,000
22. VI. 10	15,000	17. VII. 10	14,000
29. VI. 10	39,000		

The cell count was at all times small and very uniform.

Ass III. First sample 3 weeks after foaling.

Date	Cells per c.c.	Date	Cells per c.c.
26. VII. 10	12,000	27. IX. 10	20,000
9. VIII. 10	7,000	19. X. 10	Deposit so large that no count was possible
16. VIII. 10	12,000		
25. VIII. 10	24,000	25. X. 10	10,000
30. VIII. 10	16,000	9. XI. 10	26,000

The milk of this ass was distinguished by the white deposit referred to, being in greater quantity than in the case of any of the other asses. On one occasion (19. X. 10) the deposit was quite $\frac{1}{8}$th inch deep in the rotation tube, and precluded any hope of counting the cells.

Ass IV. First sample 3 days after foaling.

Date	Cells per c.c.	Date	Cells per c.c.
22. IX. 10	16,000	7. X. 10	42,000
26. IX. 10	16,000	11. X. 10	23,000
30. IX. 10	44,000	14. X. 10	22,000
4. X. 10	24,000	25. X. 10	10,000

On account of the recent foaling, samples were examined every fourth day, but no effect at all on the count was observed. On several occasions, notably from the first to the fourth sample, much of the heavy white deposit was present. From 7. X. 10 nearly all the cells were large in size and resembled the so-called "colostral bodies." The cell count was always small and fairly constant.

A certain number of stained preparations were examined with the following results:

No. of animal	Date	Nature of cells present
Ass I	23. II. 10	Mostly large uni-nuclears and multi-nuclears.
,,	14. III. 10	Cells very scanty—a few vacuolated cells and small uni-nuclears.
,,	24. III. 10	Large number of multi-nuclears, with a few large and small uni-nuclears and vacuolated cells.
,,	31. III. 10	Cells scanty, a few large and small uni-nuclears, multi-nuclears and vacuolated cells.
,,	7. IV. 10	Cells scanty, as in last specimen.
,,	20. IV. 10	As in last specimen.
Ass II	17. VI. 10	Cells scanty, mostly large uni-nuclears.
,,	22. VI. 10	Very few cells. A few large and small uni-nuclears.
,,	29. VI. 10	Very few cells. Mostly small uni-nuclears, with some multi-nuclears and a few large uni-nuclears.
,,	6. VII. 10	Cells practically absent.
,,	12. VII. 10	Cells very scanty; a few small uni-nuclears.
Ass III	—	The slides contained so few cells that the examination was of no real value.

All these samples of asses' milk were distinguished by low cell counts and the cells themselves stained with difficulty. That is quite in accordance with our conclusions as it is evident that these cells are in such cases only cast off slowly and are consequently in a degenerate condition, hence their poor staining capacity and indefiniteness.

The Goat.

For the samples of goats' milk we are indebted to the kindness of W. Edmunds, Esq., M.D., who having three goats at the Brown Institution allowed us to have samples weekly from these animals.

The description of the goats is as follows:

Goat I. Normal Anglo-Nubian. Born March 23rd, 1906. Kidded about March 30th, 1910. Weight 47½ kilos.

Goat II. English. Born in 1903, or 1904. In November 1906, the thyroid gland was excised. Kidded April 18th, 1910. Weight 44½ kilos.

Goat III. Born April 1904. Both thyroids excised January 10th, 1907. Kidded March 21st, 1910. Weight 39 kilos.

All these goats were in splendid condition and quite healthy with the exception of a temporary indisposition in the case of Goat II. (See below.)

Goat I.

Date	No. of cells per c.c.	Date	No. of cells per c.c.
21. VI. 10	182,000	12. VII. 10	110,000
28. VI. 10	152,000	17. VII. 10	143,000
6. VII. 10	194,000	26. VII. 10	209,000

The cell count was very regular and low, but was interfered with by the presence of much débris and the fact that many of the cells were very small.

The microscopical examination of five slides gave the following results:

Date	Nature of cells present
21. VI. 10	Many vacuolated cells. Other cells mainly small uni-nuclears, some of the normoblastic type.
28. VI. 10	As in the last specimen.
6. VII. 10	Mostly small uni-nuclears and some vacuolated cells.
12. VII. 10	Large and small uni-nuclears.
17. VII. 10	Mostly small uni-nuclears, with a few large uni-nuclears and vacuolated cells.

Goat II.

Date	No. of cells per c.c.	Date	No. of cells per c.c.
21. VI. 10	2,230,000	12. VII. 10	382,000
28. VI. 10	7,510,000	17. VII. 10	218,000
6. VII. 10	280,000	26. VII. 10	286,000

The very large cell count of the first two samples is noteworthy and cause for it was sought. The goat was perfectly healthy and the count was evidently not normal, as it fell the following week to a lower figure and remained low. On careful inquiry it was found that this goat had arrived after a railway journey at the Brown Institution on 8. VI. 10 in rather poor condition and had had diarrhoea till the 13th. Since that date she improved rapidly in condition and the illness could only be ascribed to the railway journey. This is the only cause that can be assigned for the increased cell count and according to our views is by no means improbable, and would illustrate again the effect of external causes on cell proliferation in the udder.

Three slides were stained and examined.

Date	Nature of cells
21. VI. 10	Chiefly small uni-nuclears, many of the normoblastic type, but man large uni-nuclears and multi-nuclears and a few vacuolated cells.
12. VII. 10	Mostly small uni-nuclears.
17. VII. 10	As in the first specimen.

Goat III.

Date	No. of cells per c.c.	Date	No. of cells per c.c.
12. VII. 10	311,000	2. VIII. 10	316,000
17. VII. 10	521,000	9. VIII. 10	538,000
26. VII. 10	184,000	16. VIII. 10	680,000

The cell count is, as will be seen, fairly uniform, and calls for no comment. Stained preparations were practically identical in nature, the cells consisting mostly of multi-nuclears with some small uni-nuclears (many of the normoblastic type) and a few large uni-nuclears.

The milk of these three goats showed exactly the same types of cells as have been met with in the case of other animals. The fairly uniform count (with the exception of the two samples of Goat II already referred to) is probably the result of the easy and regular life of the animals. It is to be noted that the excision of the thyroid gland has no perceptible effect on the cell count.

Human Milk.

By the kindness of the Medical Officer of the Queen Charlotte Hospital, Marylebone Road, a few samples of human milk were obtained and examined. It was not, in the nature of the case, possible to obtain successive samples, and each is dealt with separately.

Sample I, taken 11. IV. 10, seven days after birth. First child. Breasts normal and progress satisfactory.

Cells per c.c. 2,960,000. Stained preparations showed that a large number of the cells stained poorly and were therefore rather ill-defined. The majority of the cells were small uni-nuclears and multi-nuclears, with a few large uni-nuclears and vacuolated cells.

Sample II, taken 7. VII. 10, five days after birth. First child. Breasts and lactation normal.

Cells per c.c. 252,000, but the cells were in clumps and probably the number is too low. Total deposit large. The cells were chiefly large uni-nuclears, many having semi-lunar or horse-shoe nuclei. Some small uni-nuclears, but practically no multi-nuclears.

Sample III, taken 8. VII. 10, six days after birth. First child Breasts and lactation normal.

Cells per c.c. 2,640,000. Deposit large and the count was rendered difficult by the presence of a large number of very small indefinite cells, which are not included.

The stained preparations showed that many cells were ill-defined, but almost all were large and small uni-nuclears. Many large uni-nuclears had semi-lunar or horse-shoe nuclei. Practically no multi-nuclears.

Sample IV, taken 7. VII. 10, two days after birth. Fifth child. Breasts and lactation normal. Total deposit large.

Cells per c.c. 7,440,000. The cells were large and well-defined and in marked contrast to the foregoing samples. They were of all types, large and small uni-nuclears, multi-nuclears and vacuolated cells and some eosinophiles.

Sample V, taken 11. VII. 10, three days after birth. Number of children not stated. Breasts and lactation normal. Milk very yellow.

Cells per c.c. 563,000. Again the cells were well-defined and consisted of large and small uni-nuclears and some multi-nuclears and many vacuolated cells.

Sample VI, taken 11. VII. 10, six days after birth. Fifth child. Breasts and lactation normal. The milk which was small in quantity was very thin and watery and there was a deposit of casein. A reliable count was impossible, but there were a large number of cells which in stained preparations were similar to those of Sample V.

The number of samples is too small for any generalisations, but it must be noted that in the three samples obtained from multipara, the cells were much better defined and more varied in nature than in the case of the other three samples from primipara. The cells themselves were of quite the same nature as in other milks. One cannot pass by the very high cell counts obtained in one or two cases of these *human* milks without a thought of the objection, so often raised in the case of cows' milk, that the presence of "pus" in milk, if not actually harmful, is "sentimentally" repulsive!

SUMMARY.

The general consideration of these results only tends to confirm the conclusions already arrived at. A uniform type of life evidently tends to a fairly uniform excretion of tissue cells from the udder. The case of Goat II also emphasises the effect of outside causes in increasing temporarily this excretion, while some of the samples of human milk show plainly that very high cell counts are not by any means necessarily connected with any diseased or disordered condition of the mammary gland.

We again emphasise the view we have already stated, viz. that in the cow the udder must be looked upon as an organ which has by breeding and selection been brought to an artificial condition of milk secretion and that this has been accompanied by a stimulation of the tissues to cell proliferation and that this proliferation may quite easily be caused to become abnormally great, leading to the appearance of an increased number of cells in the secretion. In support of this view we lay great stress on the fact constantly noticed by us, viz. that when the cell count is high for *any* reason, the cells themselves are always well-defined, showing little signs of degeneration, and also stain in a much more characteristic and definite manner, a fact which is difficult to explain if they are to be considered to be blood elements.

REFERENCE.

HEWLETT, R. T., VILLAR, S., and REVIS, C. (1910). On the Nature of the Cellular Elements present in Milk. *Journal of Hygiene*, Vol. x. pp. 56—92.