

ON THE NATURE OF THE CELLULAR ELEMENTS  
PRESENT IN MILK. PART IV. THE HISTO-  
LOGICAL APPEARANCES OF THE UDDER.

(FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.)

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(With Plate III.)

IN former reports by us, considerable attention was devoted to a study of the number and nature of the cellular elements in milk. We found that cells, sometimes in enormous numbers, are present in all milk (cow, goat, ass, man), that considerable variations in number occur without apparent cause, and that the presence of the slighter forms of non-suppurative mastitis does not seem to influence definitely the number of these cells.

From a study of a large number of stained films of the cellular elements present in milk, the cells were divided into six groups: (1) *Large uni-nucleated* cells, probably epithelial cells derived from the secreting layer of the gland tissue; (2) *multi-nucleated cells*, regarded as being the "germinal cells" of Winkler; (3) *small uni-nucleated cells*; (4) *eosinophile cells*; (5) *vacuolated cells*, degenerate, fat-bearing or colostrum-like cells; (6) *cells of indeterminate nature*. No cells resembling polymorphonuclear leucocytes were detected, though the "multi-nucleated" cells might well be mistaken for them.

The present report deals with the histological appearances of the udder, sections of which have been studied in the hope that some light might be thrown on the nature and source of the cellular elements in milk.

Winkler (*loc. cit.*) is particularly emphatic that leucocytes are practically never found in milk (under normal conditions) and are never found in the lumina of the alveoli of the glandular tissue of the udder, and quotes Michaelis as expressing a similar opinion. Winkler regards the majority of the cells in milk as being epithelial cells detached from the glandular layer of the alveoli. He distinguishes a layer of "germinal cells" under the epithelial layer of the gland. These were first described by Kolessnikov in 1877, and a similar layer in the submaxillary gland was described by Heidenhain. These "germinal cells" are regarded by Winkler and others as embryonic epithelial cells; they grow upwards, enlarge, and develop into and replace the cells of the epithelial layer, as these are detached or become senile. They are delicate, rounded or ovoid cells with a well defined nucleus, which is sometimes double owing to division taking place, with a fair amount of cytoplasm.

The udders which have been examined are those of: (1) a normal goat; (2) a cow (Cow No. 6) which developed a slight non-suppurative mastitis; (3) another cow (Cow 37), which also developed a slight non-suppurative mastitis; (4) three other cows, which exhibited more or less tuberculous infection, but not of the udder.

The tissues were obtained perfectly fresh, and pieces were fixed in (a) Müller's fluid, (b) a saturated solution of mercuric chloride with acetic acid, (c) formalin, 10 per cent. After fixation, the tissues were well washed in water and hardened in alcohol, and sections were prepared after embedding in paraffin and stained with Ehrlich's haematoxylin and eosin. On the whole, the tissues fixed in Müller's solution gave the best results.

The following are the results of the examination :

#### *Udder of goat.*

The alveoli are on the whole small and contracted; the glandular epithelium is regular and cubical to columnar in shape. Free cells in the lumina of the alveoli are conspicuously absent. The aspect of the gland is that of the quiescent stage.

*Udder of cow No. 6.*

Cow No. 6 was one of the six cows of Dairy Farm C, the milk of which was critically examined as to the number and nature of the cellular elements present, and reported on in our second report. This cow while under observation developed a slight thickening of the right hind quarter, with lessened yield of milk, about August 8th 1909. By August 25 she had apparently recovered. She was slaughtered on February 14th 1910.

Sections from the four quarters were separately examined. Microscopically, there is little to distinguish one quarter from another—the size of the gland alveoli, the epithelial layer and the general appearance of the epithelium and of the inter-glandular tissue differ but little in appearance in the four quarters. In the right hind quarter, which was slightly abnormal clinically, there is no increase in the inter-glandular tissue, and no sign of infiltration with leucocytes or round cells.

*Left Quarters.* Alveoli moderately distended. Practically no free cells in the lumen of the alveoli. Multi-nucleated cells in the germinal layer practically absent.

*Right Fore-quarter.* Similar to the left quarter, except that a few free cells (uni-nucleated) are present here and there in the lumen of the alveoli.

*Right Hind quarter* (the affected quarter). On the whole the alveoli are more dilated than in the other quarters; they are probably at their maximum distension. Many of the alveoli contain a homogeneous substance or a net work of fibrillated material, presumably the coagulated remains of the milk. A few large and small uni-nucleated cells are present free in the lumen of some of the alveoli. A few of the uni-nucleated cells described as of the "normoblastic" type are present in the inter-alveolar tissue in close association with the alveoli, suggesting plasma cells.

*Sinus.* In sections taken from another portion of the gland adjacent to the milk sinus very interesting details are shown. The alveoli are dilated and the glandular epithelium is swollen and the cells to a large extent are vacuolated. The picture as a whole suggests that this portion of the gland is at the height of its functional activity. Numerous alveoli contain from a few to many free cells in their lumina. These free cells are of several of the types described by us in the films prepared from milk, and confirm the views expressed as to the nature of some of

these cells. There are the large uni-nucleated cells, obviously detached glandular epithelial cells. There are cells in all stages from these to the large vacuolated ones, which are thus manifestly degenerate and sodden glandular epithelial cells. There are also some of the small uni-nucleated cells, and many of the multi-nucleated cells with two to four nuclei. A fair number of small uni-nucleated cells can be seen in the tissue under the glandular epithelial layer. The whole picture is strongly confirmative of the views of Michaelis and Winkler on the presence of the "germinal" layer of cells, and that the multi-nucleated and many of the small uni-nucleated cells present in milk are derived from this germinal layer as previously surmised by us.

*Udder of cow 37.*

Cow No. 37 was one of the several recently calved cows of Dairy Farm D, the milk of which was critically examined as to the number and nature of the cellular elements present, and reported on in our second report (*loc. cit.*). This cow was examined and reported on by Mr Villar on four occasions. On December 3rd 1909 Mr Villar found that she had a "fleshy" udder, and that the right hind quarter was slightly larger and more firm than the corresponding left quarter, though the milk was apparently normal in quality and quantity. She "ran her milk" from the left hind quarter. On December 24th 1909, the right hind quarter was quite normal, but the left hind quarter was now obviously swollen, though the milk was apparently normal in quality and quantity, and her temperature was 102.6 degrees. The condition was regarded as a non-specific interstitial mastitis. On January 19th 1910, Mr Villar found that the right hind quarter was normal, but in the left hind quarter the mastitis was slightly more marked; the quarter was somewhat harder but not larger and the first milk drawn from it was flaky and yellower than normal. The cow's temperature was still 102 degrees, and she coughed, and was obviously not healthy. On February 19th 1910, she was reported much better. She was slaughtered on March 11th 1910.

*Fore Quarters.* Alveoli distended. Epithelium very regular. Many free cells in some of the alveoli; they are mostly large and small uni-nuclears. A small number of multi-nuclears (2-4 nuclei) also present, but no cell resembling a polymorphonuclear leucocyte. In a section from HgCl<sub>2</sub>-hardened material a number of these multi-nucleated cells were seen in the alveoli.

*Left Hind Quarter* (probably the most abnormal). The alveoli throughout are small and contracted. There may be some cellular infiltration in the inter-alveolar tissue, but it is difficult to be certain of this on account of the general contracted condition of the gland, *i.e.* this infiltration may be apparent only and due to a closer packing of the tissue between the alveoli. A few of the alveoli contain free cells of the small uni-nucleated and multi-nucleated types.

*Right Hind Quarter.* A condition similar to that of the preceding specimen is present, but is more marked. The glandular alveoli are to a large extent obscured, and there is an undoubted infiltration of the inter-alveolar tissue with round cells. In the alveoli which persist, a good many free multi-nucleated cells (2-3 nuclei) are present.

The three last udders examined were obtained from the slaughterhouse through the kindness of Mr McPhail. They were all from cows suffering from more or less tuberculous infection, without, however, any clinical sign of disease of the udder.

*Cow with tuberculous infection of the supra-mammary lymphatic gland.*

The gland is largely fatty; no tubercles present. The glandular alveoli are relatively scanty and contracted. Here and there a small uni-nucleated cell is present free in the lumen of an alveolus. The gland is obviously atrophic and losing its functional activity.

*Cow with generalised tuberculosis.*

The glandular alveoli are moderately distended and the glandular epithelium is swollen and vacuolated, suggesting that lactation is either at an early stage or has recently terminated. Many free cells are present in the lumen of the alveoli; these are of the large uni-nuclear and multi-nuclear (2-4 nuclei) type. Large vacuolated cells are also present. The basement membrane is well seen, and many small uni-nuclear cells with deeply staining nuclei (*i.e.* the "germinal" cells of Winkler) are present in close approximation with the epithelial cells.

*Cow with tuberculous infection of the supra-mammary gland.*

The glandular alveoli are contracted and free cells in their lumina are absent. The gland is largely fatty and atrophic, and losing its functional activity.

## CONCLUSIONS.

This examination of these several udders has shown the extreme paucity of polymorphonuclear leucocytes in the inter-alveolar tissue, and so far as can be seen their complete absence in the lumina of the alveoli. In the sub-epithelial layer, cells corresponding to the "germinal" cells of Winkler have been detected and lend support to his conclusions respecting the origin of the epithelium from these cells.

Within the lumina of the alveoli, cells of the (1) large uni-nuclear, (2) small uni-nuclear, (3) multi-nuclear and (4) vacuolated types have been found in some of the specimens, and a study of their appearances confirms our previous views on the nature of these cells, viz. that the large uni-nuclears and vacuolated cells are epithelial cells, and that the small uni-nuclears (generally) and the multi-nuclears are cells of the "germinal" layer. None of the eosinophile type has been detected, and their origin therefore remains doubtful.

The results of this examination confirm the opinion we have already expressed that the cellular elements found in milk, either normally or in ordinary catarrhal or interstitial non-suppurative mastitis are *tissue* cells, and that "pus cells," in the ordinary acceptance of the term, do not appear in milk under these conditions.

## REFERENCES.

- HEWLETT, R. T., VILLAR, S. and REVIS, C. (1909-10-11). On the nature of the Cellular Elements present in Milk. *Journal of Hygiene*, ix, 271-278 ; x, 56-92 ; xi, 97-104.
- WINKLER (1908). Die Milchbildung und die Mikroskopische Milchprüfung. *Zeitschrift für das landwirtschaftliche Versuchswesen in Oesterreich*, 1908, p. 562.

## DESCRIPTION OF PLATE III.

Fig. 1. Section of glandular alveolus, showing a large uni-nuclear and small uni-nuclear cells free in the lumen.

Figs. 2, 3. Sections of glandular alveoli, showing multi-nuclear cells free in the lumina.

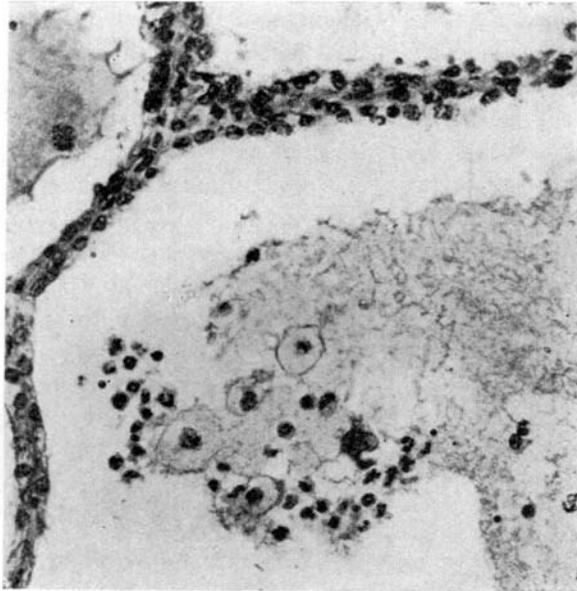


Fig. 1.



Fig. 2.

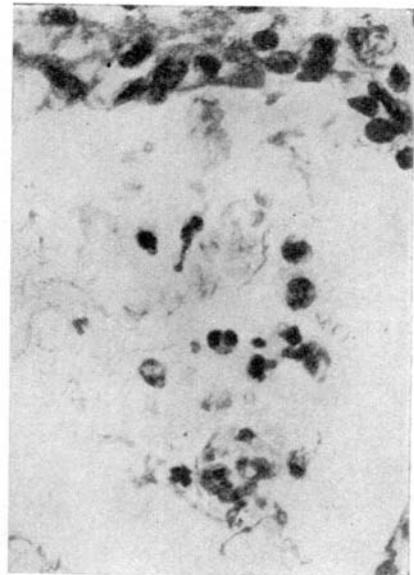


Fig. 3.