VARIATIONS OF SOMATIC CELLS AND NEUTROPHILS IN MILK THROUGHOUT LACTATION[1]

C. L. DUITSCHAEVER
Department of Food Science
AND
G. C. ASHTON
Department of Mathematics and Statistics
University of Guelph
Guelph, Ontario.

(Received for publication October 12, 1971)

ABSTRACT

Total and differential cell counts were obtained for alternate weekly morning and evening milk from 11 Holstein cows in six different lactations. Milk from quarters suspected of mastitis were examined for presence of pathogens. Weekly cell counts for each cow showed large variations throughout lactation. The neutrophil count closely paralleled the total cell count. The average neutrophil percentage varied from 65 to 96%. No relationship was observed between cell count or type and length of lactation, age of cow, and milk yield. In addition to mastitis, unspecified stresses seemed to cause irregular sudden increases in somatic cells. Except during severe stresses, total cell counts were about 200,000 per milliliter, of which 65 to 90% were neutrophils.

Microscopic examination of unprocessed milk always reveals somatic cells. The number of these cells has long been used as an indication of irritation or inflammation of the mammary gland. Somatic cell numbers tend however to vary sharply over short periods depending on several factors, including time of sampling. Marked variations in cell counts have been reported to occur during a single milking *(18)*, at intervals during 24 hr periods *(17)*, and from day-to-day and week-to-week *(3, 15)* within the same cows. Acute infections of the mammary gland are highly associated with an increase in leucocytes. There is, however, little information on the occurence and significance of different cells, particularly of neutrophils for the duration of an entire lactation. It has been suggested *(8, 19)* that of the somatic cells only the neutrophils should be counted, because they indicate pathological disturbances in the mammary gland.

The purposes of our study were: *(a)* to amplify earlier findings on cell count variations associated with time of sampling by investigating weekly variation of somatic cells throughout a complete lactation, and *(b)* to determine the presence of neutrophils and whether certain numbers of these cells are indeed indicative of pathological conditions of the mammary gland.

[1]Supported in part by a research grant (A-4423) from the National Research Council, Canada, and by the Ontario Department of Agriculture and Food.

MATERIALS AND METHODS

Animals

Eleven Holsteins from the University dairy herd were used. They freshened in late August and early September and represented six different lactations. All cows met the health requirements of the veterinary control program at the start of the experiment. Three cows out of the eleven had been treated for mastitis in previous lactations.

Sampling routine

A drip sample (100 ml) was collected once a week from each cow from the metering device (approved by the D.H.I.A.) on the pipeline milker. Weekly sampling alternated between morning (7 a.m.) and evening (4 p.m.) milkings. Two tablets of the preservative Lactab *(5)* were added to the proportionally collected sample and stored at 5 C for 4 to 18 hr before making cell counts. In a previous publication *(5)* it was shown that preserved and fresh milk samples gave comparable counts. Total milk yield at sampling was also recorded.

Cell counting procedure

Differential somatic cell counts were performed using the Millipore membrane technique described previously *(4, 5)*. This procedure was shown to be superior to the Breed-type smear method *(5)*. Thirty fields per sample were counted. The microscopic factor was 81,000 (81,000 × no. of cells per field = no. of cells per milliliter milk).

Bacteriological examination

When there was clinical evidence of inflammation of the udder, or when there was a two-fold increase in somatic cells for any individual cow, 0.1 ml from 30 ml of aseptically drawn foremilk from each quarter was streaked on blood agar and on phenol red mannitol agar and incubated for 24 and 48 hr at 37 C. Colonies were selected and divided into three groups: gram-negative rods, gram-positive rods, and gram-positive cocci. *Staphylococcus aureus* was identified by growth on phenol red mannitol agar and by the coagulase reaction. *Streptococcus* was identified by lack of catalase activity. Gram-positive and gram-negative rods were not found.

RESULTS AND DISCUSSION

Weekly cell count variation during a complete lactation

The rise and fall in cell numbers during a complete lactation could best be shown by graphs. Total somatic cells and number of neutrophils per 30 microscopic fields of the alternate morning and evening milk from each cow were plotted against weeks of lactation (Cow 9, 524, and 552 shown in Fig. 1). Large variations in cell counts were evident throughout the lactation in morning and evening milk. There was no indication of a regular 4-week cyclical rise and fall in cell numbers as was reported by Cullen *(3)*. The neutrophil count showed the same pattern of variation as the total cell count. When there was a rise or fall in total cell count, neutrophils generally responded accordingly. We interpreted this to mean that fluctuations in cell count were mainly increases or decreases in neutrophils. This was true of all cows except Cows 524 (Fig. 1) 72, and 75 (not shown). In those instances, the cells consisted mainly of small acidophilic epithelial cells whose morphology has been described previously *(4)*. Occasionally, these cells appeared also in increased proportions in milk of other cows. It was not known what condition in the mammary gland sometimes caused relative high numbers of these cells. The average sample-to-sample variation around the mean cell count measured as the coefficient of variation (Table 1) was smaller for the evening milk than for the morning milk which was opposite to what was generally indicated by the peaks and valleys in the graphs (Fig. 1). These two phenomena of variability are not contradictory because they represent two types of variation, sample-to-sample versus peak-to-valley. Whatever the reason for the two types of marked variation, the average cell counts over the complete lactation were higher for the evening milk in 9 cows.

Disparity between mean cell counts of morning and evening milk did not seem to be explained by differences in yield alone (Fig. 2). Of the 11 simple correlations computed between cell count and milk yield, eight were negative and not significant. Of the three positive ones only one was statistically significant and here yield accounted for less than 25% of the variation in cell count. There was thus little evidence of a relationship between cell count and milk yield. The pattern of greater peak-to-valley variability in evening milk might be related to exposure of the cows to different stresses during the day which might influence body cell secretion into the milk.

It is generally accepted that milk from cows in the terminal part of a lactation has more somatic cells, the majority of which are epithelial cells, resulting from the normal involution of the udder *(2, 3, 7, 10)*. These observations were not supported by the present study. Our findings agreed with those of Schipper *(15)* who reported no change in cell numbers with length of lactation. Although milk yield gradually decreased as the lactation progressed, it was not necessarily accompanied by a concurrent increase in somatic cells (Fig. 2), nor did a differential cell count show a higher proportion of epithelial cells. The observed irregular changes in cell counts might have been a response to chance infection, physical stress, various environmental conditions and management, particularly milking technique rather than to physiological causes. The invariably high proportion of neutrophils seemed to point to such a response.

Blackburn *(2)* showed that the somatic cell count increases with the lactation age of a cow. The effect of number of lactations was difficult to assess in our work, since four of the older cows developed mastitis in the course of the experiment and the milk had high cell counts for at least 6 weeks after successful treatment with antibiotics. Except for these periods of severe stress, the cell count was not appreciably higher than in the younger cows, except for Cow 552 which had a long history of mastitis. This suggests that increased cell counts in older cows are dependent on severity of pathological conditions which may have occurred during successive lactations rather than on a physiological process associated with stage of lactation.

The cell count in some cows was constantly higher or lower than in others under the same conditions of management and hygiene. Such differences were also reported by Schipper *(15)* and could be related to genetic factors as suggested by Afifi *(1)*.

Influence of infection or stress on cell count

Except for Cows 54, 524, and 552, there was no previous history of mastitis in the herd. During this experiment clinical mastitis was diagnosed and treated on 6 occasions, once in Cow 9, 74, 539, 553, and twice in 552. Although clinical evidence of inflammation was present, together with marked increases in neutrophils, the causative organism could not always be isolated. Only in four out of the six cases were either *Staphylococcus aureus* or *Streptococcus* or both found at the same time, in aseptically drawn foremilk of the affected quarter. Subsequent samples became negative by the 4th milking. A high neutrophil count of more than 1 million per milliliter sometimes persisted for 5 to 10 weeks after treatment with antibiotics. On several occasions during the lactations of any one cow the neutrophil count exceeded 1 million per milliliter although there was no clinical evidence of inflammation of the udder or visible abnormality of the milk, nor could any pathogens be isolated. The massive influx of

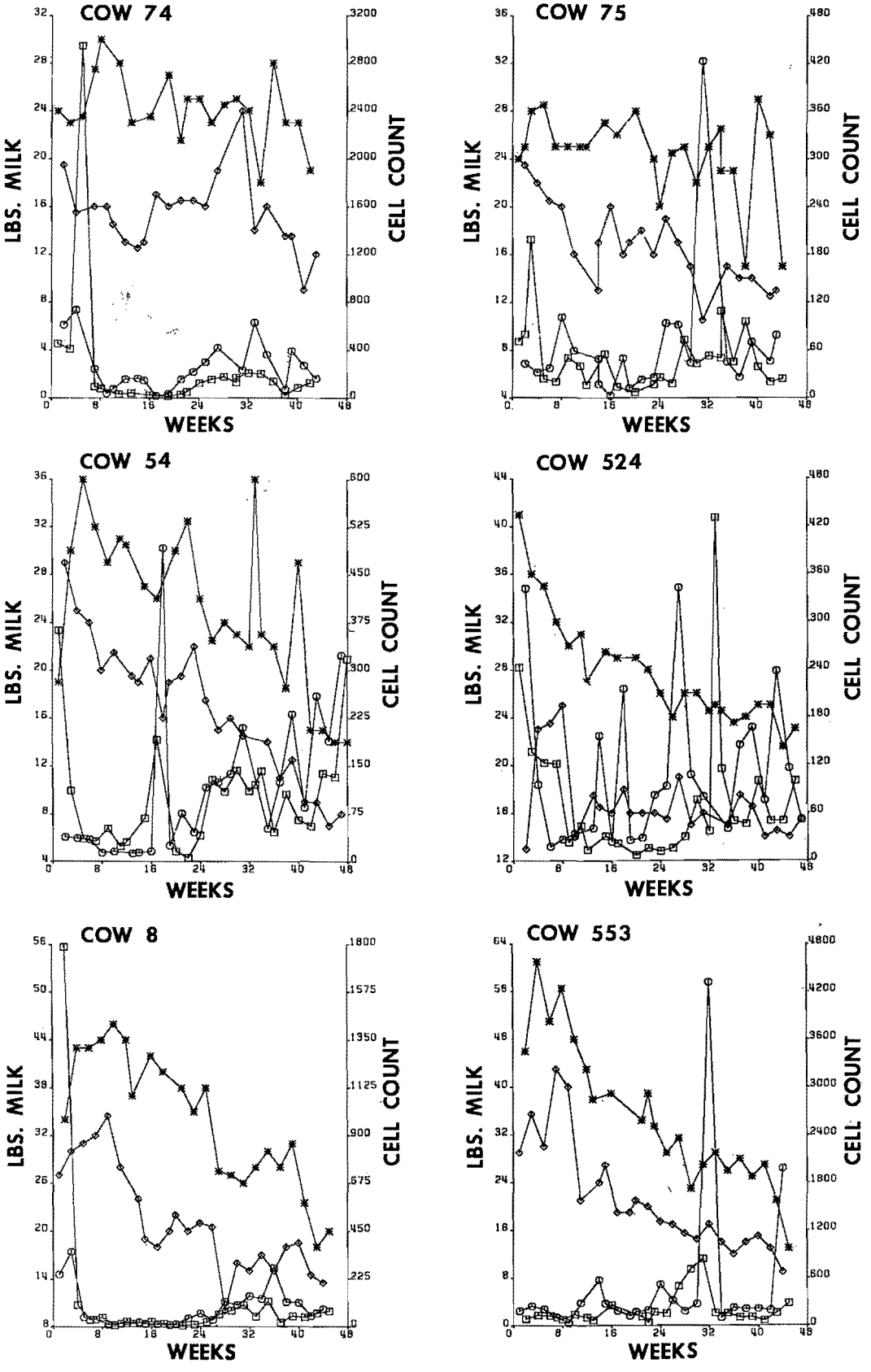

Figure 2. Weekly cell count (number of cells/30 microscopic fields) and milk yield for the whole lactation period. Yield of milk: lines with (*) = morning milk, lines with diamonds = evening milk. Total cell count: lines with squares = morning milk; lines with circles = evening milk.

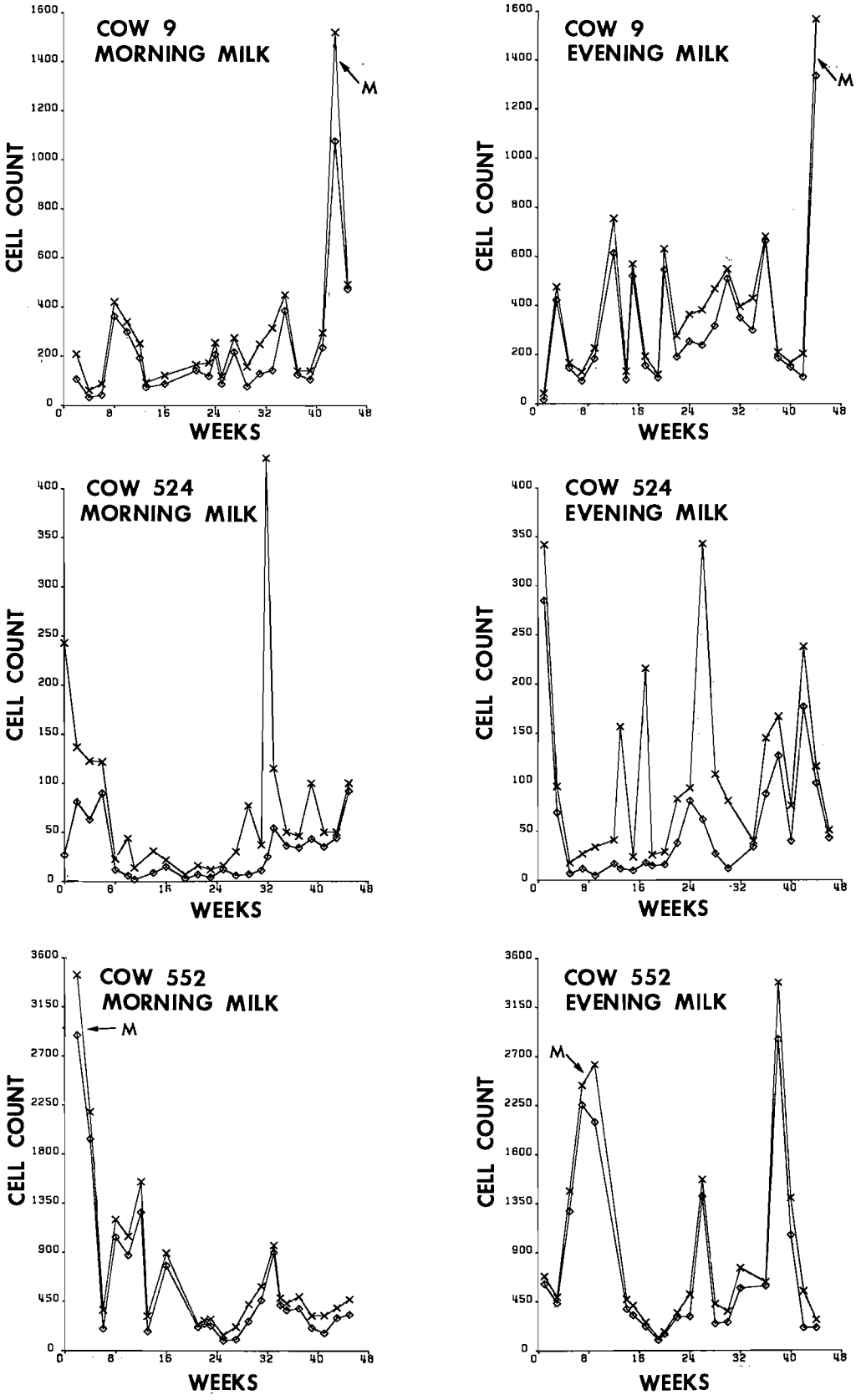

Figure 1. Weekly cell count (number of cells/30 microscopic fields) for the whole lactation period. Lines with (×) = total cell count; lines with diamonds = neutrophil count; M = mastitis diagnosed.

TABLE 1. MEAN CELL COUNT, COEFFICIENT OF VARIATION (SAMPLE-TO-SAMPLE) AND PERCENTAGE OF NEUTROPHILS FOR EACH COW FOR AM AND PM MILKING.

Lactation	Cow	Mean cell count				Neutrophils	
		AM	C.V.	PM	C.V.	AM	PM
		($\times 10^3$/ml)	(%)	($\times 10^3$/ml)	(%)	(%)	(%)
1	72	240	136	280	101	73	74
1	74[a]	280	235	478	79	88	92
1	75	97	84	110	132	65	66
2	54	190	83	180	104	79	81
3	524	140	121	210	86	75	80
3	39	470	81	750	60	95	97
4	9[a]	519	104	810	59	93	95
4	539[a]	660	146	810	78	93	95
5	8	110	301	130	106	80	82
6	552[a]	2270	103	1700	98	95	96
6	553[a]	620	102	670	190	93	95

[a]Mastitis diagnosed during lactation

neutrophils in the lactating mammary gland may have removed the invading infectious agents through phagocytosis before any symptoms of dysfunction of the gland developed. It may also be that the sudden increase in neutrophils resulted from physical stress or trauma. Usually, such a response was short and rapid recovery and return of the gland to normal occurred.

Neutrophil count

Observations at the extreme peaks in the graphs, whether associated with diagnosed udder infections or not, generally showed an influx of neutrophils which constituted invariably more than 90% of the total cells. Neutrophilia, in these instances, was clearly associated with acute abnormalities. However, relative neutrophilia was also observed in normal (or at least presumably nonpathological) secreting glands during the entire lactation. Only rarely was the neutrophil count <20% of the total, but varied from 50 to >90% at any time during the lactation. The mean cell count and the percentage of neutrophils per cow for their whole lactation period are in Table 1. Cows affected at one time or another with clinical or subclinical mastitis had the highest average cell count. That such condition had occurred was also reflected in a higher neutrophil percentage (more than 90%).

Blackburn *(2)* reported an average of 56% of polymorphs in uninfected quarters and 61 to 75% in infected quarters, depending upon the type of organism. Paape and Tucker *(9)* found 66 to 69% of granulocytes in their fraction-collected milk. These were lower neutrophil percentages than we observed. The invariably high proportion of neutrophils in normal milk was intriguing, particularly since concetrations of more than 20% were considered by Galli and Guallini *(6)* as certain infection. A preexisting neutrophilia may actually serve the cow well by increasing the resistance against infection as shown by Schalm et al. *(12, 13)*.

CONCLUSION

Large irregular sample to sample variation occured in the total somatic cells and neutrophils of alternate morning and evening milks of these 11 cows. Frequently variability in counts was further reflected in marked peaks and valleys, particularly for the evening milks. There was little evidence of a relationship between cell counts and milk yield. The somatic cell count of milk from cows free of clinical mastitis were within the generally accepted levels of 300,000 to 500,000 per milliliter. A high proportion of neutrophils (70 to 95%) was common even in the absence of diagnosed clinical mastitis.

ACKNOWLEDGMENT

The authors express their appreciation to Miss Maureen Slade for her capable technical assistance.

REFERENCES

1. Afifi, Y. A. 1968. Genetical influence on leucocyte counts in the milk of cows. Netherlands Milk Dairy J. 22:3.

2. Blackburn, P. S. 1968. The cell count of cow's milk and the microorganisms cultured from the milk. J. Dairy Res. 35:39.

3. Cullen, G. A. 1968. Cell count throughout lactation. Physiological variation in the cell count of cow's milk during lactation. Veterinary Res. 83 (5):125.

4. Duitschaever, C. L., and A. G. Leggatt. 1967. Cells in bovine milk: Differential staining in suspension; collecting, counting and examining on millipore membrane. Stain Technol. 42 (4):183.

5. Duitschaever, C. L., and G. C. Ashton. 1968. Comparison of counts using a Breed-type smear and millipore membrane methods on fresh and preserved milk samples. J. Dairy Sci. 51:665.

6. Galli, A., and L. Guallini. 1966. Richerche sul valore diagnostico dei polinucleati neutrofili nel latte mastitico. Arch. Vet. Ital. 17 (6):435.

7. Luhtala, A. T., T. Siirtola, and M. Antila. 1969. The cell count in milk using an electronic counter. Suom. Elain. 75 (3):142.

8. Munch-Petersen, E., and L. Mulgrave. 1969. Quarter vs. bulk milk samples for the determination of staphylococci and leucocytes. Australian J. Dairy Technol. 24 (4):127.

9. Paape, J. M., and H. A. Tucker. 1966. Somatic cell content variation in fraction collected milk. J. Dairy Sci. 49:265.

10. Pearson, J. K. L. 1969. The advantages of assessing the somatic cell content of bulk milk in national herd. J. Soc. Dairy Technol. 22 (3):147.

11. Plastridge, W. N. 1958. Bovine mastitis: A review. J. Dairy Sci. 41:1141.

12. Schalm, O. W., J. Lasmanis, and E. J. Carroll. 1964. Effects of pre-existing leucocytosis on experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. Amer. J. Veterinary Res. 25:83.

13. Schalm, O. W., J. Lasmanis, and E. J. Carroll. 1966. Significance of leucocytic infiltration into the milk in experimental *Streptococcus agalactiae* mastitis in cattle. Amer. J. Veterinary Res. 27:1537.

14. Schalm, O. W., and J. Lasmanis. 1968. The leucocytes: Origin and function in mastitis. J. Amer. Veterinary Med. Ass. 153:1688.

15. Schipper, C. J. 1963. Het aantal cellen in melk van individuele koeien en enkele methoden voor het opsporen van uierontsteking. Veet. en Zuivelber. 4:165.

16. Schultze, W. D., and J. W. Smith. 1964. Cell count variations in milk from bacteria-free and clinically normal individual quarters. J. Dairy Sci. 47:696.

17. Smith, J. W., and W. D. Schultze. 1967. Variation in cell content of milk associated with time of sample collection. I. Diurnal variation. J. Dairy Sci. 50:1083.

18. White, F., and E. A. S. Rattray. 1965. Diurnal variation in the cell content of Cow's milk. J. Comp. Pathol. 75:253.

19. Young, B. Y. 1968. The periodic acid-Schiff reaction as an aid in differentiating cell types in milk samples. Veterinary Record 82 (8):226.

REPORT OF THE COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS, 1970-1971

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of cooperating with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for the fabrication, installation, and operation of food equipment and to present to the membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The purpose of this cooperative program is to aid industry in improving the design, construction and installation of equipment so that it will lead to easy cleaning and proper functioning when placed into service in food establishments. It is the Committee's further purpose to cooperate with industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment.

The following report outlines the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

NATIONAL SANITATION FOUNDATION (NSF)

The Committee was represented at the 1971 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and prior to the meeting, the Committee reviewed and submitted comments on each draft of these proposals. Since the meeting, the Committee has also reviewed and submitted comments on proposed changes to standards.

Standard for soda fountain equipment

Prior to the recent revision of the Standard for Soda Fountain Equipment, properly labeled bobtail soda fountain equipment has been exempted from complying with the requirement for separate drainboards, since this small equipment normally is installed in places dispensing food in and with single service articles and with adequate facilities to wash and sanitize any multi-use equipment or utensils. Consequently, the following addition to Item 5.21 of Standard No. 1 was approved by the public health representatives:

> "This provision for separate drainboards shall not apply to bobtails; provided however a label stating the follow-in shall be affixed in a conspicuous position on each unit: This unit is intended for use with single-use service and the sink and drain section DOES NOT COMPLY with Standard No. 1 as it relates to multi-use customer service."

Standard for food service equipment

The current provision limiting the size of cutting boards to 24 × 36 inches and not heavier than 50 lb. was reviewed by the public health members and was amended to permit a 36-inch maximum dimension in any one plane and a weight not to exceed 50 lb.

Standard for spray-type dishwashing machines

The Foundation staff brought to the Joint Committee's attention that the current edition of Standard No. 3 on Spray-Type Dishwashing Machines failed to provide adequate specifications for some machines, as to spray patterns and proportion of spray jets for the lower and upper wash arms. The Joint Committee deemed that more specificity was needed in order to enable the manufacturer and evaluator to carry out their responsibilities to the user and consumer and in-

(Continued on Page 206)