THE CELL COUNT OF MILK AND RAPID TESTS FOR MASTITIS

W. G. WHITTLESTONE and H. DE LANGEN
Ruakura Agricultural Research Centre, Hamilton

SUMMARY

The literature on rapid tests for mastitis is reviewed. The results of a study of the relationships between the leucocyte count, DNA concentration and the California mastitis test (CMT) reaction are discussed. It is suggested that the CMT reaction for mastitis be based on the objective estimation of viscosity and that the basis for the test be the chemical determination of DNA in milk rather than the leucocyte count.

INTRODUCTION

TESTS FOR MASTITIS other than those dependent on a clinical examination of the animal in the field may be put into three categories.

(1) Bacteriological tests based on an examination of the flora of the milk from the affected quarter.

(2) Tests based on the fact that inflammation subsequent to infection, or due to any other cause, is associated with a rise in the cell count of the milk.

(3) Changes in the chemistry of the milk resulting from infection or trauma.

In this paper no consideration will be given to bacteriological tests. In considering the other two categories, it is important to bear in mind the fact that there is a substantial difference in the nature of these two classes of tests. An increase in the cell count of the milk may be regarded as a normal physiological response to the challenge of an infection or a traumatic change. On the other hand, changes in the chemistry of the milk, such as an alteration in pH or electrical conductivity, an increase in chloride or a decrease in lactose, represent substantial changes in the mechanism of the secretory tissue, and may, in a real sense, be thought of as secondary changes following the success of an invasion by bacteria or damage brought about by some other cause.

THE EVOLUTION OF RAPID MASTITIS TESTS

It is useful to consider the third category of tests first, because historically they played an earlier role in attempts

to discover field tests for mastitis. One of the earliest, simple diagnostic techniques was the bromthymol blue test. This is essentially a pH indicator test. When inflammation occurs in the udder, from whatever cause, there is a tendency for the pH of the milk to increase. Another test which was not so popular because of the difficulty of carrying it out except in the laboratory is the chloride test; again inflammation is associated with a marked increase in the chloride content of the milk.

These tests may be understood by an examination of the relative chemistry of blood and milk. The pH of blood is about 7.4, while that of normal milk is 6.6. Blood contains 0.35% of chloride, while milk contains 0.11 when normal. There is, of course, no lactose in blood but 4.9% may be found in milk, while the sodium level of milk is only 0.05%, but in blood reaches the level of 0.34%. Now, milk is iso-osmotic with blood; should any damage take place to the secretory tissue, resulting in a loss in the production of a specifically mammary product such as lactose which makes a large contribution to the osmotic pressure of the milk, there will be a diffusion of smaller ions and other molecules into the udder to bring the osmotic pressure of the milk up to that of the nearby blood. Thus, a loss in lactose as a result of the reduction in the secretory efficiency of the mammary gland results immediately in an increase in chloride. This increases the electrical conductivity of the milk and forms the basis for the once-popular "milk abnormality tester". This was a simple conductometer which could be taken into the field and which made possible the almost instantaneous measurement of the electrical conductivity of freshly drawn milk samples.

Early work at Wallaceville (Whittlestone and Palmer-Jones, 1944) showed that the electrical conductivity test had a satisfactory relationship with the leucocyte assessment but that pH changes were not reliably related to increases in cell content. While it is likely that the conductivity test would have been a valuable diagnostic tool, its cost and the difficulty of maintaining the apparatus in a satisfactory condition have resulted in its going out of favour.

On theoretical grounds, there is a strong case for basing diagnostic tests on the increase in leucocyte count, which is associated with irritation or inflammation from whatever cause. This is a physiological response to damage to the udder, and is thus likely to be more sensitive than any

response which takes place as a result of a change in the mode of action of the secreting tissue.

Methods for estimating in the laboratory the number of white cells in milk are very well known and will not be discussed here. Whiteside (1939) found that there was a close relationship between the cell count of milk and the viscous reaction which is obtained when five drops of milk are mixed with one drop of 4% caustic soda on a glass slide. The mixture of alkali and milk appears flakey and viscous to a degree which is associated with the cell count and shows a distinct response to counts in excess of 200,000 per ml. This test is not suitable for use in the cowshed because it requires good light and care in observation. Murphy (1942) improved the Whiteside test and made it, if anything, slightly more sensitive, while Schalm *et al.* (1956) modified the Whiteside test further and adapted it to field use by using tubes containing a suitable amount of caustic soda solution. Another test based on the leucocyte content of milk is that of Negretti (1958), known as the anti-formin test. The reagent is a mixture of sodium carbonate, calcium chlorate, and caustic soda. This, when added to milk, gives a flakey, gelatinous reaction similar to that of the Whiteside test. It is said, however, to be more reliable than the latter.

An important step forward was made when Schalm and Noorlander (1957) and McKenzie and Cameron-Mackintosh (1958) showed that the addition of a simple surface-active agent to milk containing leucocytes produces a flakey, gelatinous response rather like that shown in the Whiteside test. The former workers used a sodium salt of an alkyl-aryl-sulphonate, while the latter used sodium teepol.

Schalm and Noorlander introduced a most convenient paddle in which the test is carried out. Four depressions in a plastic paddle are used to collect a squirt of milk from each of the cow's teats. By tipping the paddle sideways, an equal volume of milk is left in each depression and to this volume an equal amount of reagent is added and the whole paddle swirled approximately ten times. Normal milk remains liquid with no evidence of a precipitate. The "trace" reaction is defined as one in which a slight precipitate forms which can be seen by tipping the paddle back and forth. Such a trace tends to disappear with continued movement of the liquid. A "one" reaction is a distinct precipitate but there is no tendency towards gel formation in the liquid. A "two" reaction shows an immediate thickening with a definite suggestion of gel formation. As the mix-

ture is swirled the gel tends to move towards the centre of the cavity. A "three", or strong positive reaction, gives a very distinct gel which during swirling forms a convex mass in the centre of the paddle.

One criticism which has been directed at this technique is that it is responsive to cells regardless of type. However, Blackburn *et al.* (1955) have shown that differential counts distinguishing polymorphonuclear leucocytes, lymphocytes and other, mainly epithelial, cells have no diagnostic advantages over total cell counts. This means that any indirect method of estimating cells is quite satisfactory as a diagnostic method. The Whiteside, anti-formin and surface-active-agent test, now widely known as the California or rapid mastitis test (CMT), are essentially indirect methods of estimating the cell content of milk.

The most recent addition to this group of diagnostic tests is one described by Paape *et al.* (1964). This is based on the Feulgen-DNA reaction. Schiff's reagent based on fuchsin in bisulphite solution is well known as a stain for structures containing DNA. Paape *et al.* based their test on this reagent, which it was found, when added to milk, gives a purple colour, the intensity of which seems to be related to the cell count. However, in view of the extremely low quantities of DNA present, even with a high cell count, it seems improbable that this colour reaction is due to DNA. This point will be discussed later.

Another well-known laboratory test for the presence of leucocytes is the catalase test which is based on the addition of hydrogen peroxide to the milk to be tested. The volume of oxygen produced as a result of the catalytic decomposition of the peroxide is measured. Leudecke (1964) has shown that the percentage of oxygen evolved in the catalase test closely follows the CMT score and the leucocyte count. Mean figures were as in Table 1.

TABLE 1: RELATIONSHIP BETWEEN CMT SCORE, LEUCOCYTE COUNT AND CATALASE TEST (LEUDECKE, 1964)

CMT score	0	Tr	1	2	3
Leucocyte count (1,000)	51	96	340	1.600	7.300
Catalase O_2%	5.4	8.0	19.8	52.4	82.5

The mechanism by which the addition of caustic soda or a surface-active agent to milk brings about the precipitate and a gel formation is not clear. One view is that the destruc-

tion of the leucocyte cells gives rise to an interaction between the cell protein, calcium from the milk, and the alkali to form a gelatinous mass. Another suggestion, that of Carrol and Schalm (1962), is that the reaction is due to the release of DNA from the cell nucleus. These workers have shown that the addition of any nucleated cells to normal milk to which the CMT reagent is added gives rise to a characteristic change in physical properties which may be quickly destroyed by the addition of DNA-ase. Furthermore, the addition of DNA-ase to milk (but not RNA-ase) will prevent the typical CMT reaction even when the milk contains a large number of leucocytes.

There are certain difficulties about accepting this explanation. A milk showing a plus one CMT reaction contains approximately one million leucocytes per ml. This corresponds to about three micrograms of DNA. It is very difficult to believe that this quantity of the substance, regardless of its enormous molecular weight, could cause an increase in viscosity of 40%. To the writers it would seem more likely that this marked change is one brought about by the catalysis of a polymerizing reaction. The release of active enzymes into the milk could undoubtedly bring about marked changes.

The Schiff reaction referred to above as a test for the presence of DNA in milk does not seem likely to be useful as a direct estimate. Widstrom (1928) has shown that the maximum sensitivity of this reagent corresponds to five hundred micrograms per ml. This makes it grossly insensitive for the detection of any change in the DNA level of milk which is brought about by contamination with leucocytes. The colour reaction shown by Paape *et al.* (1964) would appear to be due to some other constituent of abnormal milk. It could not be due to the DNA content of the leucocytes.

Before proceeding to an examination of the writers' results, it is worth while looking at some improvements which have been brought about in the California or rapid mastitis test. Paape *et al.* (1962) describe a reagent which is based on a mixture of an alkyl-aryl-sulphonate, caustic soda and methylene blue. This is referred to as the Michigan mastitis test (MMT). It combines the action of the Whiteside and California tests. The same authors have produced a cheap "home-made" mastitis reagent based on crude caustic soda and a popular liquid household detergent, to which is added a small quantity of a navy blue dye. The MMT and CMT are equally useful and have a coefficient of variation of 13%.

The home-made or lye mastitis test (LMT) is somewhat more variable. It has a coefficient of variation of 20%, but this is quite good enough for farm use in detecting sub-clinical mastitis.

The judgement of the degree of reaction of any of the tests based on viscosity changes is inevitably subjective. Thompson and Postle (1964) describe a test which is based on an estimate of the viscosity of the milk. Tubes fitted with caps in which 3/64th inch holes are drilled are used to mix reagent and milk. They are inverted for a fixed period of time to allow the mixture to pass through the small hole and then tilted back to the normal position and the amount of liquid left behind is measured. This is a function of the viscosity of the mixture. An excellent correlation was found between the square root of the leucocyte count and the height of the liquid left behind (the correlation coefficient was 0.91). The reagent used in this case was 1.25% alkyl benzene sulphonate. Two millilitres of milk and two of reagent were mixed in the tube and allowed to flow for fifteen seconds before the height of the remaining liquid was measured.

The Brabant mastitis reaction test is similar to that described by Thompson and Postle. Matschullat (1963) describes this test. It is based on the addition of milk to a suitable reagent containing sodium lauryl sulphonate, the mixture being placed in a small glass tube with a capillary at its lower end. 0.6 ml of milk and 0.4 ml of 3% sodium lauryl sulphonate solution are mixed and the time for this volume of liquid to flow through the capillary is measured in seconds. This has been found to be a valuable can test for examining milk at the factory.

Kiermeier and Keis (1964) describe a rather more precise method of measuring the viscosity changes which take place in the California mastitis test. They used a falling ball viscometer of the type used for the determination of the viscosity of lubricating oils. It is used at a temperature of 20°C. 20 ml of milk and 30 ml of the California mastitis test reagent are mixed and added to the viscometer. The time for the ball to fall through this mixture is taken and an excellent relationship has been found between the California mastitis test reading and viscosity. Furthermore, there is a very satisfactory relationship between the viscosity measurement and the cell count. The test is essentially a laboratory one in its present form but, modified, it could be used as a field test.

DNA DETERMINATION AS A MEASURE OF CELL CONTAMINATION

The presence of leucocytes in milk as it arrives at the dairy factory may be viewed from two points of view. First, it may be taken as an indication of the incidence of subclinical mastitis in the herds concerned. It may also be regarded from the public health point of view as a form of contamination of the milk. In its crudest terms, milk containing leucocytes is milk contaminated with pus. While there is no serious public health risk associated with the consumption of milk containing leucocytes, there is nevertheless a reaction against it. It may be said that milk contaminated by small amounts of cow dung would not do any harm after pasteurization. Again there is an essentially aesthetic reaction against such contamination. It is probable therefore that, in the future, one quality standard which may be insisted on is a reasonable leucocyte count.

All cell counting procedures, regardless of how carefully they are carried out, tend to have large personal errors. This is inherent in such procedures. On the other hand, the measurement of viscosity following the addition of a reagent like the California mastitis test reagent is a relatively precise procedure when carried out in the laboratory. It is highly probable, therefore, that a well standardized CMT would be more precise than a cell count. However, in all publications to date, the CMT response has been calibrated against cell counts, and thus the inter-relation has been subjected to the errors associated with cell counting. The results to be described have arisen from the fact that the estimation of DNA in milk is now a procedure which is not too difficult when carried out in the laboratory.

Normal milk contains no measurable DNA so that the level of this substance in milk may be taken as an index of its contamination with cells of whatever origin, and because its determination is reasonably precise it could form an adequate basis for a legal quality standard. Table 2 summarizes a number of reactions which have been used for the determination of DNA. It is clear that the method involving indole and hydrochloric acid as developed by Ceriotti (1952) has adequate sensitivity for the determination of DNA in milk contaminated with leucocytes. Ceriotti's method has been slightly modified and applied to the routine determination of DNA in milk samples which have also been subjected to leucocyte count and the CMT reaction. Details of the chemical method will be published elsewhere.

TABLE 2: COLOUR REACTIONS FOR THE DETERMINATION OF DNA

Reaction	*Sensitivity Min. (μg/ml)*	*Max.*	*Reference*
Cysteine + conc. H_2SO_4	50	500	Sturnf, 1947
Diphenylamine	50	500	Seibert, 1940
Tryptophane + perchloric acid	100	500	Copen, 1944
Indole + HCl	2.5	15	Ceriotti, 1952
Carbazole + HCl	500	5,000	Gurn & Flood, 1941
Schiff's reagent	500	5,000	Widstrom, 1928

TABLE 3: RESULTS AS OPTICAL DENSITY (O.D.) OF MILK SAMPLES FREE OF CELLS WITH AND WITHOUT THE ADDITION OF STANDARD DNA
(Means of duplicates)

O.D. of Blank	*DNA Added (μg)*	*DNA Found (μg)*
0.001	5	5.0
0.000	5	4.8
0.003	5	5.1
0.002	10	9.9
0.001	10	10.1
−0.001	10	9.8

TABLE 4: OPTICAL DENSITY READINGS FOR THREE CONCENTRATIONS OF STANDARD DNA SOLUTION AND ONE MILK SAMPLE WITH A LEUCOCYTE COUNT OF 1,800,000 CELLS/ML
(12 determinations were carried out on each sample.)

Sample	*Maximum*	*Minimum*	*S.D.*	*Mean*
DNA standard				
5 μg/ml	0.132	0.125	0.128	0.007
10 μg/ml	0.268	0.250	0.258	0.006
15 μg/ml	0.395	0.372	0.379	0.007
Milk				
1,800,000 cells/ml*	0.405	0.379	0.397	0.008

* Mean of 3 counts.

Briefly, the technique consists of adding sufficient potassium chloride to the milk sample to ensure that on centrifuging all of the leucocytes will go into the cream layer. Chilling of the centrifuge tubes makes it possible to remove the milk fat plug quantitatively. The milk fat is dissolved in 1: 1 alcohol: ether mixture, warmed, centrifuged, and to the residue trichloracetic acid is added and the mixture heated. After cooling and centrifuging, an aliquot of the hydrolysed DNA solution is taken and to this the indole hydrochloric acid reagent is added. After mixing, heating, and subsequent cooling, the solution is extracted twice with purified chloroform. The remaining aqueous solution is then centrifuged and the optical density determined against a blank consisting of water plus reagents at a wavelength of 490 mμ.

Table 3 sets out the recoveries from known DNA added to milk. As can be seen, the results are very satisfactory. Table 4 sets out optical density readings for twelve estimations for each sample at the DNA levels given. Again the results are very good.

A thorough investigation of the reliability of Ceriotti's method for the determination of DNA in milk has been carried out at this station and the results, the details of which will be published elsewhere, have shown that this is a very satisfactory basis for the determination of the degree of contamination of milk by leucocytes. It is, therefore, a sound basis on which to establish the calibration of such tests as the California mastitis test.

THE INTERPRETATION OF THE CALIFORNIA OR RAPID MASTITIS TEST

Leidl and Schalm (1961), carrying out tests on bulk milk from cans, show that a score greater than one indicates a cell count above 500,000 cells per ml. Their figures show a spread of count in the five test categories such that category "0" is overlapped by $-+$ and $+-$ (the categories being 0, $-+$, $+-$, 1, and 2), while category $+-$ overlaps $-+$ and 1. Similarly, in the comparison given by Schalm (1962) between CMT score and cell count there is an overlapping between adjacent categories.

Figure 1 sets out the distribution of cell count in the five categories of the CMT score 0, 1, 2, 3, and 4. In order to simplify statistical work, the category "trace" of the original California mastitis test has been given as 1, 1 as 2, and so on. As can be seen, there is a tremendous overlapping of the categories indicating that in practice the use of a scale

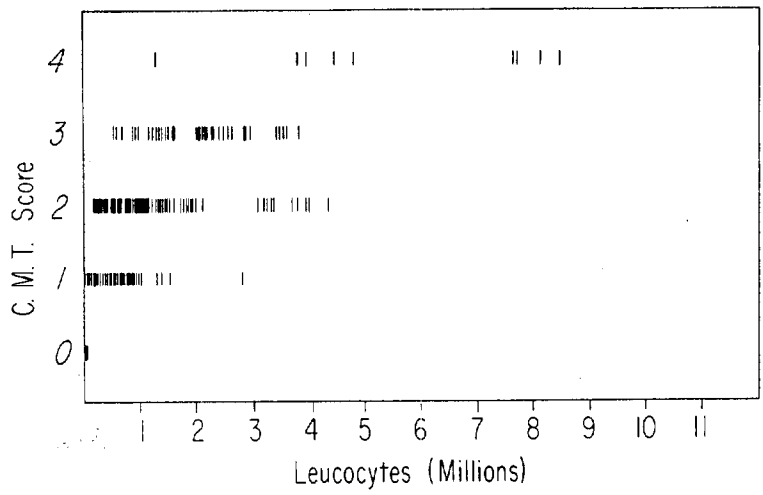

FIG. 1: *Distribution of cell count in five categories of CMT score.*

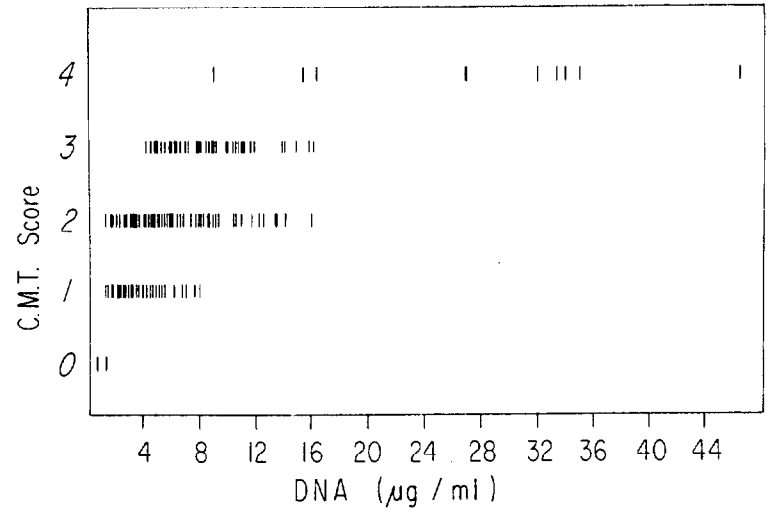

FIG. 2: *CMT scores related to micrograms DNA per millilitre.*

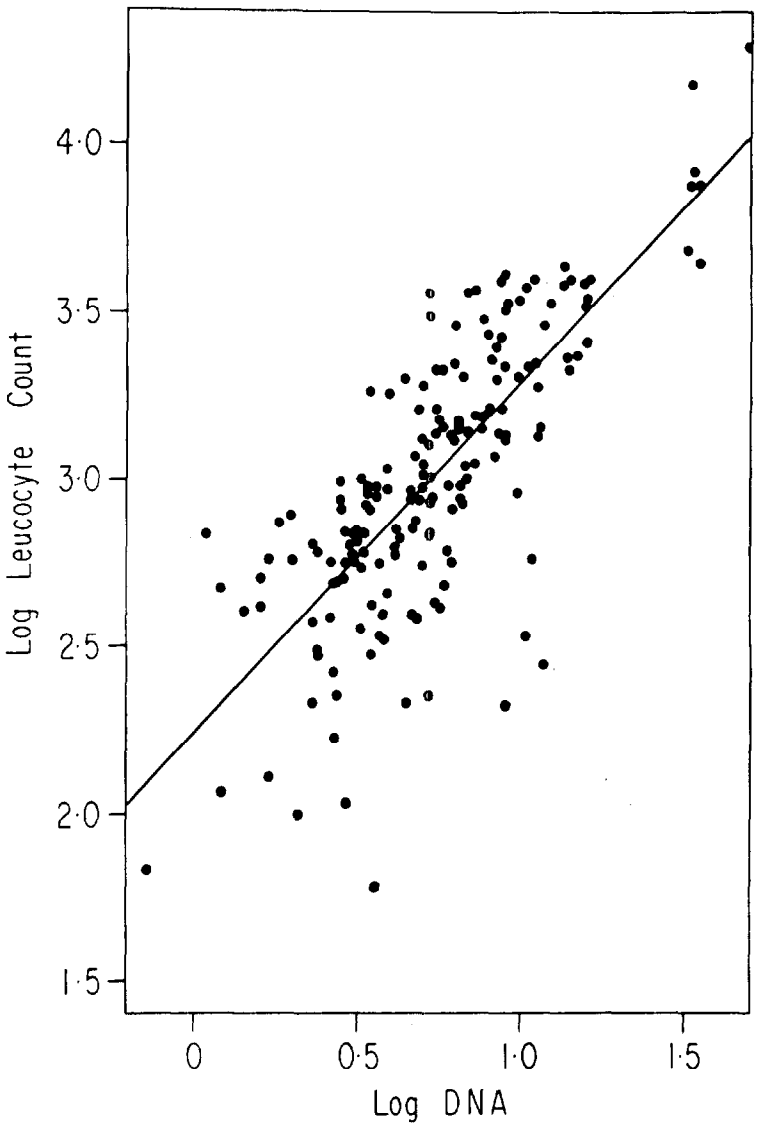

FIG. 3: *Leucocyte count related to DNA content of the milk.*

from 0 to 4 is redundant. Figure 2 sets out the CMT scores against the number of micrograms of DNA per millilitre. The error in measuring the amount of DNA is quite small and, as it is proportional to the number of contaminating cells in the milk, it may be taken as a base; thus Fig. 2 gives some idea of the inherent error in the CMT score reading. Again it can be seen that there is a great overlapping of categories. Figure 3 is a "scattergraph" relating leucocyte count to the DNA content of the milk. It can be seen there is a very large spread in the distribution of the leucocyte counts as plotted against micrograms of DNA. The spread is an indication of the basic error in leucocyte counting. The Appendix amplifies these defects of the CMT score.

It is clear from the above that both leucocyte count and CMT scoring are subject to very large errors. When an attempt is made to standardize the CMT reaction against leucocyte count, one has a combination of two lots of errors. It is, therefore, suggested that the DNA level of milk be taken as the basic measurement against which to calibrate other tests. Furthermore, it is suggested that, if the rapid mastitis test is adopted as a quality test at dairy factories, it be based on a measurement of the viscosity change which occurs in the milk. This is objective, readily determined, and does not involve expensive apparatus.

Finally, it is recommended that, when used as a field test for examining the incidence of sub-clinical mastitis, the rapid mastitis test be read in three categories — zero or free from cells, doubtful, and positive. This would mean that, on the original classification of reactions, trace and 1 would be read as doubtful, and 2s and 3s would be read as positive. Such a system of reading would reduce the problems of comparing the results of one worker with another and, as can be seen from the foregoing results, would not cause a very great loss of information.

ACKNOWLEDGEMENTS

The writers wish to acknowledge the technical assistance of Miss Lynda Franks who carried out much of the laboratory work involved.

REFERENCES

BLACKBURN, P. S.; LAING, C. M.; MALCOLM, J. F. (1955): *J. Dairy Res.*, *22*: 37.
CARROLL, E. J.; SCHALM, O. W. (1962): *J. Dairy Sci.*, *45* (9): 1094.
CERIOTTI, G. (1952): *J. biol. Chem.*, *198*: 297.
KIERMEIR, E.; KEIS, K. (1964): *Milchwissenschaft*, *19*: 65.

LEIDL, W.; SCHALM, O. W. (1961): *Tierarzliche Umschau, 16*: 219.
LEUDECKE, L. O. (1964): *Proc. Amer. Dairy Sci. Assn.*, June 22–24, p. 45.
MCKENZIE, D. A.; CAMERON-MACKINTOSH, E. (1958): *J. Soc. Dairy Technol., 11*: 178.
MATSCHULLAT, A. (1963): *Molterei-und Kaserei, 14*: 1550.
MURPHY, J. M. (1942): *Cornell Vet., 32*: 439.
NEGRETTI, F. (1958): *Atti. Soc. ital Sci. Vet., 12*: 654.
PAAPE, M. J.; SNYDER, W. W.; HAFS, H. D. (1962): *Quart. Bull. Michigan Ag. Exp. Sta., 45* (2): 255.
PAAPE, M. J.; HAFS, H. D.; TUCKER, H. A. (1964): *J. Milk Food Technol., 27*: 228.
SCHALM, O. W. (1962): *Syllabus on the Bovine Mammary Gland in Health and Disease.* Univ. of California, Davis, p. 41b.
SCHALM, O. W.; NOORLANDER, D. O. (1957): *J. Amer. vet. Med. Assn., 130*: 199.
SCHALM, O. W.; PIER, A. C.; GRAY, D. M.; NOORLANDER, D. O. (1956): *Calif. Vet.*, Jan./Feb.
THOMPSON, O. I.; POSTEL, D. S. (1964): *J. Milk Food Technol., 27*: 271.
WHITTLESTONE, W. G.; PALMER-JONES, T. (1944): Unpublished.
WHITESIDE, W. H. (1939): *Canad. Pub. Health J., 30*: 44.
WIDSTROM, G. (1928): *Biochem Z., 199*: 298.

APPENDIX

The following tables set out the relationships between leucocyte count and CMT score, CMT score and DNA concentration, and leucocyte count and DNA.

The statistical material was supplied by Miss J. G. Miller, Superintendent, Biometrics Section, Department of Agriculture, to whom the authors are greatly indebted.

1. RELATIONSHIP OF CMT SCORE AND LEUCOCYTE COUNT

	Frequency Tables CMT Score			
Log leucocyte counts	1	2	3	4
1.25 to 1.50	—	—	—	—
1.50 to 1.75	—	—	—	—
1.75 to 2.00	2	—	—	—
2.00 to 2.25	4	1	—	—
2.25 to 2.50	4	6	—	—
2.50 to 2.75	10	16	—	—
2.75 to 3.00	17	37	8	—
3.00 to 3.25	7	31	12	1
3.25 to 3.50	1	8	17	—
3.50 to 3.75	—	10	5	4
3.75 to 4.00	—	—	1	4
4.00 to 4.25	—	—	—	1
4.25 to 4.50	—	—	—	1

CMT Score	*Mean log leucocyte count*	*Mean leucocyte count ('000)*	*S.E.* —
0	1.64	44	0.36
1	2.70	499	0.30
2	2.99	971	0.26
3	3.24	1,603	0.32
4	3.80	6,258	

2. RELATIONSHIP OF CMT SCORE AND DNA VALUE

	Frequency Tables CMT Scores			
Log DNA	1	2	3	4
below 0	—	—	—	—
0 to 0.2	2	2	—	—
0.2 to 0.4	9	6	1	—
0.4 to 0.6	19	27	—	—
0.6 to 0.8	11	42	11	—
0.8 to 1.0	4	22	16	1
1.0 to 1.2	—	9	13	1
1.2 to 1.4	—	1	2	1
1.4 to 1.6	—	—	—	7
1.6 to 1.8	—	—	—	1

CMT Score	*Mean log DNA*	*Mean DNA*	*S.E. of log DNA*
0	−0.02	0.95	
1	0.52	3.34	0.20
2	0.70	4.98	0.22
3	0.92	8.28	0.19
4	1.42	26.56	0.22

3. RELATIONSHIP OF LEUCOCYTE COUNT AND DNA

There was an approximately linear relationship between log DNA and log leucocyte count. Within each CMT score the regression was highly significant and all the regression coefficients did not differ significantly. The overall regression is:

$y = 1.06\ x + 2.23$

where

y is log leucocyte

x is log DNA

this transforms back to $\dfrac{\text{leucocyte count}}{(\text{DNA})\ 1.06} = 10^{2.23}$

If the DNA value is considered to have no error, and the regression relation indicates the "true" log leucocyte count of the sample, then any observed log leucocyte count has a standard error of 0.28 around this true value. The index (1.06) is virtually 1.0 implying proportionality between DNA and leucocyte count.

DISCUSSION

DR R. E. MUNFORD: I am not surprised that Dr Whittlestone did not find a good relationship between DNA content and leucocyte counts since, among other things, the variable incidence of polynuclear cells would contribute to this lack of relationship. I am surprised that Dr Whittlestone obtained satisfactory results with Ceriotti's method, I would suggest he might like to look at one other method — Burton's adaption of the diphenylamine method which is possibly more suited to widespread application.

DR W. G. WHITTLESTONE: The work of Blackburn and co-workers (1955) which showed no advantage in using a differential count compared with a straight leucocyte count implies that there is not an enormous variation in the types of cell present. In any case, we are interested only in nucleated cells which have similar amounts of DNA in them. I think the error in leucocyte counting swamps the variations (in DNA content) from one cell type to another. Thank you for the suggestion to try Burton's method. We have found Ceriotti's method quite workable, but it does take great care to get reliable results.

H. G. REES: *Is there likely to be a variation in DNA level present in a milk sample examined at the cowshed and that present in the same sample examined 24 hours later at the factory?*

DR WHITTLESTONE: It is possible that the DNA level could change owing to the action of deoxyribonuclease in the milk. The leucocyte count could also change owing to enzyme action. However, they would tend to change together so that the DNA level would still be a useful index of cell contamination.

DR D. G. EDGAR: *Please review the evidence for the presence of leucocytes in milk being a cause of loss of milk production in the cow.*

DR WHITTLESTONE: Schalm and Noorlander of the University of California studied the relationships between production and the C.M.T. score for several thousand cows in Sacramento County, California, and found a very significant decline in production with rising C.M.T. score. This work is discussed by Professor Schalm in his lectures on the bovine mammary gland and by Noorlander in his book *Milking Machines and Mastitis.*