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# The Secretion of Abnormal Milk by Quarters free from Known Pathogens.

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CONTENTS.



## (I) Introduction.

In his conclusion to a very comprehensive survey of the literature of bovine mastitis up to the end of 1935, which includes a critical evaluation of the various direct and indirect tests for mastitis, Munch Petersen (1938) pointed out that nowhere in the literature perused did he find evidence of adequate work on the fundamentally important point as to what constitutes

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normality on the one hand and mastitis on the other. He stressed the outstanding need for accurate, detailed and frequent examination of the udder and milk of cows in their first lactation period, in order to establish adequate criteria on which normality or departures therefrom may be judged.

The efficiency and reliability of many of the indirect tests for mastitis, which are based on changes in the composition of milk, have within recent years been viewed with increasing suspicion. Hastings and Black (1937) pointed out that it would be unwise to conclude simply on the basis of abnormal chemical reaction of the milk that a cow is diseased. Van Rensburg (1941) drew attention to the unreliability of many of these tests on account of the tendency of some quarters that are apparently free from infection and clinical evidence of mastitis, to secrete milk which deviates appreciably from the standard usually accepted for normality. It appears that similar misgivings were raised in Munch Petersen's mind, since he finally detailed six points in connection with mastitis on which he considered adequate and reliable data were required. His first, and presumably most important point was " the normal udder and its secretion, including studies of cell content and flora ".

It is becoming increasingly evident that the production of abnormal or poor quality milk is not a characteristic peculiar to diseased udders, and that milk which falls below the usual standards for normality is frequently derived from quarters free from infection and clinical mastitis. Therefore the many adverse influences to which the normal disease-free udder and its secretion are subject are not merely matters of academic interest to research workers, but are of vital practical importance from the genetic, commercial, legal, economic and public health aspects, since these affect everyone concerned in the production and consumption of milk.

On the breeder in the first instance rests the responsibility for producing dairy animals which are free from hereditary defects likely to exert a detrimental influence on the quality of the milk secreted by them. While it will be shown that environmental conditions to which cows in milk may be exposed are to a large extent responsible for the production of inferior quality milk, convincing evidence will be produced to indicate that hereditary defects also play a very important rôle. Only a thorough knowledge of the nature of these undesirable characteristics will enable the breeder to practise systematic scientific and selective breeding which is the only method that will ensure the rearing of dairy animals which, when maintained under proper conditions of management and health, will consistently yield milk conforming to the prescribed standard.

The dairyman is usually the individual who has to receive the hardest blows that may result from the offering for sale of milk which is of inferior quality. Certain definite standards have been prescribed and failure of the milk supplied to the public to conform to these legal requirements render him liable to prosecution with the attendant publicity, loss of custom and sometimes even the loss of his licence. While inferior milk is frequently the result of fraudulent practices or due to the prevalence of mastitis in the herd, these two factors are not always present, and to the layman the cause remains obscure. In such cases the unfortunate producer, with his lack of knowledge of the many other factors that may be involved, regards himself as the innocent victim of unduly harsh legislation. Wider knowledge of the many environmental factors which may depress milk solids will enable the producer to reduce substantially the detrimental effects of those conditions by more rational and scientific methods of herd management.

When the consumer buys milk he does so in good faith, believing that it is clean, safe, of normal composition, and possessing in full measure all those constituents to which milk owes its high nutritive value. From the nutritional and public health aspects the water which forms approximately 87 per cent. of normal milk is valueless, and all its nutritive value is concentrated in the milk solids. The deviations from the normal which occur in the composition of milk are usually characterised by a decrease in the solids and an increase in water, soluble salts, enzymes, body cells and extraneous material. It is obvious that, from the commercial aspect as well as nutritional considerations, when the consumer purchases 100 gm. of normal milk, he actually only obtains about 13 gm. which is of nutritive value, the balance being water. Any depression below the legal standard in the milk solids for whatever reason consequently results in the consumer obtaining an adulterated article.

## (II) Objects and Scope of the Present Investigation.

In an endeavour to contribute towards the elucidation of those aspects of bovine mastitis on which Munch Peterson considers that fundamental knowledge is still lacking an experimental herd consisting originally of 20 heifers was established at Onderstepoort early in 1939.

One of the objects of the series of experiments that were thus undertaken was to ascertain to what extent variations are likely to occur in the composition of milk of the individual quarters of cows maintained under ordinary conditions of hygiene and feeding and free from disease of the udder.

To be complete a study of this nature rendered necessary a close observation over the animals and repeated analyses for some of the most important of the constituents of the milk over a number of lactations.

Before, however, these investigations had progressed very far it became obvious, not only that the variations shown in the composition of the milk from the normal quarters were more widespread than was anticipated, but also that the secretion of abnormal milk by quarters apparently normal in all respects and free from infection with known pathogenic bacteria was by no means an uncommon occurrence. Both from the point of view of mastitis research and of the practical, economical and public health aspects this observation was deemed to be of such importance as to warrant a more detailed investigation into the secretion of abnormal milk by quarters other than those affected with mastitis. This forms the basis of the present work which must be regarded not as a complete entity, but rather as a portion of a very comprehensive programme of mastitis research undertaken at this Institution.

The objects and scope of the present investigation may thus be briefly summarised as follows:—

(1) To determine to what extent abnormal milk is secreted by quarters which have been kept under close observation, the milk from which has been subjected to regular analysis from the onset of the first to the end of the fourth lactations, and which have never been infected with any of the pathogenic bacteria known to produce disease of the udder.

(2) To ascertain as far as is possible what factors are mainly responsible for the abnormalities observed, and the manner in which

the various influences exert their harmful effect on the udder and its secretions. The effects of the following aetiological factors were thus considered.—

(i) Advancing age and successive lactations.
(ii) Seasonal and climatic changes.
(iii) Stage of lactation.
(iv) Individuality and genetic characteristics.
(v) Variations in the composition of milk from the different quarters.
(vi) Conformation and structure of the udder.
(vii) Non-specific mastitis.
(viii) Micro-organisms.

Wherever necessary the significance of the differences noted was determined by statistical analysis of the data.

The whole problem of abnormal milk secretion centres round the composition of normal milk, the standard prescribed for milk, the anatomy of the udder and the physiology of milk secretion. Brief consideration is accordingly given to these relative factors as a preliminary to the main investigation.

## (III) Experimental Procedure.

### (*a*) *The Animals.*

The widespread prevalence of streptococcus mastitis does not permit of a reliable investigation into other factors which may be concerned in the secretion of abnormal milk being carried out on ordinary dairy herds, since it would be difficult to determine to what extent the results may be influenced by disease of the udder. Even negative results to the recognised bacteriological tests do not provide a definite assurance that the animals concerned are free from infection as it is well known that cows may harbour mastitis micro-organisms for considerable periods in the udder without secreting these in the milk. Freedom from the effects of known pathogenic bacteria can be assumed only if a complete history of the animals from the commencement of the first lactation is available, and frequent and regular tests for mastitis over a long period have been passed. This work conforms with these conditions.

The data forming the basis of this investigation were obtained in the course of regular examination of quarter samples of milk from a mastitis free herd. This herd originally consisted of 20 grade Friesland heifers of moderate type such as may constitute the average South African dairy herd. Six of the animals died of intercurrent disease while four others were transferred from the clean herd to a mastitis group to be utilised for studying certain aspects of that disease. The data used in this work were accordingly derived from the results obtained from the remaining ten cows. These were numbered as follows: 7904, 7905, 7909, 7910, 7912, 7913, 7914, 7919, 7921 and 7922. The heifers were all between two and three years of age and were pregnant when purchased in March and April, 1939.

Cows 7904 and 7909 only completed three lactations during the period covered by this investigation (April, 1939 to October, 1943). The right hind-quarter of 7905 revealed an infection with streptococcus agalactiae during the second half of the fourth lactation. She was, therefore, removed

from this herd, and the records for the second half of that lactation are thus not considered in this work. The remaining seven cows all completed four lactations.

### (*b*) *Herd Management.*

When purchased all the animals in the experiment were tested for tuberculosis and contagious abortion with negative results. They were then isolated in a paddock, of approximately 100 acres, on the laboratory farm Kaalplaats which adjoins Onderstepoort. They were kept in isolation there throughout the period under review, being only brought out of the paddock for milking and dipping. The milking shed—to which no other animals had access—and the dipping tank are situated about 100 yards from the entrance to the paddock. Stabling was never resorted to, the cows grazing and sleeping out in the paddock throughout the year and being only brought into the shed for feeding and milking in the morning and evening.

All animals in the herd were subjected to annual tests for tuberculosis and contagious abortion. No doubtful or positive reactions were ever obtained. Freedom from pathogenic bacteria was ensured by submitting quarter samples of milk to cultural examination on blood agar, and this was supplemented by sugar fermentation tests in suspected cases. In addition, microscopic examination of incubated milk was carried out at four-weekly intervals. The method used was described by van Rensburg (1941). Whenever the indirect tests raised any suspicion of infection additional cultural and microscopic examinations were applied.

Clinical examination of the udders in the manner described by van Rensburg (1937) was also carried out at four-weekly intervals or more frequently when conditions warranted this. While the clinical symptoms of non-specific mastitis were present in the few cases to be described later, none of the forty quarters at any time showed induration or abnormalities other than the usual injuries and superficial wounds which are inevitable in any dairy herd, and the effects of which on the composition of the milk were negligible.

### (*c*) *Feeding.*

In feeding as in selection of the animals an attempt was made to adhere as closely as possible to conditions obtaining on the ordinary South African dairy farm. No effort was made to increase the yield or the quality of the milk by high feeding. During summer they had access to an unlimited supply of green grass in the paddock and the only supplementary food given was the concentrates twice daily at milking time. The latter consisted of the following mixture:—

40 lb. crushed mealies;
20 lb. wheat bran;
20 lb. peanut meal;
6 lb. sterilised bonemeal;
2 lb. salt.

Ten pounds of this mixture were fed per head per day.

In winter very little grazing was available in the paddock, and the ration was accordingly supplemented with lucerne and teffhay, approximately 10 pounds being allowed per animal per day.

### (*d*) *Milking.*

The cows were milked at 6.30 a.m. and 3.30 p.m., and milking was done entirely by hand by two natives. Only four different milkers were employed during the whole period. The cows were, therefore, not subjected to the disturbances which invariably accompany frequent changes in the personnel handling them, and which may have an adverse effect on both the yield and composition of the milk. The four quarters of the udder were treated throughout as separate individual entities, and the milk from each quarter was drawn, weighed, tested and recorded separately. For this purpose a unit consisting of four one-gallon tins contained within a metal frame with a handle was found most convenient.

Fig. 1.—Milking outfit.

While a certain measure of cleanliness was observed in the milking shed no special hygienic precautions were taken to prevent the spread of any infection of the udder which might have been introduced into the herd at any time. There was thus no washing of the udder prior to milking, no washing of the milker's hands prior to milking each cow, and no antiseptics of any kind were used except when samples were drawn for bacteriological examination.

### (*e*) *Tests Performed.*

Determinations of the following factors, which will be considered in greater detail in another section, were made at regular intervals, namely:—

(1) Solids-not-fat.
(2) Fat.
(3) Chloride.
(4) Lactose.
(5) Chloride-lactose index.
(6) Cellular content.

Up to December, 1941, tests for fat and fat free solids were carried out at weekly intervals on the bulk milk of each quarter over a period of 48 hours, this involving the examination of two morning and two evening samples from each quarter every week. Owing to the difficulty experienced in maintaining a full supply of the necessary equipment under war conditions, the interval between the tests was extended from one to two weeks at the beginning of 1942. The average percentages for fat and solids-not-fat given in the appendix for the four-weekly periods were calculated from the results of the weekly and fortnightly tests. Therefore each percentage recorded for any of these two constituents represents the mean obtained from 16 analyses in the relative four weeks during the first half of the investigation and 8 in the second half.

For obvious reasons it was not possible to examine with the same frequency for chloride, lactose and cell content. Tests for these constituents were performed on afternoon samples once every four weeks.

It is obvious from the above that the tests for chloride, lactose and cell content were not always performed on the same samples of milk as the tests for fat and fat-free solids. This accounts for the apparent breakdown of the inter-relationship betwen the non-fatty solids and the other constituents which may be observed in a few of the records.

### (*f*) *Collection of Samples.*

It is well known that there is an appreciable difference in the composition of the foremilk, midmilk and strippings. Therefore, to eliminate as far as possible any errors which may be produced by the indiscriminate collection of samples for the various tests, the same routine was followed throughout in the collection of the samples.

The milk required for the determination of fat and solids-not-fat was taken from the milk of each quarter immediately after this had been weighed and recorded. The afternoon samples were kept in a cool room. The temperature of the morning and afternoon milk was brought to 60° F. before the tests were carried out.

The other constituents were determined on afternoon samples once every four weeks. For this purpose the teat orifice was first cleansed with a pledget of cottonwool soaked in alcohol, and 20 ml. foremilk was then withdrawn into a sterile bottle for cultural examination. Next another 20 ml. was milked also into a sterile bottle. A portion of this was used for preparing the smears for the cell counts, and the remainder was incubated for microscopic examination for mastitis streptococci. The quarters were then milked out completely and 100 ml. of this milk from each quarter was collected to be analysed for chloride and lactose.

### (*g*) *Analytical Methods.*

Fat was determined by the Gerber method. The solids-not-fat percentage was calculated by Richmond's formula from the fat percentage and the specific gravity of the milk, which was obtained at 60° F. by means of a Quevenne lactometer.

For the determination of chloride 20 ml. milk was used. Proteins were first precipitated by mixing this with an equal volume of 20 per cent. trichloracetic acid solution; 20 ml. of the milk filtrate was then used for determining the chloride by precipitating as AgCl and determining excess $AgNO_3$ against KCNS as described by Groenewald (1935).

Owing to the large number of determinations to be made for lactose (at four-weekly intervals over a long period), it was decided to modify the method so as to arrive at a figure sufficiently accurate to indicate a fluctuation from that which was indicative of the normal. The procedure adopted was to add 50 ml. of milk from each sample to 1·5 ml. mercuric nitrate solution in a flask; then to shake and filter. A 200 mm. polarimeter tube was then filled with the clear filtrate and read immediately. The final calculation of lactose was made according to the formula:—

$$M = \frac{r}{1 \cdot 05}$$ as described by Richmond (1920)

and revised in Standard Methods of Milk Analysis (1934) of the Association of Official Agricultural Chemists.

The chloride-lactose index was calculated from Koestler's (1922) formula:—

$$\text{CL I} = \frac{\text{Chloride per cent.} \times 100}{\text{Lactose per cent.}}$$

Cell counts were made according to the method devised by Prescott and Breed (1910). The smears were fixed in alcohol and stained with Gurr's Giemsa.

### (IV) Composition of Milk.

Davies (1936) supplies the following average percentage composition of cow's milk:—

| | |
|---|---|
| Water | 87·32 |
| Fat | 3·75 |
| Sugar | 4·75 |
| Casein | 3·00 |
| Other Proteins, albumin and globulin | 0·40 |
| Ash | 0·75 |

The Ash fraction is composed of: $K_2O$, 0·19; $P_2O_2$, 0·18; CaO, 0·15; Cl, 0·11; $Na_2O$, 0·07; $SO_2$, 0·03; Mg, 0·02; and FeO, 0·0017 per cent.

The following substances are also present in milk in variable amounts, namely:—

Enzymes: Catalase, diastase, peroxidase, lipase and phosphatase.

Vitamins: A, B, C, D, E and G.

Body Cells: Epithelial and blood cells.

N.P.N. substances such as thiocyanic acid, creatinine, ammonia and urea.

#### *Milk Solids.*

For practical purposes the milk solids are divided into two portions, namely fat and solids-not-fat, the latter consisting of the sugar, protein and ash, and a standard is prescribed for each of these two classes.

Since milk solids even in high class herds maintained under the best conditions of feeding and hygiene are subject to many variations any attempt to ascertain the average composition must embrace a maximum number of samples obtained and tested under all the different conditions

which normally affect the composition of milk. Davies (1936) has summarised the results obtained by various authorities in both Europe and America, and the average derived from all the available data for all breeds was 3·71 per cent. fat and 8·99 per cent. solids-not-fat.

Various correlations between the different solids have been worked out, though the results obtained by the different workers do not always correspond. Tocher (1926) found a linear correlation between fat and solids-not-fat, but in the data collected by Cranfield, Griffiths and Ling (1927) there appears to be an indication that samples abnormally low in solids-not-fat are associated with a fat percentage above the average. They pointed out that there is a fall in fat percentage with the decline in solids-not-fat to about the average (8·8 per cent.) but that below this point there appears to be a rise in fat as the non-fatty solids decline still further. Richmond (1920) is of opinion that any decrease of solids-not-fat below 9 per cent. is chiefly due to a deficiency of milk sugar. According to Turner (1936) proteins and ash increase but lactose declines slightly when fat increases and vice versa, while under drought conditions fat and protein are slightly above normal but lactose and vitamins A and G are deficient.

While in practice the determination of fat and solids-not-fat by the recognised method is sufficient to show whether the milk conforms to the prescribed standard or not, the information thus obtained is on its own not of very great assistance in any investigation which aims at determining the causal factors in the secretion of milk of inferior quality. The scope of this work was accordingly extended so as to include some of the lesser constituents of milk which are known to be very susceptible to various adverse influences, and would accordingly greatly assist in determining the different aetiological agents. The factors which were thus studied in addition to the fat and solids-not-fat are chloride, lactose, chloride-lactose index, and cell content of the milk from the individual quarters.

### *Chloride.*

The chlorides in milk consist mainly of sodium chloride and are derived directly from the blood, probably as the result of exudation through the alveolar epithelium of the udder. Rast recommended the tasting of milk for saltiness as a test for mastitis as early as 1854. Since then the determination of chloride in milk has been widely recommended for the detection of diseased quarters. Black and Voris (1934) obtained data from the analysis of 134 samples of milk during an entire lactation period, and found that of the inorganic constituents, sodium and then chloride showed the greatest variation. They concluded that sodium chloride was the most variable constituent in milk. Van Rensburg (1941) pointed out that the marked tendency of the chloride in milk to vary, frequently for no apparent reason, rendered this an unreliable test for mastitis.

The normal variations in the chloride of cows' milk have been studied by Caulfield and Riddell (1935) who found (i) that there was no significant difference in the chloride content of the milk of different breeds; (ii) that individuality had a marked influence, the milk from individual cows varying from 0·07 to 0·29 per cent. chloride; (iii) that chloride content is highest at the onset of lactation but declines rapidly in the first 10 to 20 days after which there is a general upward trend throughout the remainder of the lactation period; (iv) that variations from month to month were not marked, and (v) that daily variations were extremely small while that from the one milking to the next was insignificant.

Davies (1938) ascertained that: (i) Evening milk has lower chloride than morning milk; (ii) Fries milk is higher in chloride content than that of Shorthorns and Jerseys, though he admits that the Fries cows used in his experiment were giving poor quality milk; (iii) milk from successive lactations increases in chloride content; (iv) drought periods in summer which were accompanied by a decrease in solids-not-fat caused an increase in chloride and changing from winter feeding to spring grass lowers chloride and raises non-fatty solids; (v) chloride increases slowly when milk yield is relatively high. After this, that is in about the seventh month of lactation, it rises rapidly; (vi) milk of cows in good health was very regular in chloride content; (vii) although chloride rises at the end of lactation the total amount secreted decreases with advancing lactation owing to the reduction in total milk yield.

### *Lactose.*

Lactose constitutes the sugar of milk. According to Davies (1936) glucose may be present in very small amounts in normal milk but there is no evidence of an increased amount of it in milk of abnormal composition containing infiltrated blood constituents (globulin and sodium chloride). Lactose occurs in milk only, and is not found in blood or any other biological fluid. It is one of the three specific products of the mammary gland, being synthesized in the alveolar epithelium from its precursor, glucose, in the blood.

Lactose and chloride are two of the most important constituents to be considered in any investigation into the causes of the secretion of poor quality milk, since they are among the first to be affected by influences likely to produce any disturbance in the normal secretory activity of the mammary gland.

The osmotic pressure of the milk of the cow is the same as that of her blood plasma, namely 6·6 atmospheres, and in the case of the milk this pressure is almost entirely due to its lactose and other soluble salts, such as sodium chloride. The relationship between the concentration of lactose and sodium chloride in milk is, therefore, a reciprocal one, the lactose increasing when the salt decreases and vice versa. It is, therefore, obvious that the osmotic pressure of milk is maintained by an interchange between ionised chlorides and lactose. Davies (1936) has pointed out that from osmotic consideration one molecule of common salt (2 ions) is isotonic with two molecules of lactose, which weigh almost twelve times as much as a molecule of salt. When, therefore, circumstances arise which produce a decreased output of lactose in the alveoli of the udder the secretory mechanism of the gland is capable of effecting a compensatory increase in chloride whereby the osmotic pressure is maintained at a constant level.

### *Chloride-Lactose Index.*

The inverse relationship between chloride and lactose has induced many workers to attempt to correlate these constituents of milk in an endeavour to devise a method of detecting adulteration and other abnormalities in milk.

Mathieu and Ferre (1914) suggested that the lactose (grams per litre) plus the lactose equivalent of the chlorides ($NaCl \times 11 \cdot 9$) in grams per litre, does not fall below 70. They have termed this figure the " Constante Moleculaire Simplifice " (C.S.M.). Samples of milk with C.S.M. values below 70 are suspected of containing added water.

Sundberg (1931) arrived at the equation: Lactose = 7·07 – 18 chloride (per cent.), in which case 0·392 per cent. would be the chloride content of a lactose-free milk and 7·07 the lactose content of a hypothetical chloride-free milk.

From the calculation of a large number of samples Davies (1936) found that the formula: Lactose (per cent.) = 6·26 – 13·5 Cl (per cent.), is representative of the chloride-lactose relationship for the amounts generally found present in milk.

Koestler (1922) suggested the formula: Chloride-lactose index = $\frac{100 \times \text{chloride per cent.}}{\text{Lactose per cent.}}$ for detecting milk from cows with diseased udders or giving otherwise abnormal milk. Any sample giving an index value above 3 is regarded as abnormal.

According to Davies (1936) lactose is the factor mainly involved in low solids-not-fat. In view of this and the inverse relationship between lactose and chloride a determination of these two constituents is of considerable value in assessing the quality of the milk secreted by a quarter. After approximately 250 examinations which included tests for chloride, lactose and casein Vanlandigham *et al* (1941) concluded that for detecting chemical changes the determination of the casein number is no more desirable than the determination of either chloride or lactose, and that the determination of chloride and lactose with the calculation of the chloride-lactose index is to be preferred to either chloride or lactose alone.

### *The Cells in Milk.*

Though body cells cannot be regarded as constituents of normal milk, a variable number of such cells are always present. They consist of leucocytes derived from the blood circulating through the udder and epithelial cells resulting from desquamation of the epithelial lining of the alveoli and tubules.

The quality of the milk is to a large degree dependent on the normal functioning of the mucosa of the alveoli and tubules in which the milk is synthesized. The quantitative and qualitative changes in the cell picture may, therefore, furnish a very reliable indication as to the state of and the activity within the alveoli and tubules.

Many workers have tried to prescribe a limit above which any increase in the number of cells would characterise the milk as abnormal. While most have adopted 500,000 cells per ml. as the standard, many have pointed out that the milk which must be regarded as normal in all other respects frequently shows a much higher count. Thus Cooledge (1918), Copeland and Olsen (1926), Breed (1929) and Wayne and Macy (1933) obtained average cell counts of 930,000, 657,000, 868,000 and 1,250,000 per ml. normal milk respectively. In an examination of the foremilk of first-calf heifers entirely free from mastitis micro-organisms Little (1938) found that of 1,010 samples, 95 per cent. had less than 300,000 cells per ml. He points out, however, that, while such a low count may be considered normal for the foremilk of first-calf heifers early in their lactation, other examinations had shown that in older cows with normal udders the foremilk occasionally had a count of between 500,000 and 1,000,000 cells. Wilde (1938) studied the cell content of normal milk of 13 cows and found that the number of leucocytes varied from 32,000 to 1,280,000 and of epithelials from 10,000 to 40,000 per ml.

Eckl (1937) supplied the following differential count for the cells of normal milk:—

Lymphocytes ... ... ... ... ... ... ... ... Average 29·66 per cent.

Monocytes,— transitional forms and epithelials ... ... ... ... ... ... ... Average 12·62 per cent.

Neutrophiles ... ... ... ... ... ... ... ... Average 57·72 per cent.

He found that only 2 per cent, of normal quarters contained more than 80 per cent. neutrophiles. Samples from infected quarters showed a definite change. There was either an increase in neutrophiles or frequently also a simultaneous increase of both mononuclears and epithelials.

An abnormal increase in the number of leucocytes in milk usually indicates that the quarter concerned is mobilising its forces to oppose a threatened invasion by pathogenic bacteria or their products, whereas an increase in the number of epithelials is evidence that desquamation of the epithelial lining of the alveoli and tubules has been intensified, usually as the result of infection with pathogenic bacteria or on account of the presence of foreign and irritating substances in the alveoli and tubules. Such an increase in epithelials may, however, also be produced by physiological factors such as stage of lactation, abnormally long retention of milk and advancing age.

A study of the cytology of the milk was undertaken more for the purpose of determining whether an inflammatory state was present or not in the quarters, when milk, which was abnormal in other respects was secreted, rather than with the object of determining the degree of normality of the milk.

## (V) Milk Standards.

The Geneva Congress has defined milk as " the integral product of entire and uninterrupted milking of the female milk cow in good health and well nourished and not overworked. It ought to be collected in the proper manner and contain no colostrum ".

Milk, however, is not a commodity of uniform composition but is subject to a greater variety of environmental and genetic influences than any other biological fluid. Moreover, it is an article of food which lends itself better than any other to adulteration by the unscrupulous without the risk of detection by the consumer. It is, therefore, essential that legislative measures be instituted to protect the public not only against fraudulent practice on the part of dishonest persons engaged in the production and retailing of milk but also against the sale of milk that is of inferior quality for whatever reason. Most countries have accordingly framed regulations governing the sale of milk and prescribing standards to which milk offered to the public must conform. A comparison of these standards with the average composition of normal milk shows that in the promulgation of the regulations due cognisance was taken of and allowances made for the many variations in composition to which normal milk is subject.

According to Richmond (1899) the Society of Public Analysts in Britain adopted as the lower limits for fat and solids-not-fat 3 per cent. and 8·5 per cent. by weight respectively. These limits have been accepted as satisfactory by the great majority of analytical chemists of that country. Subsequently " presumptive legal standards " were prescribed by the Sale

of Milk Regulations of 1901 at 3 per cent. fat and 8·5 per cent. solids-not-fat. Milk below these standards is presumed to be not genuine milk until the contrary is proved. Adulteration is presumed in such samples which are regarded as either having a portion of the fat extracted or as containing added water.

After the members of the Association of Official Agricultural Chemists in the United States of America had devoted several years to the collection of data, the following definition of milk was decided on, and the standards mentioned were adopted as official for that country: " milk is the fresh clean lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within 15 days before and 10 days after calving, and containing no less than eight and one-half (8·5) per cent. of solids-not-fat and not less than three and one-quarter (3·25) per cent. of milk fat ".

In the Union of South Africa Government Notice No. 575 of 28th March, 1930, framed under the Foods, Drugs and Disinfectants Act, No. 13 of 1929, prescribed that ,, no person shall sell, as milk, milk to which any substance has been added or from which any part of any of its constituents has been removed, or which contains less than 3 parts per cent. of milk fat or less than 8·5 per cent. of milk solids-not-fat. Milk complying with the foregoing standard is referred to in these regulations as ' normal milk '. The foregoing standards do not apply to milk sold for manufacturing purposes on the basis of its milk-fat content or its total milk-solids content ".

In a restricted sense the term " abnormal " is confined to milk which is of inferior composition in virtue of the fact that it is derived from diseased quarters. It will, however, be observed from the above that no differentiation is made on a basis of the state of health or of disease of the udder, it being presumed that all milk offered for consumption is obtained from healthy udders. In this investigation the terms " normal " and " abnormal " are used in their true meaning to indicate that the milk conforms with the standards laid down for normal milk or that it fails to attain those standards irrespective of the cause of the failure. The terms " abnormal ", " poor " and " inferior " are thus regarded as being synonymous.

## (VI) Anatomy of the Udder and Physiology of Milk Secretion.

The bovine mammary gland consists of four functionally independent cutaneous glands. The two quarters on the right side are completely divided from the two on the left by the median suspensory ligament, but the two individual quarters on each side show no distinct line of demarcation between them. The division between the fore and hind quarters on the same side appears to be a very thin connective tissue septum which is irregular in outline and does not permit any mingling of the secretion of the two glands.

*Suspension of the Udder.*—The chief means of support is provided by the lateral and median suspensory ligaments and the skin. The lateral suspensory ligaments are fibrous bands which originate superiorly from the prepubic and subpubic ligaments. They pass ventrally over the lateral surface of the udder, widening anteriorly to envelop the gland. Ventrally the lateral ligaments fuse with the median suspensory ligament on each side thus forming a sling in which the udder is suspended.

The median suspensory ligament is formed by two bands of white fibrous and elastic connective tissue which originate from the abdominal tunic and are reflected ventrally from the linea alba between the two halves of the udder.

The lateral suspensory ligaments are chiefly fibrous and non-elastic while the median ligament contains many elastic fibres. Consequently filling of the udder stretches the median ligament and causes the teats to protrude obliquely outward and downward. The elastic median suspensory ligament thus serves to hold the udder in close proximity to the abdominal wall.

Numerous lamellae are given off from the inner surface of the lateral suspensory ligaments and penetrate the glandular tissue in a medio-ventral direction to become incorporated in the interstitial tissue. There is thus formed a network or cradle-like frame for the support of the glandular portion of the udder.

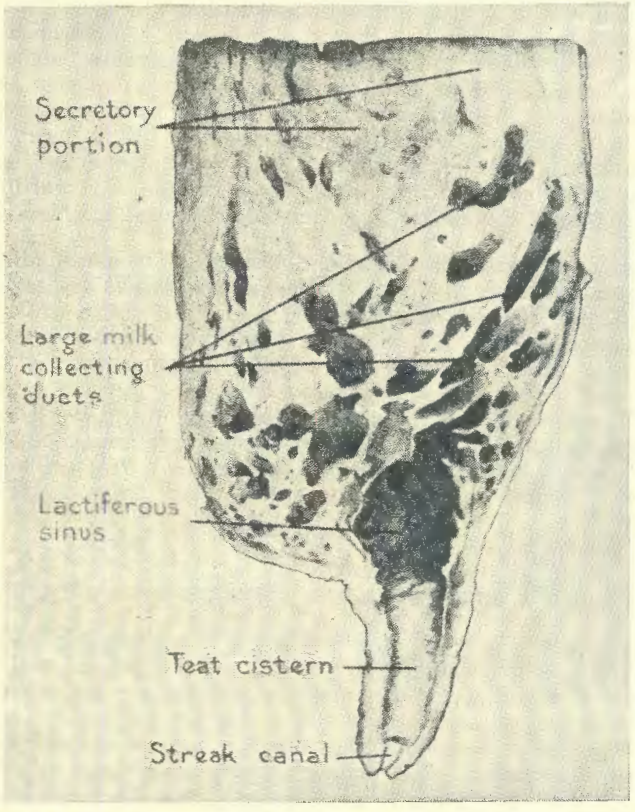



Fig. 2.—Section through quarter and teat.

*Collecting System.*—Commencing at the lower extremity one finds that the teat opening leads into a small duct (ductus papillaris, streak canal, " stricht kanaal ") $\frac{1}{4}$ to $\frac{3}{8}$ inches long surrounded by a sphincter which prevents leakage of milk from the udder. The streak canal opens above into the teat cistern or papillary sinus which again communicates superiorly with the larger storage cistern of the gland (receptaculum lactis, lactiferous

sinus). Opening into this large gland cistern are the large milk collecting ducts (lactiferous ducts) which vary in number, the average being about ten. These ducts are not of uniform calibre, but may change suddenly from very narrow ducts to large sinus-like enlargements.

*Secretory Apparatus.*—The large lactiferous ducts branch repeatedly, the branches getting smaller and smaller until finally the smallest branches or tubules end in dilatations (acini or alveoli). An alveolus consists of a single layer of epithelial cells attached to a basement membrane (membrana propria) and has a diameter of 0·1 to 0·3 mm. The ducts also have constrictions where they join the larger ducts or where they pass through the connective tissue septa which divide the secretory tissue of the gland into lobules and lobes. A lobule comprises a group of alveoli and a group of lobules again constitutes a lobe. The secretory portion may be likened to a bunch of grapes in which the grapes and stem are hollow. The grapes would represent the alveoli while the hollow stem of the individual grapes would represent the small tubules and the main stem one of the collecting ducts. The lobules are separated by a thin connective tissue septum containing the vascular network through the walls of which the glandular tissue derives the constituents necessary for the synthesis of milk.

The teat and gland cisterns and all the large milk-collecting ducts are lined with a two-layered epithelium, but the smaller ducts and alveoli have only a single layer On account of this similarity between the lining of the tubules and that of the alveoli and because the stroma surrounding the tubules contain less fibrous tissue and more blood vessels than the stroma supporting the larger ducts many workers now believe that the secretion of milk is not confined to the alveoli. but may also take place in the tubules.

*Blood and Nerve Supply.*—Blood is conveyed to the udder by the external pudic artery which originates from the prepubic. It passes through the inguinal canal and then immediately forms an S-shaped curve which allows a certain degree of extension with the filling of the udder. It is then continued as the mammary artery. After giving off a branch to the supramammary lympathic gland it divides into posterior and anterior branches which ramify in the posterior and anterior quarters respectively, dividing and sub-dividing and ultimately ending in arterioles and capillaries forming a network surrounding the alveoli.

Blood leaves the udder by three routes, namely, (*a*) through the inguinal canal via the external pudic vein which is a satellite of the external pudic artery and follows a similar course back into the abdominal cavity; (*b*) over the ischial arch via the perineal vein, and (*c*) along the abdominal wall via the subcutaneous abdominal vessels, commonly called the milk veins. They penetrate the abdominal wall at a point opposite the xiphoid cartilage and unite with the internal thoracic veins.

The nerve supply is partly spinal and partly sympathetic in origin. Cutaneous nerves are formed from the ventral branches of the first, second and third lumbar nerves and supply the skin and superficial tissues of the udder. The inguinal nerve also rises from the ventral roots of the same lumber nerves and in addition receives a branch from the spermatic nerve. It passes through the inguinal canal and supplies numerous branches to the glandular tissue, milk collecting system and teats.

The sympathetic nerves are formed from fibres derived from posterior mesenteric and sacral plexuses of the autonomic systems. These fibres join the nerve trunks of the inguinal nerves and innervate the udder to control all smooth muscle tissue.

*Synthesis of Milk.*—Two facts of fundamental importance in the formation and secretion of milk are (*a*) that all the constituents of milk must be brought to the udder in some form or other by the blood, and (*b*) that the synthesis of milk takes place only in the alveoli and, probably to a lesser extent in the tubules. Most of the constituents of milk such as water, albumin, globulin and the mineral salts occur in the blood and reach the alveolar epithelium by a process of diffusion out of the blood stream, through the capillary walls, into the liquid in the small spaces between the alveolar cells from where the required substances are taken up by the cells.

The three most important constituents of milk namely fat, lactose and casein are, however, specific products of the mammary gland and do not occur as such in the blood. They are synthesised in the alveolar epithelium from their precursors in the blood. It is now generally believed that the milk fat is derived by chemical transformation of fatty acids of the blood neutral fats, that the lactose is obtained from the circulating blood sugar, and that the source of casein might be the amino-acids and the inorganic phosphates of the blood.

The exact manner in which milk is formed in the cells is not known, and the most plausible explanation is probably that given by Blackwood and Stirling (1932), which states " the nature of the transudation from the blood stream to the mammary gland may be best explained by the hypothesis of selective absorption of milk precursors at the membranes separating the secretory cells from the blood supply. It is considered that during milk secretion a transudate is separated from the blood having the same osmotic pressure as the blood, and that during the synthesis water is returned to the blood to maintain tonicity of the cell fluid. At the end of synthesis the gland will, therefore, be filled with milk isotonic with blood ".

It is believed by many that the enzymes (lipase, amylase, etc.) are vitally concerned in the synthesis of milk, and Espe (1938) states that there seems to be little doubt that the enzymatic action plays an important part in the synthesis of the constituents of milk in the cytoplasm of the epithelial cells of the mammary gland, and that other protoplasmic activities may, and probably do, play an important rôle in the milk secretion.

The formation of milk in the cells produces a gradual rise in the intracellular pressure with the result that the cells, which are normally low and cuboidal, increase in length, and fat globules and other milk constituents collect in the free end of the cell facing the lumen of the alveolus ready to be excreted into the lumen. It was at first thought that this act takes place as the result of diffusion through the cell wall, but more recent evidence suggests that a rupture of the cell wall takes place and that this again reunites before the next cycle recommences. Hammond (1936) believes that some of these cells become non-functional as lactation advances.

*Intra-mammary Pressure.*—One of the most important factors influencing the composition of milk and the rate of its secretion is the milk pressure within the gland. It has been estimated that the intra-mammary pressure at the base of the teat, just before the commencement of milking varies from 25 to 30 mm. of mercury, though this may be higher in individual cows. This pressure may be increased by 25 per cent. or more when the cow " lets down " her milk. During the course of milking the pressure drops and when the udder is completely empty, the pressure is nil. In the interval between milkings the gradual accumulation of milk in the gland storage system is accompanied by a steady increase in the intra-mammary pressure, and the longer the interval the higher the pressure.

The rate of milk secretion in unit time is governed by the intra-mammary pressure which again is determined by the amount of milk present in the udder in relation to the capacity of the storage system. Ragsdale *et al* (1924) have estimated that if the amount of milk secreted in the first hour is 100, the amount secreted each succeeding hour is about 95 per cent. of that secreted the previous hour. If the milking interval is extended sufficiently long the secretion of milk will be completely inhibited. Petersen and Rigor (1932) found that a pressure of 25 mm. mercury resulted in almost complete cessation of secretion; 10 mm. pressure permitted a quarter normal secretion and 20 mm. only about one-sixth normal secretion.

The inhibitory effect of pressure upon rate of secretion is of great practical importance and offers a satisfactory explanation for the increase in milk production obtained by more frequent milking of high producers.

Besides retarding the rate of milk secretion, increasing intra-mammary pressure also produces a gradual decrease in the total solids of the milk secreted, and according to Petersen and Rigor (1932) this is mainly due to a decrease in fat and lactose. At the same time there is an increase in chloride in order to maintain the osmotic relationship between blood and milk.

Hammond (1936) states that the effect of milk pressure on composition of milk is produced in two ways, namely (*a*) by inhibition of the actual secretory process, and (*b*) by affecting the ease with which fat globules pass down the capillary ducts into the cistern, since the fat globules are held up by capillary attraction in the finer ducts to a greater extent than the more fluid constituents of milk. Turner (1935) again advances the theory that increasing pressure is responsible for a gradual change in the mode of milk secretion. Instead of discharging the products of secretion from the cell by rupture of the cell membrane the pressure in the lumen of the alveoli becomes sufficient to prevent rupture of the cells. The result is that only those constituents which can pass through the semi-permeable membrane are discharged, probably at a reduced rate, while the fat which is in suspension cannot leave the cell. It is obvious that the richer milk obtained by a more frequent milking must be due to the maintenance of a comparatively low pressure within the udder not allowing the latter to become overstocked.

"*Letting down*" *of milk.*—The "letting down" or "holding up" of milk has always been regarded as a wilful act on the part of the cow. Recent evidence, however, has shown that a cow cannot voluntarily hold up her milk, though she may fail to let it down. It is now known that the major portion of milk at the time of milking is in the alveoli and tubules from which it has to be expelled when it is "let down".

Ziegler (1941) and Swanson and Turner (1941) have described the presence of cells beneath the secretory epithelium of the alveoli and in the inter-lobular spaces, which have the appearance of smooth muscle fibres. The walls of the duct system also contains similar smooth muscle cells, but no tendency was observed for these cells to form muscle sphincters which would upon contraction, impede the flow of milk.

It is now generally accepted that the letting down of milk is due to a reflex, and according to Petersen (1942) stimuli from the cutaneous nerves of the udder and teat such as may be provoked by touching these parts act on the pituitary causing it to secrete a hormone, oxytocin, which produces the contraction of the smooth muscle fibres described above. The milk is thus forced out of the alveoli and tubules into the larger milk tubes and

cisterns. It takes about 40 seconds for the oxytocin to get to the udder after it has been secreted by the pituitary. This gland continues secreting oxytocin for about seven minutes after the stimulation. If, therefore, milking is not completed in that time some of the milk is retained in the alveoli and tubules. Hence the importance of speed in milking.

*Hormonal control of milk secretion.*—Apart from oxytocin several other hormones are concerned in the initiation and maintenance of milk secretion.

The oestrogenic hormones of the ovary promote mammary growth. In some species their action seems to be limited to the promotion of growth of the mammary duct system, the additional influence of progesterone elaborated by the adrenals being necessary for full development of the alveolar system. According to Folley (1940) the administration of oestrogen also increases fatty and non-fatty solids in milk.

The administration of thyroxine secreted by the thyroid produces a marked increase in quality and quantity of milk, but this is temporary and the milk generally returns to the normal level ten to fourteen days after injection.

The anterior lobe of the pituitary produces a lactogenic hormone (prolactin) which initiates functioning of the secretory cells and is the activating hormone of the udder at parturition. Extensive investigation in the bovine has shown that in this species considerable temporary increases in milk yield and in fat percentage can be obtained by injection of anterior pituitary extracts.

## (VII). The Secretion of Abnormal Milk: Literature.

The adoption of legal standards for milk is apparently the result of careful investigation by the authorities concerned in the different countries, and was presumably made after sufficient evidence had been obtained to ensure that the standards prescribed are well below the average composition of milk and that the prescribed minima can be obtained by dairy herds free from disease and maintained under proper conditions of hygiene, feeding and management.

Davies (1936) obtained an average of 3·82 per cent. fat and 8·91 per cent. solids not fat from the results given by 13 different investigators in England, Scotland, Germany and the United States. The number of samples examined by these individual workers varied from 127 to 330,000. The average obtained from such a large number of samples from cows in different countries and kept under varying conditions must be regarded as being very representative.

The generous gulf permitted between the average given by Davies and the standard prescribed should be quite sufficient to allow for variations which cause a depression in milk solids and which are produced by factors difficult to control, such as season and stage of lactation. Notwithstanding this big margin numerous complaints are made in practice to the effect that the minimal standard cannot be attained. It appears that either the effect of the factors known to depress the milk solids are more marked than was at first supposed or that there must be other influences at work.

That bovine mastitis is an important factor in the causation of a depression of milk solids is amply demonstrated by the results obtained by many investigators.

In 1936 the National Institute for Dairy Research in Britain began a comprehensive investigation into the problem of low quality milk particularly with reference to deficiencies in non-fatty solids. This revealed that individual herd samples showed wide variations in solids not fat, the extremes being 6·25 per cent. and 10·25 per cent. Although the yearly averages of all the samples for the first two years of the investigation were 8·74 and 8·78 per cent. respectively, it was found that in the first year 13·11 per cent. and in the second year 9·15 per cent. of the total number of herd samples were below the legal presumptive minimum of 8·5 per cent. solids not fat. After analysing a huge mass of data which revealed that 70 per cent. of the animals yielding under the 8·5 per cent. standard were suffering from chronic infectious mastitis it was concluded that the most important factor influencing the non-fatty solids content of milk is mastitis.

Foot and Shattock (1938) investigated the incidence of mastitis in cows giving low solids not fat in 29 herds comprising about 800 cows. They found that 19 per cent. of cows yielded milk below 8·5 solids not fat and that 6·15 per cent. of these were infected, though they point out that the total infection probabaly amounted to 70 per cent.

Rowland and Zein-el-Dine (1938) examining milk from 247 individual quarters of 62 cows found that the average solids not fat percentage of samples of fat free milk from uninfected quarters of the different breeds was Shorthorn 9·36, Friesian 9·11, Ayrshire 9·2 and Guernsey 9·82 while the samples from infected quarters averaged only 8·44, 8·38, 8·28 and 9·49 (only one sample) respectively. Of 121 infected samples 76 (63 per cent.) were below the minimum standard while only 10 (9 per cent.) of 114 uninfected samples were deficient in solids not fat. They concluded that milk samples with a physiological low solids not fat content are relatively rare and that in such cases the deficiency is not pronounced.

The need for research in South Africa was emphasised by Davel (1929) who pointed out that the chemical composition of our milk appears to be too low in fat and solids not fat, and that experimental work at Government institutions had revealed that numbers of cows, both purebreds and grades, gave milk which was unusually low in solids not fat. Hardy (1929) also declared that comparatively little research appears to have been carried out in connection with the problem of low solids not fat in South Africa, though recent investigations have tended to show that a good many cows produce milk with low solids not fat just as some do milk low in fat content.

In the same year (1930) in which the minimum standard was prescribed for South Africa a Maritzburg dairyman was prosecuted for selling milk which was below the standard. This was made a test case, in which Government officials showed that the deficiency indicated in this case was by no means singular and that even at a certain Government institution where the best conditions prevail, solids not fat falls below 8·5 per cent. in a large number of cases. As a result of this case the Union Department of Public Health instructed all Medical Officers of Health to suspend prosecutions of this nature for a period of five years to give dairymen an opportunity of replacing their deficient cows, unless it was proved that adulteration had taken place.

Smit (1929) analysed the records kept since 1926 of a purebred Fries and a purebred Jersey herd. His analysis showed that the individual milk of both breeds fell more often below the minimum for solids not fat than for fat. Schutte (1929) analysed the records of 73 Fries and 16 Shorthorn cows

entered for milking competitions during 1917 to 1926 and found that 27·40 per cent. Frieslands and 6·25 per cent. Shorthorns gave milk below 8·5 per cent. Schutte also investigated the solids not fat content of milk at three Government institutions and found that at the first the average solids not fat content was 0·1442 per cent. below the minimum legal standard; the second had an average of 8·5063 per cent., while at the third the statistical measures for the purebred Fries herd were constantly lower than those of the other two.

At the time the above investigations were made the prevalence of mastitis in South Africa and the significance of this disease in producing low solids not fat were not appreciated. In 1936 nine cows from one of the institutions studied by Schutte were sent to Onderstepoort on account of the poor quality of their milk. When examined at this station all nine were found to be infected with chronic streptococcus mastitis. Similarly the herd at the institution quoted in the Maritzburg case, when tested for mastitis a few years later, showed an incidence of nearly 100 per cent. In discussing the prevalence of mastitis in South Africa van Rensburg (1939) suggested that this disease is one of the principal causes of the deficiency in solids not fat in this country.

Notwithstanding the important rôle played by mastitis in the production of poor quality milk, there is increasing suspicion that, apart from known genetic and environmental factors and disease of the udder, there must be other influences that are also concerned in the production of abnormal milk. Mastitis investigations have perhaps done more than anything else to focus attention on the prevalence of abnormal secretions from udders which are free from known mastitis producing micro-organisms. This phenomenon has attracted the attention particularly of those workers in the field of mastitis research, who combine bacteriological methods of diagnosis with biochemical examination of the milk.

In discussing the chemical composition of milk low in solids not fat Davies (1937) pointed out that although udder disease, end of lactation, and hot dry summer weather tend to reduce this content, many apparently healthy cows under good management, produce milk with abnormally low solids not fat content throughout their lactation. This constituted a problem of obvious importance from the nutritional, commercial and legal aspects, and as its cause was not appreciated remedial measures were lacking.

Hastings and Beach (1937) studied the production of milk of abnormal composition in a herd of 31 cows free from *streptococcus agalactiae* during their first lactation. They analysed 3,000 samples of milk from separate quarters for chloride, catalase and pH. One or more samples which satisfied the criteria of abnormality were obtained from 23 of the 31 cows. Detailed study of the record indicated that 17 of the animals may be considered as normal and 14 as abnormal. They were at a loss to explain the persistent secretion of abnormal milk by certain cows, and considered at first that a low grade mastitis was responsible. As a result of further study, however, they inclined to the view that the cause was inherent in the animal.

In a discussion on the secretion of abnormal milk by udders free from streptococci Hastings and Peterson (1940) pointed out that no other micro-organisms were found with sufficient consistency and in sufficient numbers to be apparently responsible for the changes noted in the milk. They concluded that the causes of abnormal milk or of deterioration of the udder may be more complex than is now supposed, and they indicated the need of a more detailed and more prolonged study of the subject.

Van Rensburg (1941), in evaluating several tests for mastitis, stated that the secretion of abnormal milk by quarters, which have never been infected with mastitis streptococci or other known pathogenic organisms and which show no induration of the udder tissue, is by no means a rare occurrence. He pointed out that this tendency contributed in a large measure to the unreliability of indirect tests for mastitis.

## (VIII). General Results.

The following criteria were used for determining abnormality in milk:—

Solids not fat: under 8·50 per cent.

Fat: under 3·00 per cent.

Chloride: 0·150 per cent. and over.

Lactose: under 4·50 per cent.

Chloride-lactose index: over 3.

Cells: over 1,000,000 per ml.

The standards used for solids not fat and fat are those usually prescribed as the minimum permitted by the regulations which govern the sale of milk, No legal limits are imposed for chloride, lactose, chloride-lactose index and cells. The criteria adopted for these factors are those which are mostly used by mastitis research workers to determine whether milk is normal or not. Milk which fails to conform with these limits is generally regarded as having been obtained from udders affected with mastitis. The limit for cells is higher than that which is generally recommended but it should be pointed out that the maximum of 500,000 adopted by many workers usually applies only to leucocytes whereas in this work epithelial cells were included in the count.

The mean for each of the six factors studied is shown in Table 1. This was calculated from the results of all the analyses carried out on the milk from the 40 individual quarters of the ten cows over a period of four and a half years. In addition to the mean the table also shows the range, standard deviation and coefficient of variation for the different constituents.

Table 1.

*Mean, range, standard deviation and coefficient of variation of various factors for the whole period.*

| Factor. | Mean. | Range. | S.D. | C.V.%. |
|---|---|---|---|---|
| Solids, not fat, per cent. | 8·54 | 7·44 –10·12 | 0·2985 | 3·49 |
| Fat, per cent. | 3·99 | 1·90– 6·29 | 0·3598 | 9·01 |
| Chloride, per cent. | 0·135 | 0·025– 0·243 | 0·0162 | 12·00 |
| Lactose, per cent. | 4·69 | 2·66 – 5·90 | 0·2303 | 4·91 |
| Chloride-lactose index. | 2·91 | 0·86–8·12 | 0·5188 | 17·82 |
| Cells (thousands per ml.). | 9567 | 3–20473 | 840·8994 | 8·78 |

The most obvious conclusion that can be drawn from a consideration of the data presented in the above table is that despite the wide range shown by the results of individual tests, the average for the different factors over the whole period was well within the limits prescribed for normality in

every case. Therefore any deficiencies that were revealed by some quarters throughout the period or by all quarters at certain times or under certain conditions were more than counterbalanced by the higher tests shown by the other quarters or under more favourable environmental conditions.

This fact is of practical importance in that it suggests that, once the aetiological factors concerned in the production of abnormal milk are known, it might be possible for the dairy husbandryman to organise his business in such a manner that the bulk milk will always conform with the required standard. The methods whereby this can be achieved will be referred to later in this work.

Notwithstanding the degree of normality shown by the means of the various factors studied, the wide range displayed indicates that some if not all of these healthy quarters secreted abnormal milk either consistently or intermittently. This is confirmed by the data in Table 2 in which is summarised the total number of tests performed with the number of normal and abnormal reactions and the percentage of results that were abnormal. The latter is further illustrated by the block graph (Fig. 3).

TABLE 2.

*Total number of examinations made; number normal, and number and percentage abnormal.*

| Constituents. | Total number of observations made. | Number of times milk was | | Percentage of abnormal reactions. |
|---|---|---|---|---|
| | | Normal. | Abnormal. | |
| Solids, not fat | 1476 | 866 | 610 | 41·3 |
| Fat | 1476 | 1431 | 45 | 3·0 |
| Chloride | 1388 | 953 | 435 | 31·3 |
| Lactose | 1256 | 903 | 353 | 28·1 |
| Chloride-lactose index | 1255 | 761 | 496 | 39·5 |
| Cells | 1168 | 837 | 331 | 28·3 |

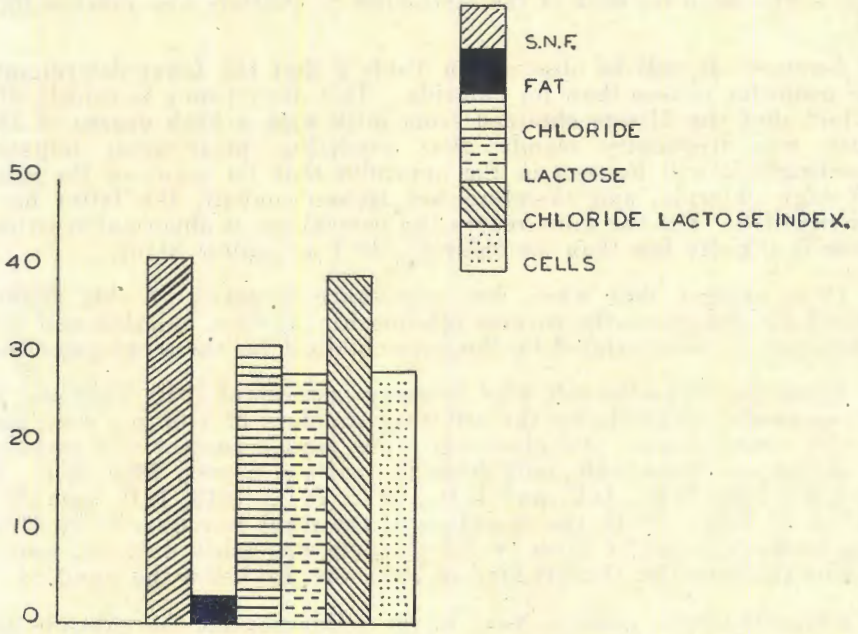
*Solids not fat.*—The largest number of deficiencies was recorded by the analyses for solids not fat, 41·3 per cent. of the total number of examinations yielding percentages falling below the legal limit of 8·50 per cent. Table 3 giving the results of the various tests conducted on the milk from each quarter shows that not one of the 40 quarters succeeded in continuously secreting milk which satisfies the criteria for non-fatty solids. The best performance in this respect was put up by the four quarters of cow 7914, each of which failed only once to reach the minimum in a four-weekly period. There was thus only 2·6 per cent. abnormal reactions recorded against each of her quarters. Besides these only one other quarter, the left hind of 7919, yielded less than 10 per cent. abnormal results to the test for solids not fat.

On the other hand one quarter (7905 RH) consistently yielded milk which was deficient in solids not fat. Throughout the whole investigation this quarter, in not a single instance, succeeded in giving an average of 8·50 per cent. or over for any four-weekly period. The remaining three quarters of this cow as well as all four of 7909 all revealed a deficiency in non-fatty

solids in 90 per cent. or more of the tests. In addition to the above the milk from the four quarters of 7921 was also deficient in solids not fat in over 50 per cent. of the tests. The remaining 28 quarters all yielded milk of normal solids not fat content in over 50 per cent. of tests.

A study of the mean solids not fat percentage of the milk from every quarter over the whole period in Table 4 reveals that the twelve quarters of the three cows, which in the majority of tests secreted milk, which was deficient in solids not fat also all failed to obtain an average of 8·50 per cent. or over for the whole period. These are in fact the only quarters which failed to reach a mean equivalent to or above the legal minimum. The means for the other 28 quarters were all above 8·50 per cent.

FIG 3. PERCENTAGE OF SAMPLES THAT WERE ABNORMAL




*Fat.*—Comparison of the data presented for fat with that of the fat-free solids reveals a far more satisfactory position as far as this constituent of milk is concerned. The mean fat percentage for all the quarters was 3·99 which is far above the legal limit of 3·00 per cent. Out of 1,476 determinations made only 45 (3 per cent.) were below the minimum standard, and 18 out of the 40 quarters consistently gave milk with a fat content above the limit.

The left forequarter of 7921 secreted milk deficient in fat in 22·5 per cent. of tests, and this is the only quarter in which more than 10 per cent. of the tests showed abnormally low fat content.

The mean fat content of every one of the 40 quarters was well above the 3 per cent. standard, the lowest (3·39 per cent.) being shown by the right fore quarter of 7909.

*Chloride.*—The analyses for chloride showed that 435 out of 1,388 (31·3 per cent.) samples of milk from individual quarters had an abnormally high chloride content. Every one of the 40 quarters at various times secreted milk with an abnormal chloride percentage (Table 3). Only two quarters (7904 L.H. and 7910 L.F.) yielded less than 10 per cent. abnormal results to the test for chloride. In 11 quarters (all of 7905 and 7909, 7912 R.F., 7919 L.F., and 7921 L.F.) the majority of the determinations made revealed an abnormally high chloride content of the milk, although the percentage of abnormal reactions to chloride was not as high as that for solids not fat. The highest percentage of abnormal reactions to chloride was 70·6 given by the right fore quarter of 7905.

The mean chloride content of all the quarters was 0·135 per cent. which is well within the limits for normality. Ten of the 11 quarters mentioned above as giving a majority of abnormal results also had a mean chloride content of 0·150 per cent. or over, the right fore quarter of 7912 being the only one of that group to obtain a mean (0·143 per cent.) within the normal range. The mean for each of the remaining 29 quarters was under 0·150 per cent.

*Lactose.*—It will be observed in Table 2 that 132 fewer determinations were made for lactose than for chloride. This discrepancy is mainly due to the fact that the filtrate obtained from milk with a high degree of abnormality was frequently cloudy thus rendering polarisation impossible. Accordingly it will be seen in the appendix that for many of the samples with high chloride, and therefore low lactose content, the latter has not been recorded. For the same reason the percentage of abnormal reactions to lactose is slightly less than for chloride (28·1 as against 31·3).

It is evident that when due cognisance is taken of this slight but unavoidable difference the inverse relationship between chloride and lactose is very clearly demonstrated by the data obtained for these two constituents.

Three quarters—the left hind in every instance of 7910, 7914 and 7922, were successful in obtaining the arbitrary standard of 4·50 per cent. lactose in every examination. An abnormally low lactose content was revealed in the majority of tests in the milk from 12 quarters, namely 7905, R.F., R.H. and L.F., 7909 R.F., L.F. and L.H., 7912, R.F., 7913, L.H. and all four quarters of 7921. With the exception of the right fore quarter of 7921 the mean lactose content of these twelve quarters was below 4·50 per cent. In addition the mean for the left hind of 7905 was also below the standard.

*Chloride-lactose index.*—Next to the solids not fat the chloride-lactose index detected the largest number of abnormal samples, namely 39·4 per cent. This index offers a better criterion for detecting abnormal milk than either chloride or lactose determination alone since it exposes all those borderline cases which, though high in chloride and low in lactose, just manage to fall within the limits prescribed for normality for these two constituents.

As in the case of fat-free solids and chloride none of the forty quarters succeeded in passing all the tests for chloride-lactose index. In only two quarters (7904 L.H. and 7914 L.H.) was the percentage of abnormal results under 10. Sixteen quarters, namely all of 7905, 7909 and 7921, and 7912 R.H., 7913 L.H., 7919 L.F. and 7922 L.F. gave abnormal results in the majority of determinations made for the chloride-lactose index, and the means for the same sixteen also exceeded the normal limit of 3.

## TABLE 3

*Total number of observations made on*

| Cow and Quarter. | Solids not Fat. | | | | Fat. | | | |
|---|---|---|---|---|---|---|---|---|
| | Total. | Normal. | Abnormal. | | Total. | Normal. | Abnormal. | |
| | | | No. | Per cent. | | | No. | Per cent. |
| 7904..... RF | 30 | 18 | 12 | 40 | 30 | 28 | 2 | 6·7 |
| RH | 30 | 21 | 9 | 30 | 30 | 29 | 1 | 3·3 |
| LF | 30 | 16 | 14 | 46·7 | 30 | 30 | 0 | 0 |
| LH | 30 | 19 | 11 | 36·7 | 30 | 29 | 1 | 3·3 |
| 7905..... RF | 36 | 1 | 35 | 97·2 | 36 | 34 | 2 | 5·6 |
| RH | 36 | 0 | 36 | 100 | 36 | 33 | 3 | 8·15 |
| LF | 36 | 2 | 34 | 94·4 | 36 | 35 | 1 | 2·8 |
| LH | 36 | 2 | 34 | 94·4 | 36 | 34 | 2 | 5·6 |
| 7909..... RF | 30 | 1 | 29 | 96·7 | 30 | 29 | 1 | 3·3 |
| RH | 30 | 1 | 29 | 96·7 | 30 | 30 | 0 | 0 |
| LF | 30 | 3 | 27 | 90 | 30 | 28 | 2 | 6·7 |
| LH | 30 | 1 | 29 | 96·7 | 30 | 28 | 2 | 6·7 |
| 7910..... RF | 40 | 28 | 12 | 30 | 40 | 40 | 0 | 0 |
| RH | 40 | 30 | 10 | 25 | 40 | 39 | 1 | 2·5 |
| LF | 40 | 32 | 8 | 20 | 40 | 39 | 1 | 2·5 |
| LH | 40 | 29 | 11 | 27·5 | 40 | 40 | 0 | 0 |
| 7912..... RF | 40 | 25 | 15 | 37·5 | 40 | 40 | 0 | 0 |
| RH | 40 | 30 | 10 | 25·0 | 40 | 39 | 1 | 2·5 |
| LF | 40 | 23 | 17 | 42·5 | 40 | 39 | 1 | 2·5 |
| LH | 40 | 27 | 13 | 32·5 | 40 | 39 | 1 | 2·5 |
| 7913..... RF | 40 | 32 | 8 | 20 | 40 | 40 | 0 | 0 |
| RH | 40 | 36 | 4 | 10 | 40 | 39 | 1 | 2·5 |
| LF | 40 | 29 | 11 | 27·5 | 40 | 40 | 0 | 0 |
| LH | 40 | 30 | 10 | 25 | 40 | 40 | 0 | 0 |
| 7914..... RF | 38 | 37 | 1 | 2·6 | 38 | 38 | 0 | 0 |
| RH | 38 | 37 | 1 | 2·6 | 38 | 38 | 0 | 0 |
| LF | 38 | 37 | 1 | 2·6 | 38 | 37 | 1 | 2·6 |
| LH | 38 | 37 | 1 | 2·6 | 38 | 38 | 0 | 0 |
| 7919..... RF | 35 | 26 | 9 | 25·7 | 35 | 35 | 0 | 0 |
| RH | 35 | 28 | 7 | 20·0 | 35 | 35 | 0 | 0 |
| LF | 35 | 20 | 15 | 42·9 | 35 | 35 | 0 | 0 |
| LH | 35 | 32 | 3 | 8·6 | 35 | 35 | 0 | 0 |
| 7921..... RF | 40 | 15 | 25 | 62·5 | 40 | 37 | 3 | 7·5 |
| RH | 40 | 13 | 27 | 67·5 | 40 | 37 | 3 | 7·5 |
| LF | 40 | 9 | 31 | 77·5 | 40 | 31 | 9 | 22·5 |
| LH | 40 | 14 | 26 | 65·0 | 40 | 36 | 4 | 10·0 |
| 7922..... RF | 40 | 30 | 10 | 25·0 | 40 | 40 | 0 | 0 |
| RH | 40 | 35 | 5 | 12·5 | 40 | 40 | 0 | 0 |
| LF | 40 | 24 | 16 | 40·0 | 40 | 40 | 0 | 0 |
| LH | 40 | 36 | 4 | 10·0 | 40 | 38 | 2 | 5·0 |

TABLE 3. *(cont.)*

*servations made on individual quarters; number normal; number and percentage abnormal*

| Chloride. | | | | Lactose. | | | |
|---|---|---|---|---|---|---|---|
| Total. | Normal. | Abnormal. | | Total. | Normal. | Abnormal. | |
| | | No. | Per cent. | | | No. | Per cent. |
| 28 | 23 | 5 | 17·9 | 27 | 23 | 4 | 14·8 |
| 29 | 26 | 3 | 10·3 | 28 | 26 | 2 | 7·1 |
| 29 | 24 | 5 | 17·2 | 28 | 24 | 4 | 14·2 |
| 29 | 28 | 1 | 3·4 | 28 | 26 | 2 | 7·1 |
| 34 | 10 | 24 | 70·6 | 32 | 12 | 20 | 62·5 |
| 34 | 12 | 22 | 64·7 | 32 | 15 | 17 | 53·1 |
| 35 | 12 | 23 | 65·7 | 33 | 12 | 21 | 63·6 |
| 34 | 13 | 21 | 61·8 | 31 | 16 | 15 | 48·4 |
| 30 | 9 | 21 | 70 | 25 | 8 | 17 | 68·0 |
| 30 | 14 | 16 | 53·3 | 27 | 15 | 12 | 44·4 |
| 30 | 9 | 21 | 70 | 25 | 11 | 14 | 56·0 |
| 29 | 12 | 17 | 58·6 | 26 | 11 | 15 | 57·7 |
| 38 | 30 | 8 | 21·0 | 37 | 30 | 7 | 18 9 |
| 38 | 34 | 4 | 10·5 | 37 | 33 | 4 | 10·8 |
| 37 | 34 | 3 | 8·1 | 37 | 31 | 6 | 16·2 |
| 38 | 34 | 4 | 10·5 | 36 | 36 | 0 | 0 |
| 38 | 18 | 20 | 52·6 | 35 | 15 | 20 | 57·1 |
| 38 | 33 | 5 | 13·2 | 37 | 30 | 7 | 18·9 |
| 38 | 31 | 7 | 18·4 | 36 | 29 | 7 | 16·7 |
| 38 | 34 | 4 | 10·5 | 36 | 32 | 4 | 11·1 |
| 38 | 30 | 8 | 21·0 | 36 | 27 | 9 | 25·0 |
| 38 | 31 | 7 | 18·4 | 36 | 31 | 5 | 13·9 |
| 37 | 31 | 6 | 16·2 | 34 | 29 | 5 | 14·7 |
| 38 | 21 | 17 | 44·7 | 32 | 16 | 16 | 50 |
| 36 | 25 | 11 | 30·6 | 30 | 24 | 6 | 20·0 |
| 36 | 31 | 5 | 16·1 | 32 | 29 | 3 | 9·4 |
| 36 | 30 | 6 | 20·0 | 31 | 28 | 3 | 9·7 |
| 35 | 31 | 4 | 12·9 | 31 | 31 | 0 | 0 |
| 30 | 21 | 9 | 30·0 | 26 | 23 | 3 | 11·5 |
| 30 | 20 | 10 | 33·3 | 27 | 25 | 2 | 7·4 |
| 30 | 14 | 16 | 53·3 | 24 | 15 | 9 | 37·5 |
| 30 | 27 | 3 | 10·0 | 26 | 25 | 1 | 3·8 |
| 37 | 23 | 14 | 37·8 | 33 | 16 | 17 | 51·5 |
| 37 | 22 | 15 | 40·5 | 32 | 15 | 17 | 53·1 |
| 37 | 16 | 21 | 56·8 | 31 | 5 | 26 | 83·9 |
| 37 | 28 | 9 | 24·3 | 32 | 16 | 16 | 50·0 |
| 38 | 28 | 10 | 26·3 | 34 | 31 | 3 | 8·8 |
| 38 | 30 | 8 | 21·1 | 32 | 32 | 0 | 0 |
| 38 | 23 | 15 | 39·2 | 31 | 18 | 13 | 42·6 |
| 38 | 31 | 7 | 18·4 | 33 | 32 | 1 | 3·0 |

115-116a

115–116 b

115-116c

TABLE 3 *(cont.)*

*percentage abnormal.*

| Chlor./Lact. Index. | | | | Cells. | | | |
|---|---|---|---|---|---|---|---|
| Total. | Normal. | Abnormal. | | Total. | Normal. | Abnormal. | |
| | | No. | Per cent. | | | No. | Per cent. |
| 27 | 19 | 8 | 29·6 | 28 | 24 | 4 | 14·3 |
| 28 | 25 | 3 | 10·7 | 28 | 25 | 3 | 10·7 |
| 28 | 22 | 6 | 21·4 | 27 | 24 | 3 | 11·1 |
| 28 | 26 | 2 | 7·1 | 27 | 24 | 3 | 11·1 |
| 31 | 5 | 26 | 83·9 | 34 | 32 | 2 | 5·9 |
| 32 | 11 | 21 | 65·6 | 32 | 31 | 1 | 3·1 |
| 33 | 5 | 28 | 84·8 | 33 | 29 | 4 | 12·1 |
| 31 | 9 | 22 | 71·0 | 33 | 33 | 0 | 0 |
| 25 | 6 | 19 | 76·0 | 29 | 11 | 18 | 62·1 |
| 27 | 7 | 20 | 74·1 | 28 | 26 | 2 | 7·1 |
| 27 | 5 | 22 | 81·5 | 27 | 19 | 8 | 29·6 |
| 26 | 5 | 21 | 80·8 | 27 | 13 | 14 | 51·9 |
| 37 | 25 | 12 | 32·4 | 34 | 31 | 3 | 8.8 |
| 37 | 31 | 6 | 16·2 | 34 | 26 | 8 | 23·7 |
| 36 | 30 | 6 | 16·7 | 33 | 30 | 3 | 9·1 |
| 36 | 31 | 5 | 13·9 | 33 | 26 | 7 | 21·2 |
| 35 | 15 | 20 | 57·1 | 30 | 7 | 23 | 76·7 |
| 37 | 29 | 8 | 21·6 | 29 | 17 | 12 | 41·4 |
| 36 | 28 | 8 | 22·2 | 31 | 17 | 14 | 45·2 |
| 36 | 28 | 8 | 22·2 | 29 | 21 | 8 | 27·6 |
| 36 | 22 | 14 | 38·9 | 31 | 24 | 7 | 22·6 |
| 36 | 29 | 7 | 16·7 | 31 | 22 | 9 | 29·0 |
| 34 | 27 | 7 | 20·6 | 31 | 24 | 7 | 22·6 |
| 32 | 15 | 17 | 53·1 | 31 | 17 | 14 | 45·2 |
| 30 | 22 | 8 | 26·7 | 30 | 21 | 9 | 30·0 |
| 32 | 27 | 5 | 15·6 | 29 | 21 | 8 | 27·6 |
| 31 | 25 | 6 | 19·4 | 27 | 20 | 7 | 25·9 |
| 31 | 30 | 1 | 3·2 | 27 | 21 | 6 | 22·2 |
| 26 | 17 | 9 | 34·6 | 23 | 22 | 1 | 4·3 |
| 27 | 18 | 9 | 33·3 | 25 | 23 | 2 | 8·0 |
| 24 | 10 | 14 | 58·3 | 23 | 20 | 3 | 13·0 |
| 26 | 22 | 4 | 15·4 | 25 | 24 | 1 | 4·0 |
| 33 | 13 | 20 | 60·6 | 29 | 12 | 17 | 58·6 |
| 32 | 12 | 20 | 62·5 | 29 | 5 | 24 | 82·8 |
| 31 | 9 | 22 | 71·0 | 29 | 7 | 22 | 75·9 |
| 32 | 14 | 18 | 56·3 | 29 | 10 | 19 | 65·5 |
| 34 | 25 | 9 | 26·5 | 28 | 15 | 13 | 46·4 |
| 32 | 24 | 8 | 25·0 | 28 | 21 | 7 | 25·0 |
| 31 | 10 | 21 | 67·7 | 29 | 17 | 12 | 41·4 |
| 33 | 27 | 6 | 27·1 | 29 | 26 | 3 | 10·4 |

← 115-116b

As indicated in the range (Table 1) this index is subject to great variation, the lowest figure obtained being 0·86 and the highest 8·12. The standard deviation was 0·5188 and the coefficient of variation 17·82.

*Cells.*—Abnormally high cell counts were found in 28·3 per cent. of the samples examined. The majority of samples derived from seven quarters, namely 7909 R.F. and L.H., 7912 R.F. and all four quarters of 7921, showed counts of over 1,000,000 cells per ml.

A total of 12 quarters consisting of the 7 mentioned above in addition to 7912 L.F. and L.H., 7913 R.H. and L.H. and 7922 R.F. showed an average cell content in the milk in excess of the prescribed limit. The left hind quarter of 7905 was the only one of the 40 which never had a count above the standard. The most noteworthy feature about the cell content is the fact that the four quarters of 7905 which revealed the largest number of abnormalities in all other respects yielded lower average cell counts than the four quarters of any other cow. The secretion of poor quality milk is, therefore, not always associated with a high cellular content nor is the best quality milk necessarily that which contains the smallest number of cells. The significance of this will be discussed later.

The cells showed far greater variation than any other constituent of milk. This is obvious from the range which fluctuated from 3,000 to 20,473,000 and the standard deviation which was 840·8994. The average cell content is, therefore, not a reliable index of the state of activity in the udder, though this does not imply that the cellular content of the individual samples may not be of considerable assistance in determining whether the quarter concerned was in a state of normal functional activity or not when the sample was taken.

The data furnished in Tables 3 and 4 clearly establish the fact that the milk derived from some quarters conforms so closely with the accepted standard for the different constituents that the quarters concerned must be regarded as normal in all respects. When they did secrete an inferior quality milk it was intermittent and due to environmental factors which were generally of a transient nature. In other quarters again the means fell so far short of the prescribed standard and the percentage of abnormal reactions to the tests employed was so high that a classification of these quarters as abnormal was justified. In a third or intermediate group the deviations from the normal were slight and were revealed in only one or two of the constituents. The few quarters concerned in this were accordingly classified as doubtful.

Another important factor brought to light by the data is that any quarter which failed to obtain an average equal to the standard prescribed for normality in any one of the constituents studied, also, with very rare exceptions yielded abnormal results in the majority of tests applied for the constituent concerned. This is well illustrated in Table 5 in which a classification into "normal" and "abnormal" was made on the following basis:—

(*a*) *Composition.*—When the mean for the relative constituent conformed with the prescribed standard this was designated by N while ABN indicated that the mean was below the limit.

(*b*) *Tests.*—When over 50 per cent. of the total number of examinations made for any constituent proved the milk to be of normal composition this was similarly shown by N in the relative column and ABN was applied to those in which the majority of tests revealed abnormality of the milk.

From the table it is evident that 20 of the 40 quarters, namely all four quarters of 7904, 7910 and 7914 in addition to 7912 R.H., 7913 R.F. and L.F., 7919 R.F., R.H., L.H., and 7922 R.H. and L.H. satisfied all criteria for normality. Four other quarters, namely 7912 L.F. and L.H., 7913 R.H. and 7922 R.F. failed only in that the cell content of each of them was above the prescribed limit. There was no other abnormality and these quarters are, therefore, also classed as normal.

In the twelve quarters of cows 7905, 7909 and 7921 the deficiencies were so marked in all respects except fat and in the case of 7905 the cells, that there was no hesitation in classing these quarters as abnormal.

The intermediate or doubtful group was provided by 4 quarters, namely 7912 R.F., 7913 L.H., 7919 L.F. and 7922 L.F. Although the means for the fat and fat-free solids of these four quarters were above the legal standards, they could not be regarded as normal in view of the deviations shown by their milk in chloride, lactose, chloride-lactose index and cells. These are the only four cows in which one of the quarters showed significant differences in the composition of the milk as compared with the other three quarters of the same udder, and this aspect will receive fuller consideration in the relative section later in this work.

*Discussion.*

Notwithstanding the fact that the means for all the factors investigated conformed with the standards prescribed for normal milk, a very large percentage of the samples examined were found to be of abnormal composition. The highest percentage (41·3) of abnormal samples were detected by the tests for solids not fat.

Although three of the ten cows persistently yielded poor quality milk from all their quarters, they could not be held solely responsible for all the abnormalities that were noted. Analysis of the data revealed that every one of the 40 quarters at various times produced milk which was below standard. This suggests that any quarter which is free from disease and is judged to be normal by a number of different standards may under certain conditions secrete abnormal milk. The scope of this work has, therefore, been extended to include a study of the principal factors which may be concerned in the production of inferior quality milk.

The record of this herd, therefore, furnishes additional evidence of the tendency of quarters which are free from known pathogenic bacteria to secrete abnormal milk, and confirms the views previously expressed, namely that the secretion of milk which is of abnormal chemical composition cannot be considered as proof that disease of the udder is present. It adduces striking confirmation of the doubts which exist in the minds of many investigators as to the unreliability of indirect tests, based on the biochemical examination of milk, to detect the presence or absence of any form of mastitis.

These tests fail not only in that they are frequently unable to detect disease in the incipient or latent stages but, what is of greater practical and economic importance, is that they are also liable to condemn as diseased quarters which are entirely free from infection and pathological changes. The determination of chloride in milk has frequently been advocated as one of the most dependable indirect tests for mastitis. According, however, to the results given in Table 3 the application of this test as a routine measure

## TABLE 4.

*Means and range of the various factors for each individual quarter.*

| Cow and Quarter. | Solids not Fat. | | Fat. | | Chloride. | | Lactose. | | Chloride-Lactose Index. | | Cells. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean Percentage. | Range. | Mean Percentage. | Range. | Mean Percentage. | Range. | Mean Percentage. | Range. | Mean. | Range. | Mean. | Range. |
| 7904..... RF | 8·54 | 7·99– 8·92 | 3·77 | 2·46–4·57 | 0·123 | ·071–·187 | 4·76 | 4·10–5·24 | 2·63 | 1·53–4·47 | 608 | 9–3006 |
| RH | 8·58 | 8·30– 9·47 | 3·88 | 2·70–4·80 | 0·112 | ·071–·182 | 4·81 | 4·10–5·05 | 2·35 | 1·43–4·45 | 514 | 12–7734 |
| LF | 8·53 | 8·23– 8·86 | 3·80 | 3·12–4·50 | 0·126 | ·076–·213 | 4·74 | 3·81–5·05 | 2·61 | 1·70–3·85 | 258 | 18–1356 |
| LH | 8·52 | 8·22– 8·86 | 3·88 | 2·65–5·03 | 0·117 | ·091–·167 | 4·79 | 4·29–5·14 | 2·41 | 1·81–3·31 | 314 | 6–1465 |
| 7905..... RF | 8·03 | 7·58– 8·57 | 3·50 | 2·96–4·65 | 0·164 | ·076–·208 | 4·42 | 3·71–5·24 | 3·74 | 1·63–5·32 | 364 | 12–1308 |
| RH | 8·05 | 7·44– 8·44 | 3·56 | 2·80–4·30 | 0·157 | ·061–·213 | 4·45 | 3·70–4·95 | 3·55 | 1·28–5·75 | 256 | 6–1136 |
| LF | 7·97 | 7·59– 8·50 | 3·55 | 2·50–4·40 | 0·164 | ·086–·213 | 4·40 | 3·62–4·95 | 3·76 | 1·81–6·16 | 398 | 3–2686 |
| LH | 8·06 | 7·62– 8·58 | 3·58 | 2·48–4·32 | 0·156 | ·091–·208 | 4·48 | 3·91–4·86 | 3·50 | 1·92–5·08 | 188 | 6–539 |
| 7909..... RF | 8·06 | 7·70– 8·60 | 3·39 | 2·95–4·11 | 0·162 | ·122–·218 | 4·37 | 3·53–4·86 | 3·79 | 2·61–5·82 | 2399 | 24–15976 |
| RH | 8·10 | 7·76– 8·80 | 3·51 | 3·00–4·09 | 0·156 | ·127–·197 | 4·54 | 4·00–4·95 | 3·39 | 2·71–4·18 | 692 | 95–7751 |
| LF | 8·07 | 7·71– 8·96 | 3·40 | 2·88–4·17 | 0·159 | ·111–·208 | 4·42 | 3·90–4·95 | 3·59 | 2·45–5·06 | 837 | 18–3438 |
| LH | 8·10 | 7·77– 8·85 | 3·50 | 2·90–4·33 | 0·156 | ·116–·203 | 4·47 | 4·10–5·14 | 3·51 | 2·50–4·72 | 1036 | 148–2414 |
| 7910..... RF | 8·63 | 7·73– 9·93 | 4·45 | 3·09–5·78 | 0·132 | ·091–·223 | 4·75 | 4·10–5·24 | 2·81 | 1·84–4·98 | 415 | 41–1207 |
| RH | 8·68 | 8·02– 9·41 | 4·51 | 2·69–5·53 | 0·119 | ·051–·157 | 4·90 | 4·38–5·33 | 2·47 | 1·07–3·50 | 903 | 59–8520 |
| LF | 8·72 | 8·23– 9·44 | 4·49 | 2·85–5·89 | 0·119 | ·071–·177 | 4·90 | 4·29–5·33 | 2·46 | 1·50–3·58 | 383 | 18–2172 |
| LH | 8·70 | 7·91– 9·57 | 4·62 | 3·07–5·91 | 0·117 | ·081–·162 | 4·98 | 4·57–5·52 | 2·36 | 1·42–3·70 | 427 | 30–1438 |
| 7912..... RF | 8·56 | 7·91– 8·89 | 4·36 | 3·24–5·50 | 0·143 | ·046–·263 | 4·44 | 3·24–5·43 | 3·45 | 1·43–8·12 | 4406 | 18–20473 |
| RH | 8·59 | 8·09– 9·00 | 4·09 | 2·96–5·06 | 0·120 | ·056–·172 | 4·81 | 4·19–5·81 | 2·51 | 1·06–3·76 | 995 | 77–3006 |
| LF | 8·54 | 7·72– 9·04 | 4·14 | 2·96–5·29 | 0·129 | ·066–·192 | 4·81 | 4·28–5·52 | 2·66 | 1·19–4·25 | 1031 | 49–5722 |
| LH | 8·63 | 7·99– 9·58 | 4·23 | 2·77–5·08 | 0·120 | ·061–·162 | 4·88 | 3·91–5·52 | 2·49 | 1·05–3·54 | 1190 | 5–9290 |
| 7913..... RF | 8·62 | 8·18– 9·19 | 3·98 | 3·20–4·99 | 0·136 | ·076–·208 | 4·62 | 3·43–5·52 | 2·98 | 1·54–5·32 | 748 | 9–2438 |
| RH | 8·72 | 8·24– 9·25 | 4·06 | 2·64–5·16 | 0·122 | ·056–·177 | 4·84 | 3·81–5·52 | 2·56 | 1·20–4·53 | 1235 | 9–4239 |
| LF | 8·70 | 8·18– 9·21 | 4·00 | 3·21–4·96 | 0·128 | ·056–·234 | 4·80 | 4·19–5·33 | 2·54 | 1·50–3·65 | 906 | 27–8225 |
| LH | 8·60 | 8·14– 9·12 | 3·86 | 3·01–4·86 | 0·147 | ·061–·218 | 4·46 | 3·63–5·24 | 3·34 | 1·20–5·20 | 1626 | 53–7515 |
| 7914..... RF | 8·99 | 8·48– 9·65 | 4·33 | 3·50–6·14 | 0·128 | ·056–·213 | 4·96 | 4·29–5·71 | 2·46 | 1·01–4·25 | 895 | 130–4083 |
| RH | 9·00 | 8·41– 9·72 | 4·42 | 3·32–6·22 | 0·118 | ·051–·208 | 4·92 | 4·29–5·90 | 2·32 | 0·86–4·84 | 852 | 47–5095 |
| LF | 9·01 | 8·48– 9·66 | 4·39 | 2·87–5·89 | 0·121 | ·061–·192 | 4·98 | 4·19–5·81 | 2·28 | 1·06–4·11 | 786 | 9–6450 |
| LH | 9·00 | 8·48– 9·66 | 4·45 | 3·64–6·29 | 0·111 | ·051–·203 | 5·09 | 4·67–5·90 | 2·02 | 0·90–3·09 | 763 | 36–5976 |
| 7919..... RF | 8·71 | 8·19– 9·61 | 4·01 | 3·32–4·96 | 0·141 | ·091–·243 | 4·85 | 4·10–5·33 | 2·84 | 1·80–5·19 | 329 | 53–1047 |
| RH | 8·73 | 8·22– 9·49 | 4·21 | 3·63–5·52 | 0·132 | ·066–·177 | 4·79 | 4·00–5·33 | 2·82 | 1·04–4·05 | 389 | 18–1811 |
| LF | 8·69 | 7·95– 9·69 | 4·00 | 3·37–4·88 | 0·150 | ·086–·217 | 4·61 | 3·63–5·24 | 3·29 | 1·77–6·00 | 394 | 41–1612 |
| LH | 8·76 | 8·27– 9·70 | 4·14 | 3·13–5·30 | 0·123 | ·091–·177 | 4·99 | 4·48–5·52 | 2·42 | 1·74–3·37 | 184 | 30–1237 |
| 7921..... RF | 8·44 | 7·88– 9·24 | 3·57 | 2·56–4·76 | 0·142 | ·086–·187 | 4·51 | 3·81–5·52 | 3·13 | 1·94–4·76 | 1494 | 195–6348 |
| RH | 8·35 | 7·93– 9·03 | 3·60 | 2·34–4·93 | 0·144 | ·081–·208 | 4·46 | 3·71–5·05 | 3·29 | 1·42–5·19 | 3290 | 107–11657 |
| LF | 8·29 | 7·67– 8·85 | 3·57 | 2·05–4·99 | 0·155 | ·076–·223 | 4·09 | 2·86–4·86 | 3·94 | 2·40–7·26 | 1912 | 379–7722 |
| LH | 8·37 | 7·94– 9·01 | 3·58 | 1·90–4·99 | 0·138 | ·081–·187 | 4·45 | 3·14–5·33 | 3·18 | 1·81–5·48 | 1517 | 142–7337 |
| 7922..... RF | 8·67 | 8·31– 9·27 | 4·06 | 3·42–4·81 | 0·129 | ·056–·182 | 4·85 | 3·91–5·43 | 2·68 | 1·05–3·80 | 1067 | 65–4396 |
| RH | 8·77 | 8·37– 9·60 | 4·11 | 2·91–5·16 | 0·128 | ·056–·177 | 4·84 | 4·57–5·24 | 2·61 | 1·17–3·72 | 875 | 41–2811 |
| LF | 8·68 | 8·20– 9·66 | 4·25 | 2·63–5·43 | 0·143 | ·058–·197 | 4·58 | 2·66–5·14 | 3·22 | 1·58–7·42 | 994 | 47–5633 |
| LH | 8·83 | 8·35–10·12 | 4·31 | 3·49–5·69 | 0·119 | ·025–·177 | 4·96 | 4·38–5·52 | 2·44 | 1·16–3·50 | 396 | 24–2024 |

## TABLE 5.

*Classification of quarters into normal* (N) *and abnormal* (ABN) *on a basis of* (a) *composition and* (b) *the results of the tests applied.*

| Cow and Quarter. | Solids not Fat. | | Fat. | | Chloride. | | Lactose. | | Chloride-Lactose Index. | | Cells. | | Classification of Quarter. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Compn. | Tests. | Compn. | Tests. | Compn. | Tests. | Compn. | Tests. | Compn. | Tests. | Compn. | Tests. | |
| 7904..... RF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| 7905..... RF | ABN | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | N | N | Abnormal. |
| RH | ANB | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | N | N | Abnormal. |
| LF | ABN | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | N | N | Abnormal. |
| LH | ABN | ABN | N | N | ABN | ABN | ABN | N | ABN | ABN | N | N | Abnormal. |
| 7909..... RF | ABN | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| RH | ABN | ABN | N | N | ABN | ABN | N | N | ABN | ABN | N | N | Abnormal. |
| LF | ABN | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | N | N | Abnormal. |
| LH | ABN | ABN | N | N | ABN | ABN | ABN | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| 7910..... RF | N | N | N | N | N | N | N | M | N | N | N | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| 7912..... RF | N | N | N | N | N | ABN | ABN | ABN | ABN | ABN | ABN | ABN | Doubtful. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | N | N | N | N | N | N | ABN | N | Normal. |
| LH | N | N | N | N | N | N | N | N | N | N | ABN | N | Normal. |
| 7913..... RF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | ABN | B | Normal. |
| LF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LH | N | N | N | N | N | N | ABN | ABN | ABN | ABN | ABN | N | Doubtful. |
| 7914..... RF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| 7919..... RF | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | ABN | ABN | N | N | ABN | ABN | N | N | Doubtful. |
| LH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| 7921..... RF | ABN | ABN | N | N | N | N | N | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| RH | ABN | ABN | N | N | N | N | ABN | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| LF | ABN | ABN | N | N | ANM | ABN | ABN | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| LH | ABN | ABN | N | N | N | N | ABN | ABN | ABN | ABN | ABN | ABN | Abnormal. |
| 7922..... RF | N | N | N | N | N | N | N | N | N | N | ABN | N | Normal. |
| RH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |
| LF | N | N | N | N | N | N | N | N | ABN | ABN | N | N | Doubtful. |
| LH | N | N | N | N | N | N | N | N | N | N | N | N | Normal. |