"SOME ASPECTS OF MILK SYNTHESIS AND COMPOSITION IN RELATIONSHIP TO THE MOTHER'S ABILITY TO BREAST-FEED HER INFANT"

by


Thesis presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Edinburgh.

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ACKNOWLEDGMENTS.
INTRODUCTION.

The problem of disordered lactation is a complex one and why some women should be unable to breast-feed their infants or only succeed in doing so for a limited period of time has never been fully understood. Many factors may account for inadequate lactation - factors concerning both mother and infant. Thus, where the mother is affected, her disability may arise from psychological or nutritional disturbances, endocrine dysfunction, or super-imposed systemic disorders. In the mammary gland itself, anatomical defects, as well as disease, may sometimes be responsible for the mother's failure to breast-feed her infant. Although many cases of unsatisfactory lactation are probably due to these factors, the clinician is frequently satisfied in placing women in one or other category after a superficial examination and sometimes without completing a sufficient number of test-feeds. Thus, the aetiology and treatment of these cases is based merely on clinical impressions. Simple tests to determine the chemical composition of milk and its bacterial content are usually omitted, though it is hard to explain such neglect, because these lines of investigation are frequently applied to cattle with sub-normal milk yields. Recently, however, the letting down of milk has been studied
and thyroid and iodine therapy has been instituted in some cases to increase the milk yield, though the results so far have not been of outstanding practical value. The complete neglect of routine investigation of mothers with inadequate lactation is most unfortunate because a fuller understanding of such a disorder might result in mothers being judged more fairly and treated more efficiently by the physician. Moreover, a greater percentage of mothers would be able to fulfil their ambition to breast-feed their infants, more mothers might enjoy better mental and physical health and more infants might pass unmolested through the hazards of the first year of life.

In an attempt to find some investigation which would help the physician to assess a woman's ability to feed her infant, research has been carried out on certain tests which might give information as to the adequacy of lactation in women. These tests involve the estimation of the chloride content and electrical conductivity of milk and the protein and cholesterol in the blood, and have been made on samples of milk and blood taken from mothers who were breast-feeding their infants adequately and others who were breast-feeding them inadequately.

The investigation on both chloride and electrical conductivity values of milk has been divided into four parts. The first part concerns the relationship of the test results to adequacy of lactation, the
second is devoted to the effects of mammary disease upon various tests, including the milk chloride and conductivity tests, the third involves the assessment of the duration of lactation by means of the chloride and conductivity tests, while the fourth part is devoted to an assessment of the value of these tests for the detection of adulterated milk.

Observations have been made on various constituents of mothers' blood and a separate section has been devoted to each class of substance discussed. The first section consists of a review of the literature on the blood glucose of women and the relationship to the sugar content of milk and milk yield, the second is composed not only of a review, but also of investigations on blood protein in lactating women with varying milk yields, and the third section is devoted to a review of the literature correlating values of blood lipids with milk yield and to the correlation of a series of blood cholesterol values obtained for lactating women with the milk yield of these women.
FACTORS INFLUENCING THE CHLORIDE CONTENT OF WOMEN'S MILK.

Although the estimation of the chloride content of women's milk has been made for at least fifty years, it was not extensively practised until after 1915, when the rapid micro-method of determining chloride in body fluids replaced the previous laborious methods involving either gravimetric methods or the Volhard titration, (Sisson and Denis, 1921). In either of the older methods, a considerable volume of milk had to be evaporated to dryness, ashed in a potassium dish at a low heat with the help of an alkaline fusion mixture and the ash then extracted with water before any estimation could be made. Such a process was tedious and time-consuming and one requiring an elaborate equipment and considerable knowledge of clinical techniques. The micro-method can be performed directly on a small sample of milk, and is quickly and easily completed. Since the introduction of modifications of the micro-method (McLean and Van Slyke, 1915) by Van Slyke and Donleavy (1919) and Sisson and Denis (1921), the estimation of chloride has been used to detect the adulteration of cows' milk or of women's milk with water, and also for the detection of human milk mixed with cows' milk.

Before investigating the practical value of
estimating the chloride content of women's milk, it is necessary to know the normal chloride value of human milk and the factors that influence it. Information on this is to be found in the literature, but, in some instances, the effect of some of the factors upon the chloride content of women's milk is doubtful. In such cases, reference will be made to the results of investigations on the chloride content of cows' milk. The various factors influencing the chloride content of milk are the mother's age, parity, health, and food intake, and these will be discussed later. The relationship between the chloride content of milk and the chloride content of the mother's blood and urine, and the reaction of the infant to milk of varying chloride content are also factors of importance in the investigation of the practical application of milk chloride estimations.

The first extensive investigation into the chloride content of women's milk was made by Sisson and Denis, who published the results obtained by examination of three hundred and twenty-seven specimens of milk in 1921. These results revealed marked variation in the chloride content of milk from mother to mother, and showed that in any one mother the chloride value of her milk might vary from day to day and throughout a feed. These writers stated that chloride values in the first month of lactation
ranged from 17 to 158 mg. per cent and in the second to fifth month from 17 to 114 mg. per cent. The values of milk obtained from mothers with inadequate lactation exceeded 50 mg. per cent. These views have been shared by other investigators, though with some reservations. Nozaki (1934), who examined eighty-one specimens of milk, and Ishii (1937), who examined nine hundred specimens of milk, drew conclusions from their results which differed from those of Sisson and Denis (1921) in that they found the chloride values for milk from mothers with adequate lactation frequently exceeded 50 mg. per cent, while chloride values for women with inadequate lactation was often less than 50 mg. per cent. These findings are, however, open to criticism, because the investigators used the Arakawa Reaction of milk to determine the adequacy of lactation. While an Arakawa positive reaction usually indicates adequate lactation and an Arakawa negative reaction generally means inadequate lactation, the relationship between Arakawa reaction and adequacy of lactation is not constant. Widdows et al (1935) also doubted whether values of milk above 50 mg. per cent indicated inadequate lactation because in their series of cases nine out of ten women with milk containing 51 to 58 mg. per cent had adequate lactation.

A review of the chloride values of milk given by
TABLE 22.

TO SHOW THE RANGE OF CHLORIDE VALUE FOR MILK TAKEN FROM MOTHERS WITH ADEQUATE LACTATION AND THOSE FOR MOTHERS WITH INADEQUATE LACTATION.

<table>
<thead>
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<th>LACTATING ADEQUATELY</th>
<th>LACTATING INADEQUATELY</th>
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<tr>
<td></td>
<td>No. Cases</td>
<td>Milk Cl. mg%</td>
</tr>
<tr>
<td>Sisson &amp; Denis 1921</td>
<td>2 - 52</td>
<td>35</td>
</tr>
<tr>
<td>Nozaki 1934</td>
<td>Over 4</td>
<td>40</td>
</tr>
<tr>
<td>Widdows et al 1935</td>
<td>2 - 42</td>
<td>80</td>
</tr>
<tr>
<td>Ishii 1937</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5 - 8</td>
<td>17</td>
</tr>
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<td></td>
<td>9 - 12</td>
<td>21</td>
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the above-mentioned authors (See Table 22, Page 7) shows that the chloride values range from 7 to 167 mg. per cent between the second to forty-second week of lactation. The average value from month to month does not vary appreciably while the values for milk from mothers with adequate lactation are generally under 50 mg. and those for mothers with inadequate lactation are generally over 50 mg. Even in earlier studies on the correlation of chloride content of milk with milk yields, a definite relationship between the two had been noted by Holt et al (1915). Their conclusions were based on the results of estimations of chloride content of milk taken from ten mothers before the tenth month of lactation and again after that period. In the earlier stage of lactation, the women had a larger milk yield than in the later stage of lactation, and with the larger yield, the chloride content of the milk was relatively low as compared with the value for the smaller milk yield.

The inverse relationship between milk chloride content and milk yield in women which was noted by the previous authors was, however, based on a small number of inadequately investigated cases. No accurate records of milk yield were made and routine weighing and test-feeding of the infant were not employed. Adequacy of lactation was gauged by whether or not the infant required complementary feeds (Sisson and Denis, 1921) or by the Arakawa Reaction of the
milk, (Nowaki, 1934, and Ishii, 1937), which has already been mentioned, is not necessarily an accurate method of assessing the ability to lactate. These investigators also based their observations on estimations of the chloride content of a single specimen of milk taken at an unspecified time and from one breast only. All these technical details tend to diminish the accuracy of their findings.

The importance of taking samples of milk from both breasts for examination lies in the fact that the chloride value of the milk from the two breasts may differ and this difference may be considerable. The comparison of milk chloride values from each breast has been most carefully made by Ishii (1937) who estimated the chloride content of specimens of milk taken from both breasts. He tested four hundred and fifty women in this way and found that the difference between the chloride values for the two breasts of any one women with Arakawa positive milk was 0 to 21 mg., while the difference between the values for women with Arakawa negative milk was 0 to 57 mg. A similar, but less satisfactory investigation in this field has been made on a series of eighty mothers by Nozaki (1934) and on one hundred and fifty women by Ishii (1938). Both these investigations support the previously quoted findings on the variation in chloride values of milk from the right and left breast.
Variations in the chloride values for milk taken from mothers of different ages, at different stages of lactation, at different seasons in the year, and at different times of the day have been investigated by several workers. The chloride content of milk is slightly higher on the average as mothers become older (Ishii, 1937) and this increase is not influenced by parity of mothers (Widdows et al., 1935). A rise in milk chloride value also occurs in hot seasons of the year (Ishii, 1937), but such increases are relatively small. Much greater variations in the chloride content of the milk from one woman may occur throughout the day, though variations of the order of three hundred per cent probably only occur in milk taken from mothers with inadequate lactation. In the terminal stage of lactation, the fluctuation in the chloride content of milk in twenty-four hours can be one thousand per cent. (Sisson and Denis, 1921). A review of the literature suggests that the chloride values tend to be greatest either in the early morning (Sisson and Denis, 1921; Macy et al., 1932, and Ishii, 1937) or in the late evening (Sisson and Denis, 1921). It has also been shown that the chloride values for fore-milk and after-milk differ and that the chloride content of the after-milk tends to be 0 to 10 mg. higher than in the fore-milk (St. Engel, 1909; Sisson and Denis, 1921; Macy et al., 1931, and Ishii, 1937), but in exception-
all cases the difference between fore and after-milk may be as great as 83 mg. (Sisson and Denis, 1921).

The chlorides content of women's milk can be affected by the mother's sodium chloride intake. This depends not only on the amount of salt taken, but also on the duration of the change in salt intake. If a mother starves for a short period, such as eight to twelve hours, the chloride content of her milk is not appreciably affected, (Bunge, quoted by Freund, 1898, and Sisson and Denis, 1921). However, when a salt-free diet is given to a series of nine nursing-mothers for eight to twelve days, as was done by Sacco (1932), the chloride content of their milk decreases. Conversely, though an increase in a mother's salt intake of ten to thirty grams for one day does not alter the chloride content of her milk (Bunge, quoted by Freund, 1898; Lesme et al, 1933, and Sugihara and Miyazaki, 1938), a significant increase in the salt content of her diet over a period of time will eventually raise the sodium chloride content of her milk. (Baldassi, 1941).

Similar observations on the salt intake of animals have been made and show that in cows single doses of sodium chloride or an increased salt intake of 2.5 to 3.7 oz. NaCl per day does not alter the chloride content of the milk (Lobstein and Wallart, 1927; Roadhouse and Henderson, 1930; Rosell, 1931; Jocks, 1934). In goats, the feeding of a diet con-
eating adequate amounts of sodium chloride eventually produces an increase in the chloride content of their milk. (Sisson and Denis, 1921). In rats too, an increase in the chloride content of their milk probably occurs when they are given a diet to which salt is added, because under these circumstances, their milk yield is materially impaired. (Heller, 1932).

The possibility of a relationship between the chloride content of blood and that of milk has been investigated in an attempt to explain the observed fluctuations in the chloride content of milk. Under normal circumstances, there is no relationship between the two. This conclusion is based on the results of an extensive investigation on one hundred and fifty women. (Ishii, 1938). From each woman, a sample of blood and one of milk were taken simultaneously. The chloride values for the specimens of blood ranged from 270 to 337 mg. per cent, and those for the corresponding specimen of milk were 14 to 95 mg. per cent. When the blood chloride values and the milk chloride values for each woman were compared, no relationship could be found.

Sisson and Denis (1921) suggested that the threshold for secretion of chloride by the breast varied from woman to woman and this resulted in the variation in the chloride content of their milk. This is probably true and is supported by the following observations.
on cattle. Thirty cows with milk of relatively high chloride content did not have a higher blood chloride than that found in ten cows secreting milk of lower chloride content. (Sato and Murata, 1937). Therefore, the difference in the chloride content of milk obtained was probably the result of a different threshold for secretion in the two groups of cows.

Under certain circumstances, however, which are merely experimental, a relationship between the chloride content of blood and that of the milk does exist. For example, it has been shown that the addition of sufficient salt to the diet of three nursing mothers ultimately increases both the chloride content of their blood and their milk, but a small increase in the salt intake or a single dose of sodium chloride only raises the chloride content of the blood, without affecting the chloride value of the milk. (Sugihara and Miyazaki, 1938). Similar observations have been made on goats. These animals require to take sufficient salt to raise their blood chloride by eighteen per cent before the chloride content of their milk increases. (Denis and Sisson, 1921).

The chloride content of milk has been correlated with the chloride content of the urine. It has been shown that the chloride content of urine from women with adequate lactation is 0.725 to 1.355 grams per litre (average 1.062 grams) and that the chloride
content of urine from women with inadequate lactation tends to be less. (Umemura, 1940). This author states that retention of chloride by the body can occur with anxiety and that increased loss of chloride may result from the administration of Vitamin B (Umemura, 1940) or salt (Baldassi, 1941). This increase loss is associated with a rise in the concentration of chloride in the urine.

The effect of feeding women's milk of high chloride content to infants has been investigated and may be harmful. Sisson and Denis (1921) state that indigestion frequently occurs in babies taking this type of milk. Courtney and Brown (1930) noticed that out of six infants receiving an inadequate amount of breast milk of high chloride content (over 50 mg. per cent), two had diarrhoea and one had pylorospasm and eczema, and they suggested that these infants' symptoms were possibly due to the abnormal composition of the milk they received. The salt content of women's milk has since been shown to influence eczema in breast-fed infants, all of whom rapidly improved, or were cured, when their mothers were given a salt-free diet. In every case, the chloride content of the mother's milk diminished after she had been on her special diet for five days and the maximum drop in the chloride content of milk occurred on the twelfth to fifteenth day.

Conversely, exudative eczema in breast-fed infants is aggravated by adding sodium chloride to their mothers'
diet. (Kerning and Hopf, 1931). Such observations strongly support the view that a mother's milk may not always suit her infant, a fact which has been repeatedly stated in the literature since the days of Hippocrates. (Still, 1931).

It is possible to summarise, from the preceding review, the factors influencing the chloride content of women's milk and their relative importance. Thus, it has been shown that the factors causing the most marked and definite variation in the chloride content are the stages of lactation and its adequacy. Relatively high milk chloride values are usually associated with women in the first week of lactation, in those in whom lactation is failing, and in women with inadequate lactation. Lower values are generally obtained in cases where lactation is fully established and normal. Should a pathological condition be present in the mother, the chloride content of the milk may rise. The extent to which this occurs is unknown, but judging from observations made on cattle, the rise in chloride content of milk is usually considerable when the mammary gland is diseased.

Small variations in chloride values of women's milk are frequently noted when milk specimens taken from each breast are tested, when specimens are taken several times a day, and when the test is made on milk taken before and after a breast feed. Although
the difference in the values obtained for milk from the two breasts is usually small, it can be considerable and, therefore, it may be wiser to test both breasts separately when making investigations on the chloride content of milk. Tests on milk specimens taken throughout the day and throughout a breast-feed have shown that the chloride content of milk may vary considerably in some circumstances, and these results indicate that a series of selected specimens of milk would be necessary to obtain adequate information regarding the chloride content of milk from a given woman.

Factors which can be neglected when interpreting the chloride value for women's milk are the age and parity of the mother, and the season of the year, because they have little or no effect upon the results. The differences noted in the chloride content of milk taken from different women and in specimens taken at different times in the same woman are probably due to the occurrence in different women of different threshold levels for secretion of chloride in milk. It is also possible that the threshold may vary from time to time in any given mother.

Evidence, therefore, suggests that the determination of the chloride content of milk might be of value in detecting mothers with inadequate lactation, provided a suitable test involving the estimation of
milk chloride could be devised. Moreover, such a chloride test might be of value in the diagnosis of infantile dyspepsia or eczema associated with the consumption of milk with high chloride content.
PRACTICAL DETAILS and CALCULATION for the ESTIMATION of CHLORIDE CONTENT of MILK.

The sample of milk for testing for chloride content was mixed gently and 0.2 c.c. of the sample taken in a pipette and discharged into a clean test tube containing 3 c.c. concentrated nitric acid. The pipette was washed out by drawing the nitric acid up into the pipette and expelling it. The washing of the pipette was repeated three times. 4 c.c. acetone (E.P.) and three drops of a saturated solution of iron alum are then added to the mixture in the test tube. 1.0 c.c. N/60, Ag NO₃ was delivered from a 1 c.c. bulb pipette into the test tube. The excess Ag NO₃ was titrated with approximately N/60 Ammonium Thiocyanate which was held in a micro-burette. The end point of the titration was indicated by the first appearance of a pink colour which lasted for at least thirty seconds after the mixture had been thoroughly mixed.

To ensure that the N/60 Ag NO₃ was of the correct strength, it was standardized from time to time against a solution of NaCl of known strength using potassium chromate as indicator. The ammonium thiocyanate, approximately N/60, was made up in a mixture of two parts alcohol and one part water. The solution was
standardized against \( \frac{N}{60} \) Ag NO₃ before and after each daily batch of titrations.

The method of calculating the chloride content of milk is illustrated by the following example:

Supposing 0.84 c.c. thiocyanate is used for the titration of the excess Ag NO₃ and 0.95 c.c. corresponded to 1 c.c. Ag NO₃; then

\[
\begin{align*}
0.95 \text{ c.c. Thiocyanate} & \quad 1.0 \text{ c.c. } \frac{N}{60} \text{ Ag NO₃} \\
1.0 \text{ c.c. Thiocyanate} & \quad \frac{1}{0.95} \text{ c.c. } \frac{N}{60} \text{ Ag NO₃} \\
0.84 \text{ c.c. Thiocyanate} & \quad \frac{1 \times 0.84}{0.95} \text{ c.c.} \\
0.88 \text{ c.c. } \frac{N}{60} \text{ Ag NO₃}
\end{align*}
\]

Therefore the amount of Ag NO₃ used is 1.0 - 0.88 c.c. 0.12 c.c.

Since 60,000 c.c. \( \frac{N}{60} \) Ag NO₃ 58.5 g NaCl

60 c.c. \( \frac{N}{60} \) Ag NO₃ 58.5 mg NaCl

1 c.c. \( \frac{N}{60} \) Ag NO₃ 0.97 mg NaCl

As 0.12 c.c. Ag NO₃ corresponds to 0.12 x 0.97 mg NaCl

0.116 mg NaCl

0.2 c.c. milk contains 0.116 mg NaCl

100 c.c. milk contains \( \frac{0.116 \times 100}{0.2} \) mg 58 mg NaCl
THE CORRELATION OF THE CHLORIDE CONTENT OF MILK TAKEN BEFORE A BREAST FEED WITH THAT OF MILK TAKEN AFTER A BREAST FEED.

In the review on the chloride content of women's milk (Pages 9 and 10), it has been stated that the chloride value of milk taken before a breast feed (fore-milk) usually differs very little, if at all, from that of milk taken after a breast feed (after-milk), the difference being 0 to 10 mg. In exceptional cases, however, it may be as much as 83 mg., but such a wide variation appears to be rare, although no statement as to its frequency has been found in the literature. An attempt has been made to determine the average difference in values between the chloride of fore and after-milk, and to decide whether such changes are significant. The possibility of a relationship between the differences and adequacy of lactation has also been investigated. It was hoped that as a result of these investigations it would be possible to decide which was the most accurate method of sampling milk, so that this method could be used in a further investigation.
The chloride value of women's milk taken before and after a breast feed. (Continued)

To determine whether there is a significant difference between the chloride value of milk taken before a breast feed and that of milk taken after a breast feed, observations were made on sixty-two women. These women had been lactating for periods varying from two to twenty weeks and some of them had adequate milk supply for their infants, while others had not. Each woman provided 4 to 8 c.c. breast milk before breast feeding her infant and another specimen of 4 to 8 c.c. taken from the same breast as the first specimen after the baby had been fed. The chloride values of these specimens were estimated in the routine way and the results are set out in the Appendix (Pages 35 to 37). The chloride values are usually greater for the second milk specimen than for the first, irrespective of whether the milk was obtained from women with adequate lactation or from those with inadequate lactation. The average chloride value for all the fore-milk specimens is 67.7 mg. per cent, and that for all the after-milk specimens is 75.2 mg. per cent. To determine whether the difference is significant, the figures have been subjected to a statistical analysis by applying two tests, the "t" test and
the "analyses of variance".

The statistical analysis has been made first by applying the "t" test to the sixty-two observations on milk taken before a feed and the sixty-two taken after a feed, thus regarding the results as two independent sets of observations. The calculation for the analysis is in the Appendix (Page 38) and the value obtained for "t" is 0.94 which indicates that the difference between the chloride value for fore-milk and that for after-milk is insignificant.

The "t" test in this analysis is not, however, altogether appropriate because the two sets of observations are not independent. Each set is derived from the same sixty-two women, each women giving a milk sample both before and after a breast feed. The individual variations are, therefore, common to both series and due allowance must be made for this when determining the significance of the difference between the two means. Moreover, the same difference between the means will be more significant when the two means are derived from two related series as in the case in question than if one mean is derived from one group of women and the other mean is derived from an entirely different group of women.

To eliminate the factor of individual variation, the "analysis of variance" has been applied to the data (Appendix Page 39).
will be found in the Appendix (Page 39).

The result gives "Z" to equal 0.996 which shows that the difference between the average values of the two series is significant. Therefore, on the average, the chloride content is greater than that for fore-milk. It, therefore, follows that in taking samples of human milk for investigation, it is important to take always the fore-milk or else always the after-milk to minimise the degree of error. Samples of fore-milk, however, are probably preferable to samples of after-milk when correlating the chloride values of milk with adequacy of lactation, because a given amount of milk can be more readily and accurately obtained before, rather than after, an infant has been suckled.
The question arises as to whether the chloride content of milk can assist in deciding whether a woman is capable of feeding her infant adequately. This would be of especial value if breast-fed infants were thought to be under-fed.

In order to decide whether the milk chloride test was of practical value, it was necessary to show whether there was a correlation between the chloride value and the ability of the mother to feed her infant. To this end, a series of five hundred and thirty-eight specimens of milk from women in the second week to the fifth month of lactation inclusive were examined; three hundred and fifty-two were taken from women with adequate lactation, and one hundred and eighty-six from women with inadequate lactation. The mothers providing the samples of milk gave two specimens from the same breast, one before the 6 a.m. breast feed and the other before the mid-day feed (12 p.m. or 2 p.m.) on the same day.

A second series of observations involving the chloride estimation of milk taken from both breasts of each woman was considered necessary, in view of a number of anomalous results obtained in the first series. One hundred and thirty-six specimens of milk
taken during the third week to fifth month of lactation were examined; seventy-two were obtained from mothers with adequate lactation and sixty-four from mothers with inadequate lactation. The mothers providing the samples gave one from each breast before the 6 a.m. breast feed and one from each breast before the mid-day breast feed. All specimens obtained from each mother were collected in separate containers and tested separately. In both series of cases, each sample amounted to about 8 c.c. which were expressed into a special container that had been thoroughly cleaned, rinsed in distilled water and dried in a drying oven.

All the mothers who provided samples were apparently healthy and had given birth to a mature infant which fixed well at the breast and was breast fed at regular intervals five or six times a day. Their infants were apparently normal at the time of the test. The choice of a mother with adequate lactation was made by noting that her infant was gaining weight while it was entirely breast fed. Women with inadequate lactation were selected by first finding a mother whose infant was healthy, but not gaining weight, or who required complementary feeds. Such infants were then test-fed for at least twenty-four hours. If the results showed that their babies did not receive two ounces milk per pound body weight per day, they were considered to be under-fed.

The method of estimating the chloride content of
FIGURE I

COMPARISON OF CHLORIDE VALUES OF MILK FROM MOTHERS WITH ADEQUATE LACTATION (*) WITH THOSE FROM MOTHERS WITH INADEQUATE LACTATION (*).
the milk is the same as was employed in the previous sections and the detailed results of the investigation are given in the Appendix (Pages 1 to 20). A summary of the findings of both series of investigations is given together in Figure I (Page 25). This figure gives the mean chloride content of the early morning and mid-day milk specimens obtained from a given breast. The values for milk chloride obtained from mothers with inadequate lactation are generally higher than those for milk from mothers with adequate lactation. In the figure, the results are divided according to the time after parturition at which the milk sample was taken. For each of these weekly or monthly stages, a level has been chosen above which lies the majority of chloride values for milk obtained from women with inadequate lactation and below which lies the majority of values for women with adequate lactation. This "critical" level for milk specimens taken in the third and fourth weeks of lactation is the seventy-five mg. NaCl. per 100 c.c. milk; for milk taken in the second month, it is at sixty mg. NaCl., and for milk taken in the third to fifth months it is at fifty-five mg. NaCl. For milk taken during the second week of lactation, the division between milk and chloride values for women with adequate lactation and those for women with inadequate lactation is not so clear-cut, and therefore, two "critical" levels instead of one are necessary for
To show the percentage of mothers with adequate lactation and milk with a chloride content below the "critical" level for their stage of lactation.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>3-4 weeks</th>
<th>2 months</th>
<th>3-5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Critical&quot; level mg. chloride</td>
<td>...</td>
<td>75</td>
<td>60</td>
</tr>
<tr>
<td>Number of mothers investigated</td>
<td>...</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>% mothers with milk with chloride content below &quot;critical&quot; level</td>
<td>...</td>
<td>87.1</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Data obtained from cases in Series I (Appendix Pages 1 to 7).
diagnostic purposes; the lower level being at 75 mg. NaCl. per 100 c.c. milk, and the higher one at 112 mg. NaCl. per 100 c.c. milk. Values below the lower "critical" level are usually associated with adequate lactation, while those above the upper "critical" level are generally associated with inadequate lactation. Values lying between the two "critical" levels are without diagnostic value.

It is possible to use these "critical" levels to assess the ability of a woman to breastfeed her infant by comparing the chloride content of her milk (taken as described previously) with the "critical" level corresponding with her stage of lactation. If the mother's milk chloride lies below this "critical" level, it is likely that she can satisfy her infant on the breast. If it falls above, she is probably not producing enough milk to meet her infant's requirements. This fact is emphasised in Table 19 (Page 26) which has been constructed from the first series of observations to show the percentage of mothers with adequate lactation in the third week to fifth month, whose milk chloride value lies below the "critical" level for the corresponding stage of lactation. Table 20 (Page 27) shows the percentage of mothers with inadequate lactation whose milk chloride lies above the corresponding "critical" level. In the case of women with adequate lactation, at least
To show the percentage of mothers with inadequate lactation and milk with a chloride content above the "critical" level for their stage of lactation.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>3-4 week</th>
<th>2 months</th>
<th>3-5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Critical&quot; level mg. chloride</td>
<td>...</td>
<td>75</td>
<td>60</td>
</tr>
<tr>
<td>Number of mothers investigated</td>
<td>...</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>% mothers with milk chloride content above &quot;critical&quot; level</td>
<td>...</td>
<td>92.9</td>
<td>92.9</td>
</tr>
</tbody>
</table>

Data obtained from cases in Series I (Appendix Pages 8 to 12).
eighty-five per cent of the values lies below the "critical level", and for women with inadequate lactation, at least ninety-two per cent lies above the level. Therefore, it is generally possible to tell whether or not a mother has adequate lactation by noting whether the results of her chloride test fall above or below the "critical level" for her stage of lactation.

For a few mothers with adequate lactation, the chloride test is misleading. Similarly, anomalous milk chloride results have been obtained from mothers with inadequate lactation. Some light has been shed on the explanation for the majority of these anomalous results by the results obtained for the chloride content of milk taken from the breasts of women in the third week to fifth month of lactation (Appendix Pages 1 to 20 and 34). In this series, twenty-two women were lactating adequately and twenty-two inadequately. It was noted that three of the women with adequate lactation and five of those with inadequate lactation had one breast functioning well and the other functioning badly, as judged by the comparison of milk chloride from each breast with the relevant "critical" chloride level. In such cases, therefore, the chloride test did not reveal whether or not these women were lactating adequately, and it was necessary to depend on the results of test-feeding of the infants to decide whether the mother's lactation was adequate.
### TABLE 34

**WOMEN WITH UNILATERAL BREAST DYSFUNCTION AS JUDGED BY THE CHLORIDE CONTENT OF THEIR MILK.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Chloride Content of breast milk</th>
<th>Critical Chloride Level</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right Side</td>
<td>Left Side</td>
<td></td>
</tr>
<tr>
<td>390</td>
<td>70.5</td>
<td>55.0</td>
<td>55</td>
</tr>
<tr>
<td>396</td>
<td>48.0</td>
<td>57.5</td>
<td>55</td>
</tr>
<tr>
<td>421</td>
<td>60.0</td>
<td>52.0</td>
<td>55</td>
</tr>
<tr>
<td>326</td>
<td>45.5</td>
<td>70.5</td>
<td>55</td>
</tr>
<tr>
<td>361</td>
<td>60.0</td>
<td>97.0</td>
<td>60</td>
</tr>
<tr>
<td>385</td>
<td>99.0</td>
<td>56.0</td>
<td>60</td>
</tr>
<tr>
<td>385</td>
<td>70.5</td>
<td>55.0</td>
<td>55</td>
</tr>
<tr>
<td>455</td>
<td>57.0</td>
<td>52.0</td>
<td>55</td>
</tr>
</tbody>
</table>

Chloride value in mg. per cent
The extent to which unilateral breast dysfunction accounts for anomalous results in the milk chloride test depends on the frequency with which unilateral breast dysfunction occurs. Judging from the series of forty-four cases, it was present in three out of twenty-two, or 13.6 per cent of mothers with adequate lactation, and in five out of twenty-two, or 22.7 per cent of mothers with inadequate lactation. This incidence is by no means insignificant and might help to explain the occurrence of fourteen anomalous results in the first investigation of one hundred and seventy mothers. Then it is to be expected that the accuracy of the milk chloride test could be considerably increased by testing both breasts.

Although it appears necessary to test-feed infants of mothers with unilateral breast dysfunction, in order to ascertain the adequacy of the milk supply, it is desirable that further investigations into such cases with the milk chloride test be carried out; for it is possible that the mean chloride value for the milk from both breasts might indicate whether a woman is lactating adequately or inadequately. Evidence supporting this possibility is supplied by the findings obtained with the eight women in the series of forty-four women with unilateral breast dysfunction (Table 34, Page 28). Six of these women had a mean milk chloride value for both breasts which lay above the "critical level" for their stage of lactation, which
would indicate they were lactating inadequately, and this was clinically confirmed in four of them. The mean milk chloride value for both breasts of two women lay below the "critical level", thus indicating adequate lactation, and in both cases this was confirmed by test-weighing of their infants.

When considering anomalous results obtained in the first series, another explanation must be kept in mind, apart from unilateral breast dysfunction. In two mothers in the same series of forty-four cases, the milk from both breasts had a chloride content below the "critical level" and yet their milk output was inadequate. Such cases are probably relatively rare as compared with unilateral breast dysfunction and, therefore, only account for a small proportion of anomalous results in the first series of cases investigated. This type of breast function may be due to hypoplasia of the gland tissue. Breast disorders of this nature cannot be detected by the milk chloride test and will, consequently, remain a source of error in such a test.

In conclusion, it can be said that the milk chloride test gives a valuable indication as to whether or not a mother is lactating adequately, particularly if both breasts are tested and the results are in agreement. If the chloride content of milk from one breast is below the "critical level", while that of milk from
the other breast is above the "critical" level, no
definite decision as to the adequacy of lactation
can be made and in such a case it is desirable that
the mothers' infants should be test-weighed.

It is possible that further investigation with
the chloride test may show that the mean value for
both breasts is of diagnostic significance. It seems
clear that in a few cases mothers may have a low
chloride value for milk from both breasts and yet
have inadequate milk yield. The type of case, however,
appeared to be of infrequent occurrence, only two being
encountered in forty-four cases. Inaccurate results
may also occur should mothers be ill when the milk
is tested or should the infant be either premature or
ill at the time of the test.
THE COMPARISON OF THE CHLORIDE CONTENT OF MILK TAKEN FROM THE RIGHT BREAST WITH THAT OF MILK TAKEN FROM THE LEFT BREAST.

In the review on the chloride content of women's milk (Page 8), it has been stated that the difference in chloride content of milk from two breasts of the same woman was usually small, the maximum difference being 21 mg. in women with adequate lactation and 57 mg. in women with inadequate lactation. These figures were given by Ishii (1937) whose series of one hundred and thirty-seven comparisons was made on milk obtained from Japanese women, and was the largest found in the literature. In the previous section, where the chloride content of women's milk is correlated with adequacy of lactation, there are chloride values for forty-four women who had both breasts tested. The difference in the chloride values for milk obtained from both breasts tended to be greater than that given in the literature. Moreover, these differences in nine out of forty-four cases were sufficient to influence the interpretation of the results in the investigation correlating milk chloride with adequacy of lactation. Therefore, the extent to which the values for milk from the two breasts differ in women in this country has been explored.
To determine whether there is an appreciable difference between the chloride content of milk taken from the right breast and that of milk taken from the left breast, one hundred and fifty-two comparisons have been made. From any one woman, at least two samples of milk were taken, one sample from the right breast and one from the left. These two samples were taken at the same time and just before the mother was due to feed her infant. The chloride content of each sample was estimated and the results have been recorded in the Appendix (Pages 41 to 47).

Facts revealed from this investigation are: (firstly) that there is usually a difference between the chloride values of milk taken from the right breast and that for milk taken from the left breast, the mean of the absolute values of the one hundred and fifty-two differences being 23.5 mg. Of the one hundred and fifty-two differences, ninety-nine, or 65.1 per cent, are less than 23.5 mg., and fifty-three, or 34.9 per cent exceed 23.5 mg. (Secondly), the average chloride value for milk taken from the right breast is 64.5 mg. per cent, and that for milk
taken from the left breast is 64.4 mg. per cent. The difference in these averages is 0.1 mg., which is insignificant.

In conclusion, it can be stated that although there is no tendency for the right breast to secret milk of a higher or a lower chloride content than that of the left, the chloride content of the milk from the two breasts usually differs. Because this difference, on the average, amounts to 36.5 per cent and did amount to 223 per cent in one individual, it is highly important when estimating the chloride content of milk from any woman to determine the chloride content of milk from both her right and her left breast. A similar conclusion is also obtained by reviewing the appropriate literature. (Page 8).
In a previous section (Pages 23 to 30) it has been shown that the chloride of women's milk is related to the adequacy of their lactation. It is, therefore, possible that a relationship exists between the chloride content of milk and the daily milk yield. This hypothesis has been supported by evidence obtained from a series of observations made on women who were breast feeding their infants. The mothers who were subjected to the investigation were apparently healthy and had infants which fixed well on the breast. Each had been lactating for between two weeks to five months and each had either one or both breasts tested. Forty-nine tests were made on mothers in the first month of lactation, and sixty-one were made on mothers in the second to fifth month of lactation. The amount of milk secreted by one breast in a day was measured by weighing the infant before and after it was put to the breast, and the procedure was repeated every time the infant was suckled the same breast during a period of twenty-four hours. Three specimens of milk were taken from each breast under investigation and tested for chloride content. Each specimen consisted of
**TABLE 21.**

**TO SHOW THE RELATIONSHIP OF THE MEAN CHLORIDE VALUE OF MILK TO THE DAILY YIELD OF MILK FROM ONE BREAST**

<table>
<thead>
<tr>
<th>No. Cases</th>
<th>Milk Chloride mg. per cent</th>
<th>Av. Milk Vol. oz. per day</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>221 - 240</td>
<td>0.0</td>
<td>)</td>
</tr>
<tr>
<td>1</td>
<td>201 - 220</td>
<td>0.25</td>
<td>)</td>
</tr>
<tr>
<td>3</td>
<td>181 - 200</td>
<td>1.3</td>
<td>)</td>
</tr>
<tr>
<td>3</td>
<td>161 - 180</td>
<td>2.2</td>
<td>)</td>
</tr>
<tr>
<td>3</td>
<td>141 - 160</td>
<td>4.8</td>
<td>)</td>
</tr>
<tr>
<td>6</td>
<td>121 - 140</td>
<td>5.2</td>
<td>)</td>
</tr>
<tr>
<td>14</td>
<td>101 - 120</td>
<td>6.3</td>
<td>)</td>
</tr>
<tr>
<td>10</td>
<td>81 - 100</td>
<td>7.15</td>
<td>)</td>
</tr>
<tr>
<td>20</td>
<td>61 - 80</td>
<td>8.35</td>
<td>)</td>
</tr>
<tr>
<td>44</td>
<td>40 - 60</td>
<td>10.9</td>
<td>)</td>
</tr>
</tbody>
</table>

Data obtained from Appendix (Pages 30 to 34)
approximately 4 c.c. milk, one of which was taken before the early morning feed (a.m.), one before the mid-day feed (noon), and one before the evening feed (p.m.), and each was taken on the day that the baby was test-weighed. The results of the test-weighing and of the chloride estimations are given in the Appendix (Pages 30 to 34). Accompanying the results are the mean values for the three chloride estimations.

From the results, it is apparent that the volume of milk at a given feed does not bear a significant relationship to the chloride content of milk taken at the beginning of the feed, but the daily yield of milk by one breast varies inversely with the chloride content of the milk. This is clearly seen in Table 21 (Page 35) which has been constructed to show the average daily yield of milk for women secreting milk with different percentages of chloride, the minimum yield being 1.3 oz. per day when the milk contains 181 to 200 mg. chloride, and the maximum yield being 10.8 oz. when the milk contains 40 to 60 mg. chloride per cent. This shows there is a close correlation between the average milk yields of groups of women and the chloride content of their milk. The milk yield of the individual women who constitute any one group may, however, differ markedly from each other in their milk yield at the time of the investigation.
FIGURE II

Comparison of milk volume from one breast in one day with the mean chloride value of the milk taken on the same day.

- Milk taken in 1st month of lactation
- Milk taken in 2nd-4th month of lactation
Therefore, this method of investigation does not form a reliable method of determining the milk yield of individual women.

To show the variation of milk yield for women secreting milk of a given chloride content, Figure II (Page 36) has been constructed from the data in the Appendix (Pages 30 to 34). In this Figure, the daily volume of milk from one breast has been plotted against the mean chloride value of the milk from the same breast. The milk volumes range from half to twenty-four ounces per day and the mean chloride values of milk from 40 to 199 mg. per cent. The variation in milk yield between women secreting milk of a given chloride value differs in extent; this variation is negligible when the chloride values are in the region of 230 mg. per cent, but becomes progressively more significant as the chloride content of the milk diminishes, the maximum variation amounting to 20 oz. when women secret milk with a chloride content of 30 to 50 mg. per cent. Thus, although an inverse relationship between the chloride content of milk and daily milk yield has been shown to exist, it cannot be utilised to determine accurately the milk yield of individual women.

Important deductions can be made, however, from this investigation; firstly, when the chloride
content of milk from one breast is 110 mg. per cent or more, it is almost certain that the daily milk yield from that breast will not exceed 6 oz. and that provided both breasts have the same functional capacity, the mother will secrete less than 12 oz. per day. This quantity is inadequate for the needs of a normal infant during the second to fourth week of life, because Holt and McIntosh (1940) have stated that 13 to 26 oz. is required during this period. Secondly, if the chloride content of milk is 90 to 70 mg. per cent during the neo-natal period, the daily milk yield from one breast is likely to be 7.1 to 8.35 oz.; therefore, from two breasts it will be 14 to 17 oz., provided the functional capacity of the two breasts is the same and this quantity is usually sufficient for infants two to three weeks old. Thirdly, mothers in the second to fifth month of lactation must have milk with a chloride content of less than 60 mg. per cent if their infants are to obtain 20 to 38 oz. breast milk, which is the amount necessary to maintain their health and normal development (Holt and McIntosh, 1940). The conclusions are the same as those obtained in the previous section where the chloride content of milk was correlated with the adequacy of lactation in women. It is apparent, therefore, that the estimation of chloride content
of milk constitutes a test of adequacy of lactation when the results are compared with the above criteria.

The estimation of chloride content of milk is certainly of value, provided the result obtained is relatively high, i.e. above 60 mg. per cent, but when the chloride values are less than 60 mg. per cent, their significance is sometimes doubtful because the variation in milk yield which accompanies such values is very great and amounts to 20 oz. There are at least two reasons for this great variation in milk yield. The first and most frequent explanation is that in eleven out of the thirty-five tests in this group of cases the demand made upon the breast was not great, because the infants suckled were under a month old (values "0" in Figure II Page 36). In such cases, the relatively low quantity of milk obtained almost certainly reflected the demands of the infant, rather than the potentialities of the breast. Low milk yields accompanying milk with low chloride values may also be due to hypoplasia of the breast. This condition, however, is not common as is indicated in a previous investigation (Page 25) where only three out of forty-four women investigated were presumed to have this abnormality, i.e. inadequate lactation, although their milk contained less than 55 mg.
chloride per 100 c.c. milk. In this investigation, six values were obtained for women in the second to fifth month of lactation, and these women were presumed to have hypogalactic breasts because, in each case, the chloride content of milk from each breast was less than 60 mg. per cent and yet the daily milk yield from these breasts ranges from 3\(\frac{1}{2}\) to 6\(\frac{1}{2}\) oz. (Figure II Page 36) an exceptionally small amount for mothers in the second to fifth month of lactation. Thus, of forty-nine tests in which the milk chloride content was less than 60 mg. per cent, six are thought to be due to hypoplasia of the breasts - a percentage of twelve. It is, therefore, apparent that such cases will rarely be a source of error when interpreting the significance of the chloride value of milk in relation to milk yield.
FACTORS INFLUENCING THE ELECTRICAL CONDUCTIVITY OF
MILK FROM WOMEN AND COWS.

From a survey of the literature on the electrical conductivity of women's milk, only two references have been obtained. The first (Koenpe, 1898) gives the conductivity measurements of nineteen specimens of milk taken from five women who were in various stages from the first week to twelfth month of lactation. The values of specimens taken in the first month ranged from 174 to $454 \times 10^{-5}$ and those taken during the second to eleventh months varied from 150 to $238 \times 10^{-5}$. In the twelfth month, only one specimen was taken and that eight days after the infant had ceased to be breast-fed. The conductivity of this specimen was $843 \times 10^{-5}$. All these measurements were made at $18^\circ C$.

Jackson and Rothera (1914) made a fuller investigation on the electrical conductivity of milk taken from twenty-two women. Their results showed that the conductivity of colostrum was $321$ to $391 \times 10^{-5}$ that for normal milk was $214$ to $315 \times 10^{-5}$, and that for one specimen of abnormal milk was $959 \times 10^{-5}$. All the values were obtained from milk tested at $25^\circ C$. They stated that milk taken in the evening had lower conductivity values than milk taken in the morning, and in five out of six cases the conductivity was higher in the after-milk than in the fore-milk.

The number of cases and the number of observations
in each of these references are too few to draw
definite conclusions regarding the electrical
conductivity of women's milk in general. The
results, however, show that conductivity values tend
to vary greatly and that the stage of lactation and
the time of day or stage of feed at which the specimen
was taken may influence the results. Further in-
vestigation might reveal more exactly the extent to
which the electrical conductivity of women's milk
does vary and the factors which influence the results.
Moreover, such an investigation might show a correla-
tion between the conductivity values and either the
chloride content of milk or the milk yield, or both.
Before enquiring into the factors which influence the
conductivity of milk and the possible relationship of
milk conductivity to the chloride content of milk and
milk yield, the present knowledge of electrical
conductivity of cows' milk was reviewed in the hope of
obtaining guidance in the electrical conductivity of
women's milk. The findings of this review are
summarised below:-

The measurement of the electrical conductivity of
cows' milk has been widely used throughout the world
for over fifty years to test the normality of milk.
Much attention has, therefore, been devoted to methods
of sampling the milk and to the influence of various
constituents of milk, of the animals' food intake,
and the cows' state of health and stage of lactation
on the conductivity value of the milk specimens.

The electrical conductivity test for cows' milk is applied either to the milk taken from one-quarter of the udder, or to the pooled milk from a head of cattle. When milk from one-quarter is tested, the specimen taken may be a sample of the total yield, a sample of the fore-milk or of the after-milk, depending on the technique favoured. Recently, McCulloch (1944) stressed the importance of testing the strippings after detecting milk from mastitis affected cattle, because such milk is likely to reveal an abnormality more readily than other fractions obtained at milking. It is, however, not sufficient to test one-quarter of an udder; all four quarters must be tested from quarter to quarter, as in disease only one-quarter may be affected. (Winckel, 1905; Krenn, 1929; Dounhofer and Moser, 1930). A comparison of the results of tests made on fore-milk with those on after-milk show that in healthy cows the conductivity of the fore-milk is higher than that of the after-milk (Peterson, 1904, and Jackson and Rothera, 1914), but where mastitis is present the opposite is true (McCulloch, 1944). Jackson and Rothera (1914) attributed the lower values found in the after-milk of normal cows to the greater fat content of the after-milk compared with that of fore-milk, and stated that if the fat were removed from both the fore and after-milk before testing, the
value of the after-milk might be either more or less than that for the fore-milk. The same authors also noted that evening specimens of milk usually had higher conductivity values than morning specimens and attributed this to the animals resting and taking little or no food during the night, while they fed and exercised during the day.

With these changes in activity, the authors believed that the osmotic concentration of the blood altered, and that this, in turn, influenced the composition of the milk.

A number of observations have been made in the temperature and the chloride content of the milk in relation to the electrical conductivity of the milk. It has been shown that the electrical conductivity of cows' milk varies with change in temperature. Therefore, the temperature of the sample at the time of the test must be carefully read and recorded. When the temperature is in the region of 15°C, the conductivity increases by 2.3 per cent for every one degree rise in temperature (Coste and Shelbourne, 1919). Messener (1934) gave the percentage increase as 2.17 for every degree rise in temperature from 0 to 15 degrees centigrade and as 2.26 for every degree rise between 15 and 30 degrees centigrade. The temperature coefficient for "pathological" milk (Muller, 1930) and for pasteurised milk (Achard and Stassano, 1925) is the same as for normal milk. Thus,
TO SHOW CLOSE CORRELATION BETWEEN MEAN CONDUCTIVITY VALUE AND CHLORIDE VALUE OF COW'S MILK.
the taking of conductivity measurements of cows' milk at high or low temperatures (Guagliarello, 1919) does not matter, provided the temperature at which the measurement is made is recorded.

Although there are numerous electrolytes in milk, the only one which has been carefully studied in connection with electrical conductivity is chlorine. Flohil (1911) has stated that chlorine in the form of chloride accounts for fifty-seven per cent of the conductivity of milk, but other authors believe it accounts for forty-nine to seventy-eight per cent of the conductivity. (Coste and Shelbourne, 1919). Even so, all the chloride in the milk does not influence the conductivity, chloride bound with organic material being neutral in this respect. (Koeppe, 1898). The dominant part which chloride plays in the conductivity of milk explains the close correlation found between these two properties. This relationship is shown in Figure III (Page 44) where the mean conductivity value of milk is given for specimens of different chloride values. (Davis et al, 1943). The figure shows that the conductivity varies directly with the chloride content of milk. Because of this observed relationship, which has been recognised for many years, the conductivity measurement is sometimes made instead of the chloride test for normality of cows' milk. (Schorstein et al, 1929).

The influence of certain non-electrolytes upon
the electrical conductivity of milk has also been investigated. The sugar present in milk has been shown to inhibit conduction (Schweers, 1932) in such a way that there is an inverse relationship between the sugar content and conductivity of milk (Jackson and Rothera, 1914). This applies to both women's milk and cows' milk. Milk protein also reduces the conductivity of milk by 10 to 17 per cent (Schnorf, 1905) or 2.5 to 2.76 per cent for every gram of protein present. (Bagarsky and Tangl, 1899, and Jackson and Rothera, 1914). Taylor (1913) stated that for every five per cent of fat present in milk the conductivity is reduced by 11.4 per cent. Davis (1936), however, considered the figure of 11.4 per cent high and partly due to experimental error. The method of measuring the fat content of milk by noting the change in electrical conductivity of milk which occurs with the removal of fat, however, is not accurate and, therefore, is of no practical importance (Thorner, 1891, and Krenn, 1932). This effect of butter fat on the conductivity of milk, together with the variable fat content of milk have led Jackson and Rothera (1914) to the conclusion that when conductivity measurements are made on milk, they should be made on fat-free specimens.

The possibility of a relationship between electrical conductivity of milk and the non-fatty
solids has been investigated. At present, the position is ambiguous because Taylor (1913) and Coste and Shelbourne (1919) maintain no relationship exists, while Krenn (1930) believes that there is an inverse relationship between the two.

It has been shown, however, that in cows there is a very definite relationship between the conductivity of milk and milk yield. It is generally accepted that the conductivity of milk is relatively high for milk taken from cows in the first three weeks of lactation and in the terminal stages of lactation, as compared with that for milk taken in the intervening period. This difference in conductivity of milk is associated with a change in milk yield, the yield being lower during the initial and terminal phases of lactation than it is during the intervening period. It has also been shown that the electrical conductivity of milk separately from the four quarters of a cow’s udder differs and that the difference is related to the amount of milk obtained from each quarter, the larger milk yield being associated with the lower conductivity. (Winckel, 1905, and Krenn, 1938). Krenn (1938) has shown the following relationship between the milk yield and the electrical conductivity of milk. For quarters yielding more than two litres milk per day, the conductivity of the milk is 42 to 47 x 10⁻⁵, for those yielding one to two litres the conductivity of milk is 47 to 54 x 10⁻⁵, and for those yielding less
than one litre the conductivity is $54 \times 10^{-5}$ at 25°C. Exceptions to the above findings, however, do occur.

Research into factors which influence the composition and quantity of cows' milk has shown that climatic conditions, diet and the health of the cows themselves may affect both the milk yield and composition. These changes must be taken into consideration when studying the electrical conductivity of milk. For example, hot, dry weather, by making the cow produce milk with an altered chemical composition, which in turn results in a rise in the electrical conductivity of the milk. Conversely, cold weather results in a decreased electrical conductivity (Jackson and Rothera, 1914). Similarly, in diseased cows with a reduced milk yield, the chloride content of the milk increases and the increase causes, in turn, an increase in conductivity. Again, defective food intake and particularly starvation can also reduce milk yield and increase the electrical conductivity of the milk. (See Pages 10 and 11). Under these circumstances, the electrical conductivity of the milk will be greater than normal. Such changes in electrical conductivity of milk, however, are not apparent unless the cattle consume a grossly unbalanced or insufficient diet over a period. (Fredholm, 1943).

It is apparent from the review on the electrical conductivity of milk that few observations have been made on women's milk and that the results vary over a
wide range. The very limited investigations in this field do not reveal any factors which might account for the variations. It is, however, possible that the factors which influence the conductivity of women's milk may be similar to those which control the conductivity of cows' milk. These can be summarised as follows: - the health of the cow and her stage of lactation, the quality and quantity of her food, and climatic variations. Under normal conditions, variations in feeding and climate have less effect upon the conductivity of milk than have the health and stage of lactation, and the periodic taking of food does not explain variations in milk conductivity. The importance of testing milk specimens from each quarter of the cow's udder because of the variation in values for each individual sample has been proved, and it is probable that it is equally important to test both breasts when investigating the conductivity of human milk and for the same reason. Moreover, in cows, two samples should be taken from each quarter, one in the morning and one in the evening, because the conductivity values of milk taken throughout the day may differ. In investigating human milk, a similar technique might prove profitable. To obtain as much information as possible regarding the electrical conductivity of milk, it is probably necessary to take specimens of both fore-milk and after-milk, for it has been noted that in cows the value of fore-milk
taken from healthy beasts tends to be higher than that for the after-milk, whereas the value for after-milk taken from cows with udder disease is higher than that for their fore-milk. A similar relationship may occur in human lactation.

The review of the electrical conductivity of cows' milk has shown such estimations may have a practical application because there is a close relationship between the electrical conductivity value and the chloride value for a given specimen of milk, and because the chloride test is known to be of considerable assistance in detecting milk from cows with udder disease. It has been shown, too, that a correlation exists between the electrical conductivity of milk and milk yield in cattle. Although this relationship is of no practical value in dairy-farming, since it is much easier and more accurate to learn the milk yield of a cow by milking her, it may be of great value in detecting sub-normal milk yields in women.
FIGURE IV A

Model 110A. Circuit Diagram.

FIGURE IV B
I. RESISTANCE BRIDGE:

The apparatus used for measuring the electrical conductivity of milk was the Taylor Model, 110A, Capacity and Resistance Bridge made by Taylor Electrical Instrument Ltd., Slough. The instrument was designed to give quick and accurate measurement of resistance. It consisted of a conventional bridge and included a "Magic-Eye" tube or Cathode Ray Indicator which showed when the bridge was balanced. Alternating electrical current at 50 cycles was used for running the apparatus. (Figure IVA Page 50).

Figure IVB (Page 50) shows a cable attached to the apparatus which was connected to the electric mains. The central lower switch turned the current on and off. Two variable controls were then adjusted to make a measurement of the resistance in a cell which will be described later and connected to the "Test" terminals on the Resistance Bridge. The indicator of the right variable control was turned to the point on the dial marked 1-120 ohms. The pointer on the large variable control occupying the upper
two-thirds of the instrument panel is moved throughout its whole range, i.e. 1 to 12 in 120 steps. Each step on the dial represents 1. The "Magic-Eye" in the upper part of the large dial showed whether the two circuits were balanced by the "V" shadow at the 12 o'clock position in the "Magic-Eye" dilating to its maximum and becoming clearly defined. The reading of the resistance was made at this point. If the circuits did not balance, no such change in the "Magic-Eye" occurred and the right-hand variable control was then turned to the next range of resistance, 120-1200 ohms. marked on the disc, and the large variable control rotated as before. Each step on the large dial then measured 10 ohms. Resistances within the following ranges: 1200-12000; 12000-120,000; 120,000-1,200,000; 1,200,000-12,000,000 could be measured by moving the indicator of the right lower control to the corresponding values and making the final adjustment with the large upper variable control to get the precise measurement. The value of each step on the scale for the large control will vary when the right lower control is moved. The value of each step will equal the maximum measurable resistance in ohms, as shown by the indicator on the right-hand
Figure V
control divided by 120. The degree of accuracy in making resistance measurements of milk was to within half a division when the resistance was within the range of 12,000 to 120,000 ohms and to within a quarter of a division when the resistance was within the range of 120,000 to 1,200,000 ohms.

The apparatus was earthed when resistance measurements of milk were made by attaching the earth wire to the earth terminal on the right-hand side of the apparatus.

THE CELL AND ELECTRODES:

The milk on which estimations of electrical resistance was to be made was placed in a cell. The type of cell chosen for this purpose was designed after the one used by Eggleton et alia (1946) for measuring the conductivity of body fluids. The cell was constructed from glass tubing half-an-inch external diameter and three-eighths of an inch internal diameter. The tubing was drawn out and bent to form a U-shaped capillary, parts of the original tubing being left at each end to form two cups (Figure V, Page 52). The measurements are indicated on Figure V, (Page 52).

The two electrodes for the cell consisted of platinum wire. One end of each electrode lay inside
Figure VI
the lower end of a glass tube four inches long and one-quarter of an inch diameter, and was held in position by the lower end of the tubing being fused round it. The free end of the platinum wire was one inch long, the last half-inch forming a loop. The end of the platinum lying inside the tube was surrounded by mercury which filled three-quarters of the tube. One end of a thick copper wire dipped into the mercury in the glass tube to a distance of two-and-a-half inches. The other end of the wire was attached to the "Test" terminal on the Resistance Bridge. There were two such electrodes (Figure VI Page 53).

The loops of the electrodes were introduced into the cell containing the fluid to be tested and clamped into position. Owing to the fact that the resistance of the cell when filled with milk was almost entirely attributable to the fluid in the capillary part of the cell, slight disturbance in the position of the platinum loops makes very little difference to the measured resistance. It is, therefore, not absolutely necessary to keep the loops at exactly the same point throughout a series of measurements, but in fact, the positions were kept fairly constant throughout the course of the investigation on milk.
It was unnecessary to platinise the electrodes from time to time because the resistance of the cell was high (100,000 ohms) and so little or no polarisation took place even with an A.C. frequency as low as 60 cycles. From time to time, the electrodes were cleaned by dipping them in concentrated nitric acid, after which they were heated in a bunsen flame. To ensure efficient cleaning of the platinum electrode, it was held in an oxidizing flame of a bunsen burner until it was red-hot. The operation was performed before measuring resistance of any fluid.

The cell was cleaned from time to time with a mixture of potassium chronate and sulphuric acid. Before the cell was filled with the fluid for testing, it was carefully cleaned with warm water, distilled water, alcohol and ether applied in that order. Care was taken to see the cell was perfectly dry after it had been cleaned. If it was not, it was rinsed a second time with alcohol and then with ether.

When the cell was in use, it contained ½ to 1 c.c. of a well mixed sample of milk, the caps of the cell being approximately half-full. It was held in a water bath with the water level half-way up the caps and the loops of the platinum electrodes submerged in the milk in the lower half of the cups. One electrode was introduced into each cup and approximately the
same position was maintained from test to test. The temperature of the water in the both was kept at or near room temperature ($18^\circ$C). Each time the resistance was measured, the bath temperature was read to the nearest $\frac{1}{10}$°C. A standard thermometer was used for this purpose. The accuracy of this thermometer was checked by submerging the mercury-containing portion of the thermometer in melting ice water. It was then noticed that the freezing point was $-2^\circ$C instead of $0^\circ$C. This error was allowed for when making calculations for electrical resistance of milk at $18^\circ$C.

With apparatus complete and milk in the cell, the measurement of electrical resistance of milk took about two minutes. If measurements are made in a shorter time, there is a risk of the temperature of the milk and the water differing. If the measurements are taken after the milk has stood in the cell an hour or so, there is the danger that the fat globules may have risen to the surface and thus cause the resistance measurement to be abnormally low.
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**AVERAGE**

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**AVERAGE**

2.0
The resistance of each milk specimen was recorded, together with the temperature of the water bath. These temperatures differ from one another and in order to compare resistances, it is necessary to express them at the same temperature. The temperature chosen was 18°C because the results of most investigations of this nature in the past have been expressed at this temperature. To quote all resistance measurements of milk at 18°C necessitates the use of a conversion factor, this factor being the percentage change in resistance with one degree centigrade rise in temperature. To obtain the factor, the resistance of a given specimen of milk was measured at various temperatures and the percentage change calculated. The results of this investigation are given on Table 23 (Page 56) which shows the percentage change in resistance for one degree centigrade rise in temperature for whole milk and also for partially separated milk. The mean percentage change in resistance per degree rise in temperature for whole milk is 2.1% and 2% for partially separated milk. The factor 2.1 is used in the following way: if a resistance "R" taken at 13°C is to be expressed at 18°C, the resistance will fall because the temperature rises, therefore the value of the
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<th>Cell Constant</th>
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</tr>
<tr>
<td>15.75</td>
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Average Cell Constant  250.7
resistance at 18°C will be

\[ R - \left( \frac{R}{100 \times 2.1 \times 5} \right) \]

In order to calculate the electrical conductivity of milk from the measured resistance of the milk in the cell, it is necessary to determine the cell constant. This cell constant is the constant which appears in the equation \( R = \frac{K}{C} \) where "C" is the specific conductivity of the liquid in the cell and "R" is the resistance of the cell when the cell is filled with the liquid with this conductivity. "K" varies with the size and shape of the cell and is conveniently determined by measuring the resistance of the cell when it is filled with a fluid with a known specific conductivity. The solutions which were used for the determination of the cell constant were \( \% \) KCl and \( \% \) KCl because they have a resistance similar to that of milk. Provided the two cell constants obtained by using the two solutions are the same, the accuracy of the procedure is satisfactory. The resistance of the cell containing these solutions are given on Tables 24 and 25 (Pages 57 & 58). According to equation \( K = R \times C \), the value "K" is obtained by multiplying the "R" of the cell by the specific conductivity of the salt solution at the temperature at which the resistance was made (Reilly and Rae 1943).
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Average Cell Constant 248.3
"K" ranged from 244 to 255 and the mean was 250.7 when using \( \text{\% K Cl} \) and 248.3 when using \( \text{\% K Cl} \). The mean of these two readings is 249.5, which is the cell constant used in all the calculations of the electrical conductivity of milk. The cell constant was repeated from time to time and no significant change was noted throughout the investigation.

To obtain the specific conductivity of milk, the equation was used in the form \( C = \frac{K}{R} \) i.e. the cell constant is divided by the resistance in ohms of the cell containing the milk. The values thus obtained are on Pages 1 to 60 in the Appendix.
A POSSIBLE ERROR IN ELECTRICAL CONDUCTIVITY MEASUREMENT DUE TO STORAGE OF MILK.

When the milk is stored, its electrical conductivity can alter and it is, therefore, necessary to know whether or not this occurs when milk is kept under constant conditions for twenty-four hours. Should an alteration be noted, the time of its onset and the extent of the change would have to be determined.

For this enquiry, fifty-two samples of women's milk were obtained and the electrical conductivity of such specimen was taken within three hours of the milk being removed from the breast. The milk specimens were then stored at a constant temperature of 12°C for twenty-four hours, after which their electrical conductivity was again measured. The results are recorded in the Appendix (Pages 48 and 49).

The average conductivity of the fifty-two fresh specimens was $212.6 \times 10^{-5}$ and after they had been stored twenty-four hours, it was $211.5 \times 10^{-5}$.

The average conductivity value, therefore, is 0.5 per cent lower after twenty-four hours. This figure is insignificant. The conductivity of individual specimens of milk may be either increased or decreased by as much as six per cent after it has been stored. This variation is almost entirely due to experimental error.
From this investigation, it can be concluded that no significant error arises as a result of making electrical conductivity measurements on women's milk which has been kept for varying periods up to a maximum of twenty-four hours, provided the specimens are kept at $12^\circ$C.
Jackson and Taylor (1914) have stated that when measuring the electrical conductivity of cows' milk, it is preferable to first remove the fat, because the fat content of milk is variable and the removal of the fat reduces the range of conductivity values. For this reason, it was thought possible that the range of electrical conductivity values for women's milk might also be smaller if fat-free milk were used for the estimations instead of whole milk. Moreover, the difference between the values obtained for milk taken from mothers with adequate lactation and those obtained from mothers with inadequate lactation might be more definite if fat-free milk were examined instead of whole milk.

To determine the advantage, if any, of using fat-free women's milk for electrical conductivity measurements, it was necessary to solve two, or possibly three, problems. The first was to devise a suitable technique for separating the fat from the milk, and the second was to investigate the percentage change in the electrical conductivity of milk when the fat was removed. If this change was appreciable, it was decided that a third investigation would be necessary to determine the range of electrical conductivity measurements of fat-free milk and decide whether this
range made the test more valuable as a means of detecting either milk from women with adequate lactation or that from women with inadequate lactation.

The first experiment, to obtain a suitable technique for separating fat from milk, depended, as in other studies of fat-free milk, on the use of a centrifuge. It was, however, necessary to determine the length of time a milk specimen required to be spun before the fat was separated. For this purpose, fifteen specimens of milk were spun for fifteen minutes at approximately two thousand five hundred revolutions per minute, after which a layer of fat lay on top of the separated milk. The separated milk was then removed from the layer of cream and the electrical conductivity of each specimen estimated. Each specimen of separated milk was again centrifuged for a further fifteen minutes and its electrical conductivity measured a second time using the same technique as for the previous conductivity estimations. The values of the conductivity measurements of milk spun for fifteen minutes and those of the same milk spun for thirty minutes are seen in Table 26 (Page 50). The difference in the average values for the two groups is 0.3 per cent, and for individual values ranges from 0 to 6 per cent. The individual variations are almost entirely due to experimental errors and the difference between the average value for the two groups is statistically
insignificant. Therefore, it is unlikely that spinning milk for more than fifteen minutes at two thousand five hundred revolutions per minute will result in a better separation of fat from women's milk, than is obtained by spinning for fifteen minutes only.

The second problem, to investigate the percentage change in electrical conductivity when fat is removed from milk, involved the examination of a series of twenty milk specimens. Each specimen was divided into two parts, one part was used first to determine the electrical conductivity of the whole milk, and then for the estimation of its fat content by the Gerber Method. The fat was removed from the second part of each specimen by spinning it for fifteen minutes at approximately two thousand five hundred revolutions per minute. The electrical conductivity and the fat content of the separated milk which results from this process were then ascertained using the same technique as was employed for the examination of the whole milk. The results of the investigations are recorded in the Appendix (Page 50). From these figures, the percentage change in electrical conductivity of milk due to the removal of fat from the milk was calculated and the results are to be found in the Appendix (Page 51). By correlating the change in the electrical conductivity of individual specimens before and after centrifuging with the amount of fat removed from the specimen
as shown by the Gerber technique, it has been possible to calculate the percentage change in the conductivity of the milk specimens due to the removal of one per cent of fat from the milk. The results of this calculation range from 0.92 to 3.84 per cent change in conductivity, with an average change of 1.93 per cent for one per cent of fat removed. (Appendix Page 51). As the fat content of the milk specimens ranges from 1.1 to 6.2 per cent, the percentage reduction in conductivity of the specimens due to their fat content will range from 1.1 to 6.2 per cent multiplied by 1.93 per cent. Therefore, the actual reduction in conductivity varies from 1.9 to 11.3 per cent. Although the results of this investigation are variable, it has shown that the percentage change in the electrical conductivity of milk due to the presence of fat can be considerable. It was, therefore, desirable to remove the fat from a large series of milk specimens before measuring their electrical conductivity in an attempt to determine whether such a removal would lessen the variation in electrical conductivity found between different specimens of human milk.

In a third series of milk specimens, the value of estimating the electrical conductivity of separated milk was assessed. The electrical conductivity of each of two hundred and ten specimens of milk taken from mothers in the first month of lactation and that of one hundred and eighty specimens from mothers in
FIGURE VII

MILK
WHOLE
SEPARATED

MILK
WHOLE
SEPARATED

ELECTRICAL
CONDUCTIVITY

600
500
400
300
200

1st MONTH
LACTATION

2nd-5th MONTH
LACTATION
the second to fifth month of lactation was measured. Both mothers who were lactating adequately and those lactating inadequately were included in the investigation. After the estimation of the electrical conductivity, each specimen was spun for fifteen minutes at approximately two thousand five hundred revolutions per minute. The electrical conductivity of the separated milk was then removed. The results of the investigation are seen in Figure VII (Page 65). The three hundred and ninety values for whole milk are plotted in two groups; one for values of milk taken from women in the first month of lactation, and the other for values of milk taken from women in the second to fifth month of lactation. The three hundred and ninety values for separated milk are also plotted in two groups; one for values for milk taken from women in the first month of lactation, and the other for values of milk taken from women in the second to fifth month of lactation. The values for milk taken from women with adequate lactation and represented by a circle (O) and those for milk taken from women with inadequate lactation are represented by a cross (X).

From a comparison of these groups of values for women's milk, it is apparent that the scatter of the results for separated milk does not differ appreciably from that attained by estimating the electrical conductivity of whole milk. The intermingling of
values obtained from mothers with adequate lactation with those obtained from women with inadequate lactation is just as great when comparing the results for separated milk with those for whole milk. These findings apply to values for milk taken from women both in the first month and in the second to fifth month of lactation. It is, therefore, concluded that there is no advantage in separating fat from women's milk before making electrical conductivity measurements.
The electrical conductivity value of women's milk taken before and after a breast feed.

The milk specimens used for the comparison of the chloride values of fore and after-milk were also used to compare the electrical conductivity of fore and after-milk. The determination of the conductivity of the specimens was made in the same way as in previous investigations. The results are in the Appendix (Pages 35 to 37). Inspection of these shows that there is a tendency for the values to be greater in the after-milk than in the fore-milk but this is not invariably so. To decide whether the difference between the average value for the fore-milk and that for the after-milk is statistically significant, two tests have been applied to the data.

The statistical analysis was made by first applying the "t" test to the data (Appendix Pages 52 to 54) and the value for "t" was 1.0 which means that the difference between the average value for fore-milk and that for after-milk is not statistically significant. The "analysis of variance" was then applied to the same data because the method was considered preferable to the "t" test for reason given on Page 22.
From the calculation (Appendix Pages 52 to 54), the value of "Z" was -0.318. This result confirms the conclusion obtained by applying the "t" test to the data, that is, the difference in the average values for fore-milk and after-milk is statistically insignificant.

Because the average electrical conductivity value for fore-milk does not differ appreciably from that of after-milk, it might be argued that it is immaterial whether specimens of fore-milk or after-milk are used for an investigation. However, because of the tendency for the values for after-milk samples to be higher than those for fore-milk, it is advisable always to take specimens of fore-milk or else always to take the after-milk. In so doing, the chances of error in sampling is less.
A review of investigations made upon the electrical conductivity of women's milk shows that electrical conductivity has not been correlated with the chloride content of women's milk. If a close correlation between the two factors did exist, it would indicate that measurements of electrical conductivity could be used as an alternative to chloride estimations for the detection of abnormal milk. The possibility of such a relationship has, therefore, been investigated.

For the investigation, each of six hundred and sixty-eight specimens of milk, taken from mothers in the first five months of lactation, was examined for both electrical conductivity value and chloride content. The resulting values are given in the Appendix (Pages 1 to 25). To determine the relationship between the electrical conductivity value of milk and its chloride content, the conductivity value for each specimen has been plotted against the chloride value of the same specimen (Figure VIII Page 69). The points thus obtained are represented in Figure by dots. The dots are packed fairly closely together within an area which runs diagonally across the graph. It is apparent from the graph that the higher the
TABLE 26.

THE CORRELATION OF ELECTRICAL CONDUCTIVITY VALUES OF WOMEN'S MILK WITH THE CHLORIDE VALUES OF WOMEN'S MILK.

<table>
<thead>
<tr>
<th>Number of Specimens</th>
<th>Chloride Content mg%</th>
<th>Average Conductivity ( x 10^{-5}) at 18°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>380</td>
<td>25 - 75</td>
<td>192.2</td>
</tr>
<tr>
<td>191</td>
<td>75 - 125</td>
<td>245.1</td>
</tr>
<tr>
<td>44</td>
<td>125 - 175</td>
<td>299.5</td>
</tr>
<tr>
<td>26</td>
<td>175 - 225</td>
<td>383.3</td>
</tr>
<tr>
<td>13</td>
<td>225 - 275</td>
<td>460.6</td>
</tr>
<tr>
<td>13</td>
<td>275 - 325</td>
<td>563.4</td>
</tr>
<tr>
<td>1</td>
<td>325 - 375</td>
<td>651.0</td>
</tr>
</tbody>
</table>

Data obtained from Appendix Page s 1 to 25.
electrical conductivity of women's milk, the greater is the chloride content of the milk. This relationship is also seen in Figure IX (Page 69), which has been constructed by plotting the average conductivity values for the specimens of milk of a given chloride content. These averages have been calculated from data used for Figure VII and they have been recorded in Table 26 (Page 70). In Figure IX a second line has been plotted which shows the relationship between the electrical conductivity of pure sodium chloride and the concentration of the sodium chloride in milligrams per cent. The strength of the solution varied from forty to three hundred and seventy-five mg. per cent. The comparison between this line and that obtained for milk values yields the following information:—Firstly, all but four of the conductivity values obtained for milk lie above those for pure sodium chloride of a similar chloride content. Therefore, milk is a better electrical conductor than pure sodium chloride. Secondly, the average electrical conductivity of milk tends to approximate to that of sodium chloride solution more closely as the percentage of milk chloride rises from twenty-five to one hundred and twenty-five mg. per cent. The average electrical conductivity of milk with a chloride content of over one hundred and twenty-five mg. per cent bears a more constant relationship to the conductivity of pure sodium chloride solution. This variable relationship between the average con-
ductivity of milk and the conductivity of pure salt is probably due to a variation in the proportions of electrolytes present in milk.

In conclusion, it has been shown that electrical conductivity of women's milk varies directly with the chloride content of that milk and, for this reason, conductivity measurements are as informative as chloride estimations when investigating the abnormality of women's milk.
FIGURE X

COMPARISON OF ELECTRICAL CONDUCTIVITY VALUES OF MILK FROM MOTHERS WITH ADEQUATE LACTATION (○) WITH THOSE FROM MOTHERS WITH INADEQUATE LACTATION (×).

LACTATION
The practical value of estimating the electrical conductivity of milk from women has been assessed by an investigation carried out along the same lines as were adopted for determining the practical value of chloride estimations on human milk. Practically all the women and milk samples used for the milk chloride investigation were also used for the investigation of milk conductivity. Two series of observations were made. In the first series, five hundred and thirty-two specimens of milk were examined; three hundred and forty-six were taken from women with adequate lactation and one hundred and eighty-six from women with inadequate lactation. In the second series of observations, one hundred and thirty-six samples were examined; seventy-two were obtained from mothers who were lactating adequately, and sixty-four from mothers who were lactating inadequately. Every mother in this second series provided four samples of milk, two from each breast.

The method of this investigation was practically the same as that used in the previous section to test the practical application of the milk chloride test, except that the conductivity estimations were made in place of chloride determinations. The technique
To show the percentage of mothers with adequate lactation and milk with an electric conductivity value below the "critical" level for their stage of lactation.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>3-4 weeks</th>
<th>2 month</th>
<th>3-5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Critical&quot; level conductivity ( \times 10^{-5} )</td>
<td>225</td>
<td>210</td>
<td>200</td>
</tr>
<tr>
<td>Number of mothers investigated</td>
<td>29</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>% mothers with milk conductivity below &quot;critical&quot; level</td>
<td>96.6</td>
<td>100</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Data obtained from Appendix, Pages 1 to 12.
employed to measure the electrical conductivity was that previously described and will be found on Pages 49 to 57 and the experimental results in the Appendix (Pages 1 to 29).

A summary of the findings of both series of cases is presented (Figure X, Page 72) in a similar way to those of the milk chloride investigation. Figure X (Page 72) shows that the conductivity values of milk taken from mothers with inadequate lactation are generally higher than those for milk taken from mothers with adequate lactation. As in the case of chloride values, it is possible to fix "critical" levels for each stage of lactation below which lie the majority of values for women with adequate lactation and above which lie the majority of values for women with inadequate lactation. The "critical" level of milk specimens taken during the third and fourth week of lactation is at $225 \times 10^{-5}$; for milk taken in the second month of lactation it is at $210 \times 10^{-5}$, and for milk taken in the third to fifth month of lactation it is at $200 \times 10^{-5}$. For milk taken in the second week, two "critical" levels are used for diagnostic purposes; the lower one at $225 \times 10^{-5}$ and the higher one at $275 \times 10^{-5}$. Values below the lower "critical" level are generally associated with adequate lactation, while those above the upper "critical" level are usually associated with inadequate
To show the percentage of mothers with inadequate lactation and milk with an electrical conductivity value above the "critical" level for their stage of lactation.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>3-4 weeks</th>
<th>2 months</th>
<th>3-5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Critical&quot; level conductivity ( \times 10^{-5} )</td>
<td>225</td>
<td>210</td>
<td>200</td>
</tr>
<tr>
<td>Number of mothers investigated</td>
<td>24</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>% mothers with milk with conductivity above &quot;critical&quot; level</td>
<td>100</td>
<td>90.9</td>
<td>91.7</td>
</tr>
</tbody>
</table>

Data obtained from Appendix, Pages 1 to 12.
lactation. Values lying between the two "critical" levels are without diagnostic significance.

The importance of the "critical" levels in distinguishing cases of good lactation from those of bad lactation is illustrated in Table 27 (Page 73). This Table shows that at least ninety-six per cent of the women with adequate lactation have conductivity values for their milk which lie below the relevant "critical" level, and ninety-four per cent of the women with inadequate lactation (Table 28, Page 74) have milk values which lie above the "critical" level for their stage of lactation. Therefore, it is generally possible to tell whether or not a mother has adequate lactation by noting whether the result of the electrical conductivity test on her milk falls above or below the "critical" level for her stage of lactation; e.g. a mother in the third month of lactation has milk with a conductivity value of $180 \times 10^{-5}$. She will almost certainly have sufficient milk for her infant at the time of the test because the conductivity value of her milk is less than $200 \times 10^{-5}$, the "critical" level for her stage of lactation.

The results of the electrical conductivity estimations made on milk obtained from a series of forty-four women who had both breasts tested during their third week to fifth month of lactation (Appendix Pages 1 to 20 and 60) have elucidated some of the anomalous conductivity results obtained in the first series. Thus, in the series of forty-four women, three women with adequate lactation and five with
### TABLE 35

**WOMEN WITH UNILATERAL BREAST DYSFUNCTION AS JUDGED BY THE ELECTRICAL CONDUCTIVITY OF THEIR MILK.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Conductivity of Breast Milk</th>
<th>Critical Conductivity Level</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>358</td>
<td>180</td>
<td>251</td>
<td>200</td>
</tr>
<tr>
<td>390</td>
<td>209</td>
<td>174</td>
<td>200</td>
</tr>
<tr>
<td>415</td>
<td>200</td>
<td>214</td>
<td>200</td>
</tr>
<tr>
<td>326</td>
<td>170</td>
<td>203</td>
<td>200</td>
</tr>
<tr>
<td>361</td>
<td>196</td>
<td>233</td>
<td>210</td>
</tr>
<tr>
<td>385</td>
<td>238</td>
<td>193</td>
<td>210</td>
</tr>
<tr>
<td>385</td>
<td>213</td>
<td>192</td>
<td>200</td>
</tr>
<tr>
<td>433</td>
<td>228</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

*Electrical conductivity values $\times 10^{-5}$*
inadequate lactation have unilateral breast dysfunction as judged by the electrical conductivity test (Table 35, Page 75). These findings are similar to those obtained in the second series of observations on the milk chloride test. In these cases, it is impossible to draw any conclusion as to the adequacy of lactation from the conductivity test alone.

The incidence of unilateral breast dysfunction bases on observations made on the series of forty-four cases is three to twenty-two, or 13.6 per cent of mothers with adequate lactation, and is five to twenty-two, or 22.7 per cent of mothers with inadequate lactation. The percentage is quite considerable and it is probable that many of the anomalous cases noted in the first series had unilateral breast dysfunction. In order to detect such cases, and thus to increase the accuracy of the electrical conductivity test, milk from both breasts must be tested.

The question arises whether in women with unilateral breast dysfunction, the mean conductivity value of milk taken from both breasts will be a guide to the adequacy of the milk supply. Because of the small number of such mothers, no definite conclusion can be drawn from the present results. However, three such women had a mean conductivity value for milk taken from both breasts lying above the relevant "critical" level, suggesting that the mothers were lactating adequately. This was confirmed in two
cases only by weighing the mother's infant. In the remaining six women with unilateral breast dysfunction the mean conductivity values in each case lay below the relevant "critical" level, so suggesting adequate lactation. In only two cases was this confirmed by test-weighing. In the other three cases, lactation was inadequate.

There was one other anomalous result in the second series, apart from those due to unilateral breast dysfunction. In this case, the conductivity value of milk from both breasts was low - below the relevant "critical" level - and yet the mother was lactating inadequately. A similar result was obtained from the chloride values of the same woman. The explanation is obscure, but the phenomenon is possibly due to hypofunction of the breast, resulting from lack of secretory tissue.

The conclusions drawn from the investigation on the electrical conductivity of milk are similar to those for the investigation on the chloride content of milk. That the electrical conductivity test is a valuable indication of the adequacy of lactation, particularly if both breasts are tested and the results from both are similar. That if the conductivity value of milk from one breast is below the relevant "critical" level while that of the other above the "critical" level, no definite decision on the
adequacy of lactation can be made. In such cases, the infants should be test-weighed.

It is possible that further investigation of the conductivity values of milk may show that the mean value for both breasts is of diagnostic significance. It seems clear that in a few cases mothers may have low conductivity values for milk from both breasts and yet have an inadequate milk yield. This type of case, however, appears to be of infrequent occurrence, only one being encountered in forty-four cases.

When assessing the significance of results obtained by this test, it is important to bear in mind that the results of the test may prove unreliable should the mother be ill when her milk is examined, or should the infant be either premature or ill at the time of the test.
THE COMPARISON OF THE ELECTRICAL CONDUCTIVITY
OF MILK TAKEN FROM THE RIGHT BREAST WITH THAT
OF MILK TAKEN FROM THE LEFT BREAST.

To determine whether there is an appreciable
difference in the electrical conductivity value for
milk from the right breast and that for milk from
the left breast, one hundred and fifty-two com-
parisons have been made. From any one woman, at
least two samples of milk were taken, one from
the right breast and one from the left breast.
These samples were taken at the same time and
just before the mother was due to feed her infant.
The electrical conductivity of each sample was
measured and the results have been recorded in the
Appendix (Pages 56 to 59).

The information obtained from the results of
the investigation is (firstly) the electrical
conductivity values for milk taken from the two
breasts of any one woman usually differ. The
mean of the absolute value of the one hundred and
fifty-two differences is $30.2 \times 10^{-5}$ . Of the
one hundred and fifty-two differences, one hundred
and one, or 66.4 per cent, were less than $30.2 \times
10^{-5}$ and fifty-one, or 14.6 per cent, exceeded
$30.2 \times 10^{-5}$ (Secondly), the average electrical
conductivity values for all specimens taken from
the right breast is slightly greater than that for
all specimens taken from the left breast, the respective values being $208.7 \times 10^{-5}$ and $203.7 \times 10^{-5}$. The difference between the two averages is five, which is very little, and to decide whether it is statistically significant, the following calculation has been made.

For the statistical analysis for determining whether the average electrical conductivity value for milk taken from the right breast is significantly different from that for milk taken from the left breast, the one hundred and fifty-two differences were regarded as a set of one hundred and fifty-two observations whose real mean should be zero, but whose actual mean was $5 \times 10^{-5}$. The standard deviation of the one hundred and fifty-two observations about zero point was calculated in the usual way by dividing the sum of one hundred and fifty-two differences by one hundred and fifty-two and then obtaining the square of the resultant figure, and was found to be 46.6. If 46.6 is regarded as the true standard deviation, it follows that the standard error of the mean of the one hundred and fifty-two observations taken at random is

$$\frac{46.6}{\sqrt{152}} = 3.2$$

Hence, the deviation is $5/3.8 = 1.3$ times the standard error. "$p"$ is equal to approximately
therefore, there is about a twenty per cent chance of the observed difference occurring.

It can be concluded that there is no natural tendency for the electrical conductivity of milk from the right breast to be higher, or lower, than that of milk from the left breast. Nevertheless, there is, on the average, a difference of 14.6 per cent between the electrical conductivity values of milk from the two breasts. Moreover, the maximum difference can amount to 120 per cent. Therefore, it is essential when investigating the electrical conductivity of milk from any woman to test samples taken from both the right and the left breast.
COMPARISON OF DAILY MILK YIELD FROM ONE BREAST WITH THE ELECTRICAL CONDUCTIVITY OF THE MILK.

In a previous section (Pages 69 to 71) it has been shown that the electrical conductivity of women's milk is related to the adequacy of their lactation. It is, therefore, possible that a relationship exists between the electrical conductivity of milk and the daily milk yield. This hypothesis has been supported by evidence obtained from a series of observations made on women who were breast-feeding their infants. This consisted of measuring the daily milk yield from one breast and of the electrical conductivity of the milk from that breast. The method of test-weighing the infant to obtain the milk volume and of obtaining three milk samples was the same as was used when comparing daily milk volumes with the chloride content of milk (Page 34). In this case, forty-seven mothers in the first month of lactation, and fifty-nine mothers in the second to fifth month of lactation, were investigated.

The results of the investigation are set out in Appendix (Pages 56 to 60). They show that the milk volume at individual feeds does not bear a significant relationship to the electrical conductivity of the milk taken at the beginning of the feed, but the average daily milk volume does
TABLE 29

TO SHOW THE RELATIONSHIP OF THE MEAN ELECTRICAL CONDUCTIVITY VALUE OF MILK TO THE DAILY YIELD OF MILK FROM ONE BREAST.

<table>
<thead>
<tr>
<th>No. Cases</th>
<th>Milk Cond. x 10</th>
<th>Av. Milk Vol. oz. per day</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>371 - 390</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>351 - 370</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>331 - 350</td>
<td>2.25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>311 - 330</td>
<td>1.75</td>
<td>Inadequate</td>
</tr>
<tr>
<td>3</td>
<td>291 - 310</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>271 - 290</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>251 - 270</td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>231 - 250</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>211 - 230</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>191 - 210</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>170 - 190</td>
<td>13.1</td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from the Appendix (Pages 56 to 60)
bear a very definite relationship to the mean electrical conductivity of the milk; this is seen on Table 29 (Page 82) the average daily milk yield for one breast for women who are secreting milk of different electrical conductivity, the minimum yield being 0.5 oz. when the conductivity of the milk is $371 - 390 \times 10^{-5}$ and the maximum yield is 13.1 oz. when the conductivity of the milk is $170 - 190 \times 10^{-5}$. This shows that there is a close correlation between the average milk yields of groups of women and the conductivity of their milk. The milk yield of the individual women who constitute any one group may, however, differ markedly from each other in their milk yield at the time of the investigation. Therefore, the method of investigation does not form a reliable method of determining the milk yield of individual women.

To show how the variation of milk yield for women secreting milk of a given electrical conductivity value, Figure XI (Page 83) has been constructed from the data in the Appendix (Pages 56 to 60). In this Figure, the daily volume of milk from one breast has been plotted against the mean electrical conductivity value of the milk from the same breast. The daily milk volumes range from 0 to 24 ounces per day and the mean conductivity values of milk from $164 - 540 \times 10^{-5}$. The
FIGURE XI

COMPARISON OF MILK VOLUME FROM ONE BREAST IN ONE DAY WITH THE MEAN VALUE FOR ELECTRICAL CONDUCTIVITY OF THE MILK TAKEN ON THE SAME DAY:

- MILK TAKEN IN 1st MONTH OF LACTATION
- MILK TAKEN IN 2nd-5th MONTH OF LACTATION

MILK IN OZ

SPECIFIC CONDUCTIVITY

0 10 20 30 40 50 60

0 10 20 30 40 50

- 70 80 90 100 110 120

- 130 140 150 160 170 180

- 190 200 210 220 230 240

- 250 260 270 280 290 300

- 310 320 330 340 350 360

- 370 380 390 400 410 420

- 430 440 450 460 470 480

- 490 500 510 520 530 540

- 550 560 570 580 590 600

- 610 620 630 640 650 660

- 670 680 690 700 710 720

- 730 740 750 760 770 780

- 790 800 810 820 830 840

- 850 860 870 880 890 900

- 910 920 930 940 950 960

- 970 980 990 1000
variation in milk yield between women secreting milk of a given conductivity value differs in extent; this variation is negligible when the conductivity value is in the region of $540 \times 10^{-5}$ but becomes progressively more significant as the conductivity value diminishes, the maximum variation amounting to 20 oz. when women secret milk with a conductivity of $171 \times 10^{-5}$. Thus, although an inverse relationship between the electrical conductivity of milk and the daily milk yield does exist, it is not an accurate way of determining the milk yield of individual mothers.

Important deductions can be made, however, from this investigation. Firstly, when the electrical conductivity of milk is $280 \times 10^{-5}$ or less, the daily milk yield from one breast will not exceed 5.56 oz. or from two breasts will not be more than 11 oz. This quantity is inadequate for the needs of a normal infant during the second to fourth week of life, because Holt and McIntosh (1940) have stated that 13 to 26 oz. are required during this period. Secondly, if the conductivity of milk is $260 - 220 \times 10^{-5}$ the daily milk yield from one breast is likely to be 6.3 to 7.8 oz. Therefore, from two breasts, it will be 12.5 to 15.5 oz. per day which would usually be sufficient for infants in the second or third weeks of life. Thirdly, mothers in the second to fifth months of lactation must have milk
with a conductivity value of $200 \times 10^{-7}$ or less if their infants are to obtain 20 to 38 oz. of breast milk per day which is the amount necessary to maintain their health and normal development (Holt and McIntosh, 1940). The conclusions are the same as those obtained in the previous section where the electrical conductivity of milk was correlated with adequacy of lactation in women. It is apparent, therefore, that the estimation of electrical conductivity of milk constitutes a test of adequacy of lactation when the results are compared with the above criteria.

The estimation of the electrical conductivity of milk is certainly of value, provided the results are relatively high, i.e. over $210 \times 10^{-7}$ but when the conductivity values are less than $210 \times 10^{-7}$ their significance is sometimes doubtful because the variation in milk yield which accompanies such values is great and amounts to 20 oz. There are at least two reasons for the variation in milk yield. The first and most frequent explanation is that in ten out of the thirty-one tests in this group of cases, the demand made upon the breast was not great because the infants were under a month old (values "0" in Figure XI Page 83). In such cases, the relatively small quantities of milk obtained almost
certainly reflected on the demands of the infant rather than on the potentialities of the breast. Low milk yields accompanying milk with low conductivity values may also be due to hypoplasia of the breast. This condition is not common as is indicated in a previous investigation (Page 75) where only one out of forty-four women investigated was presumed to have this abnormality, i.e. inadequate lactation, although the milk from both breasts had an electrical conductivity below the relevant "critical" level. In this investigation, however, no values were obtained for women in the second to fifth month of lactation who had hypoplastic breasts of this type. It is, therefore, apparent that such cases will rarely be a source of error when interpreting the significance of the chloride value of milk in relation to milk yields.
THE STATISTICAL ANALYSIS OF CHLORIDE AND ELECTRICAL
CONDUCTIVITY. VALUES OF MILK TAKEN FROM MOTHERS WITH
ADEQUATE LACTATION AND THOSE OF MILK TAKEN FROM
MOTHERS WITH INADEQUATE LACTATION.

The data for statistical analysis was obtained from examination of five hundred and five specimens of milk taken from mothers whose babies thrived on their mother's milk and two hundred and ninety-two specimens from nursing mothers whose infants required complementary feeds. Every mother provided at least two specimens of milk on a given day during the first five months after parturition. Each specimen amounted to about 8.0 c.c. and one was taken in the early morning (6 a.m.) and one at mid-day (12 p.m. or 2 p.m.) before the breast feed. This procedure was adopted when investigating a series of two hundred and nineteen mothers and also for a second series of one hundred and eighteen mothers. The second series was done to provide more extensive data which seemed desirable after the first series had been completed. There was yet a third series of observations made on one hundred and twenty-three women from whom three milk samples were taken on the same day and at the following times: - early morning (6 a.m.), mid-day (12 p.m. or
2 p.m.) and evening (6 p.m. or 10 p.m.). Seventeen of these women had been excluded in the first series of observations and one hundred and six in the second. In any one woman the specimens were always taken from the same breast. Each specimen was tested for chloride content and electrical conductivity within twenty-four hours of taking the specimen. The chloride result was expressed in mg. sodium chloride per 100 c.c. milk, and the electrical conductivity in reciprocal ohms per centimeter at 18°C. The results were grouped according to the number of weeks or months which had elapsed between the birth of the baby and the taking of the milk specimen. There were seven groups, one for each of the following periods: second, third and fourth week of lactation, and second, third, fourth and fifth months of lactation.

A statistical analysis of the data has been made in order to answer the following four questions. Firstly, is the variation obtained between the average values of each of the above seven groups significant? Secondly, do the average values of chloride and electrical conductivity for early morning, mid-day and evening specimens of milk vary significantly from each other? Thirdly, does the quality of the milk (as judged by chloride content and electrical conductivity) secreted by mothers with an adequate supply for their babies differ from that
secreted by mothers with an inadequate supply?

Fourthly, if the latter comparison reveals a significant difference between milk taken from mothers with a satisfactory supply and that from mothers with an unsatisfactory supply, does this difference continue throughout the whole nursing period?

Two methods have been employed in the analysis: they are the comparison of means involving the "T" test and the analysis of variance involving the "Z" test. For example, the "Z" test has been applied to data derived from milk specimens with the object of deciding whether the observed differences in the average values from week to week and month to month were dependent on the stage of lactation, or arose accidentally from purely random causes. For it is evident that if sub-groups were found by withdrawing values at random from the total number of observations, then the mean values of these sub-groups would show a certain range of variation. Such a variation would be shown by the "Z" test to have no statistical significance. The problem is to decide whether the difference between the values of the sub-groups in the present series of observations has occurred by chance or has resulted from a real change in breast function. The details of the tests are given in the appendix.
**QUESTION I(a):** Is the Variation in Average Milk Chloride Values over the Seven Lactation Periods Statistically Significant?

To answer this question, the data has been arranged in a series of Tables (Table I A to 6A). Each Table gives data referring to the chloride values for milk taken during the following seven periods: the second, third and fourth week and the second, third, fourth and fifth month of lactation. The Tables contain data referring to mothers with adequate and those with inadequate lactation and to milk drawn at various times of the day, i.e. early morning (6 a.m.), mid-day (12 p.m. or 2 p.m.) and evening (6 p.m. or 10 p.m.). By using this data, it was possible to determine whether the average values for milk chloride varied significantly throughout the first five months of lactation.

The average chloride values in Tables I A to 6A differ from Table to Table and within a given Table, but in every Table the averages obtained for the second, third, fourth and fifth month of lactation tend to be lower than for the second, third or fourth week of lactation. To emphasise this point, the average value for specimens taken in the first month as a whole has been compared with the average value

* See Appendix Pages 61 to 66.
for specimens taken in the second to fifth months of lactation as a whole. Fourteen such comparisons have been made and they all show that the average chloride value for the second to fifth month of lactation is considerably lower than that for the first month. These comparisons are set out in Table 8A.\(^*\)

The "L" test and the "T" test were used in analysis of data (Tables 1A to 6A). Each Z value derived from data in one of the Tables (1A, 2A, 5A or 6A) is given on Table 9A. The five per cent significance level corresponds to \(Z = 0.67\). This means that when \(Z\) is greater than 0.67 there is less than one chance in twenty that a degree of variability over the seven periods by which it is greater than the 0.67 has occurred by chance. Taking then \(Z = 0.67\) as the limit of significance, we find that in the case of milk taken from mothers with adequate supply the average chloride value varies significantly throughout the first five months of lactation. The "L" test applied to data for milk taken from mothers with inadequate lactation gives a \(Z\) value less than 0.67. This shows that the variation observed is not statistically significant. These conclusions apply to values for milk specimens taken either in the early morning, or at mid-day or in the evening (Table 9A).\(^*\)

In order to determine at what period of lactation the variation occurs, the \(Z\) test was again

\(^*\) See Appendix Pages 61 to 66. \(^*\) See Appendix Page 71.  
\(^{*1}\) "  "  " 61, 62, 65 and 66.  
\(^{*2}\) "  "  "  Page 69.
applied to the data that showed a significant variation (Tables 1 and 5)*, but only to values for the second, third, fourth and fifth month. The results are given on Table 10A*. The values for Z in these calculations are well below 0.67, the significant level. Therefore, the significant variation must be during, or at the end of, the first month of lactation. To verify this conclusion, the "T" test was used to assess the significance of the difference between the mean values for the first month as a whole and for the second to fifth months as a whole (Table 8A)*. Here, the value of "T" which corresponds to P = 0.05 is 1.96, so that when "T" is greater than 1.96 the difference may be regarded as statistically significant. The results of the calculation are shown in Table 11A which refers to the first, second and third series separately and to series one and two combined. When using data for milk taken from mothers with adequate supply, "T" value is more than 1.96 the significant level - but when data for milk from mothers with inadequate supply is used, the value of "T" is less than 1.96 except in the case of the combined series of observations for milk taken at mid-day.

The absence of a significant variation in the average values for milk taken from mothers with an inadequate supply in the first, second and third

*1 See Appendix Pages 61 to 65
*2 See Appendix Pages 69 and 70
*3 See Appendix Pages 72
*4 See Appendix Pages 73
series of observations does not mean that such a variation does not exist, but merely that no variation could be detected when the data was submitted to the "T" test. Some variation almost certainly does exist, because with the combined data a significant variation was detected (Table 11A) when the data for milk drawn at mid-day was combined (Series I and II). The variation for milk from mothers with an inadequate supply is less significant because the range of variation of individual values within any one group is greater and the number of observations less than for milk taken from mothers with adequate lactation.

**QUESTION I(b):** Is the Variation in Average Electrical Conductivity of Milk over the Seven Periods of Lactation Statistically Significant?

Electrical conductivity values were obtained from the milk specimens which had been used for chloride estimations. The arrangement of the data for conductivity of milk was the same as for chloride content. Tables 1B to 6B show the data grouped according to seven lactation periods, i.e. the second, third and fourth week, and the second, third, fourth and fifth months. The greatest difference between the averages for each period

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*1 See Appendix Page 73.
*2 " " Pages 83 to 87.
appeared to be between those for the first month of lactation and those for the later months of lactation. To emphasise this point, average conductivity values for the first month as a whole were compared with those for the second to fifth months as a whole (Table 8B).*

The "Z" test was applied to data in Tables 1B, 2B, 5B and 6B to show whether there was a significant variation in average conductivity values throughout the first five months of lactation and also whether there was a significant variation between values for the second to fifth month of lactation. The "Z" values for the calculations have been collected in Tables 9B and 10B. The values of "T" calculated from data in Table 8B are presented in Table 11B.*

The results are essentially the same as for the chloride estimations. There was a significant variation in average conductivity values for milk taken throughout the first five months from mothers with adequate lactation, but not for milk from mothers with an inadequate supply. The variation occurred either during, or at the end of, the first month of lactation. This applied to average values for specimens whether taken in the early morning, at midday or in the evening.

According to the results of the "T" test

*1 See Appendix Pages 83 and 84, 87 and 88.
*2 " " " 91 and 92.
*3 " " " 93 and 94.
*4 " " Page 95.
there was no significant variation in average conductivity for milk from mothers with inadequate lactation when using data from either the first or second series of cases separately, but when the data for the combined series was used, a significant variation was obtained from mid-day milk specimens. A significant variation was detected less frequently in milk taken from women with inadequate lactation, because the range of variation of individual values within any one group was greater and the number of observations less than for milk taken from mothers with adequate lactation.

QUESTION 2(a): Do the Average Chloride Values for Early Morning, Mid-day and Evening Specimens of Milk differ significantly from each other?

The chloride values of milk taken from the same woman at different times of the day, i.e. early morning (6 a.m.), mid-day (12 p.m. or 2 p.m.) and evening (6 p.m. or 10 p.m.) have been compared. The data used for this comparison will be found in Table 12A. It refers to mothers with (1) adequate lactation and (2) inadequate lactation; the data is tabulated to show average chloride values for the first month and for the second to fifth month (Table 12A).*

Table 12A shows that the average chloride values for a given period of lactation are generally lowest in the early morning and highest at mid-day, irrespect-
ive of whether the milk is taken from mothers with an adequate or an inadequate supply. Values for milk specimens taken in the evening generally lie between those for early morning and mid-day, but this is not invariably the case.

When the statistical significance of the differences between chloride values of milk taken in the morning and those taken at mid-day are assessed, it is possible, in the analysis of variance (Z test) to make allowance for individual factors characterising each mother, because specimens were taken from each mother at both these times. Allowance for such factors can also be made when the average values for early morning, mid-day and evening milk are compared simultaneously. The data for the first month and for the subsequent months are considered separately because of the significant difference already shown to exist between these two periods. The Z test has been first applied to data for early morning and mid-day specimens. The results are given in Table 13A. All "Z" values over 0.67 are proof of a significant variation in average chloride values throughout the day. The only significant result for milk taken from mothers with adequate lactation is the specimens which were drawn during the first month of lactation. There is no significant variation between early morning and mid-day values of milk taken from mothers

* See Appendix Page 76.
with an inadequate supply. The difference between the results of the two groups is partly due to the small number of observations and partly to the wide range of individual values obtained from mothers with inadequate lactation.

A statistical analysis has also been made of the third series of observations (Table 12A) which consists of chloride estimations on milk taken from the same women in the early morning, mid-day and evening of the same day. Eighty-one of these women were lactating adequately and forty-two inadequately. Again, the analysis of the data takes into account the existence of individual factors. The "z" test has been applied to the data given for the first month as a whole and for the second to fifth month as a whole. The "z" values found are set out in Table 14A. The Z value indicating the critical level of significance is 0.67. The values for the first month are over 0.67 and those for the second to fifth month were less than 0.67. Therefore, there is a significant variation between values throughout the day both for milk taken from mothers with inadequate and for those with adequate supply, but only in the first month of lactation.

It will be seen that the two series agree in indicating that the variation throughout the day during the first month in mothers with an adequate milk supply is significant. In the case of the mothers with an adequate supply, the variation is significant. In the case of the mothers with an inadequate supply, the variation is not significant.

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*1 See Appendix Pages 74 and 75.
*2 Page 77.
inadequate milk supply, the variation in the second series is significant, but not in the first series. In those mothers both the great random variation and the small number of mothers investigated tend to reduce the significance of the variation; a significant variation probably does exist in those mothers with an inadequate milk supply though only detected in the second series.

**QUESTION 2(b): Do The Average Values for Electrical Conductivity for Early Morning, Mid-day and Evening milk specimens Vary Significantly?**

The same specimens of milk which were used for chloride estimations have also been used for making measurements of electrical conductivity. The average values for electrical conductivity of milk taken in the early morning (6 a.m.), at mid-day (12 p.m. and 2 p.m.) and in the evening (6 p.m. and 10 p.m.) have been tabulated (Table 12B). The arrangement of the data is the same as that used in comparing the chloride values of milk, i.e. average values are tabulated for the first month as a whole and for the second to fifth month as a whole (Table 12B). The conductivity values resemble the chloride values in that they tend to be lowest in the early morning and highest at mid-day. In order to assess the significance of this difference, the "Z" test was applied to the data.

* See Appendix Pages 96 and 97.
The results of the analysis of the data by means of the "Z" test are summarised on Tables 13B and 14B and they are essentially the same as those obtained from the analysis of chlorides. In the first series of observations, there is a significant variation between the average conductivity values of milk taken throughout the day from women with adequate lactation in the first post-partum month (Table 13B). The conductivity of milk from mothers with inadequate lactation shows no significant variation. In the third series of observations, a significant value for Z was obtained from women with inadequate lactation in the first month post-partum as well as from women with adequate lactation during the same period (Table 14B). The results, however, differ from those obtained from analysis of chloride values because there is a significant variation in conductivity values for milk from mothers with adequate supply during the second to fifth month of lactation (Table 13B).

**QUESTION 3(a):** Does the Chloride Content of Milk Taken from Mothers with Adequate Supply Differ from that from Mothers with an Inadequate Supply?

The average chloride values of milk specimens taken from mothers with adequate lactation at a particular time of day have been compared with those from mothers with inadequate lactation at the same

*1 See Appendix Page 98.
*2 " " " " 99.
time of day. The average was taken for all morning specimens during the first five months of lactation. Similar averages were obtained for mid-day and evening specimens. Table 15A contains the data and shows that the average chloride values of milk taken from mothers with adequate supply are lower than those from mothers with an inadequate supply.

In both Series I and III the "T" test has been used to ascertain whether the observed differences between the milks obtained from mothers with adequate lactation and inadequate lactation are statistically significant. The results are shown in Table 16A. It will be seen that all the values for "T" are well above 1.96, the significant level for "T". Therefore, the differences between averages for milk from women with adequate and those with inadequate lactation are all highly significant and this conclusion holds, irrespective of the time of day at which the milk specimens are taken.

QUESTION 3(b): Does the Electrical Conductivity of Milk taken from Mothers with Adequate Supply Differ from that taken from Mothers with an Inadequate Supply?

The average conductivity values of milk taken from mothers with an adequate lactation has been compared with those from mothers with inadequate

* See Appendix Page 78.
*1 See Appendix Page 79.
lactation. The conductivity measurements were made on the milk specimens used for chloride estimations in the first part of this question, and the data is presented in a series of Tables (Table 15B) similar to those made for comparison of chloride values in Question 3(a). The Table shows that average conductivity values for milk taken from mothers with adequate lactation are lower than those for mothers with inadequate lactation.

The results of the "T" test are shown in Tables 16B and the results are similar to those obtained from the chloride comparisons in Question 3(a). In all cases, "T" is greater than 1.96, the significant level. Therefore, there is a significant variation between average conductivity values obtained from mothers with adequate lactation and those with inadequate lactation, provided the comparison is made between specimens taken at the same time of day.

**QUESTION 4(a): Is the Significant Difference between Average Chloride Values of Milk taken from Mothers with Adequate Supply and from those with Inadequate Supply Detectable at all stages of Lactation?**

The data for the comparison of average chloride values of milk taken from mothers with adequate lactation and from those with inadequate lactation is

* See Appendix Page 100.
found in Table 17A.*1 The averages have been extracted from Tables 1A, 2A, 5A and 6A and are arranged in seven groups: second, third and fourth week, and second, third, fourth and fifth months of lactation. In each group, separate averages for specimens taken in the morning, at mid-day and in the evening are given. A survey of these averages shows that the values for milk from mothers with adequate supply is always less than those for milk from mothers with inadequate supply when the values for the same period of lactation are compared. The hour at which the milk specimens were taken does not alter this relationship.

The significance of these differences has been assessed by means of the "T" test. The "T" test was first applied to average values for both morning and mid-day milk specimens and the results are to be found in Table 18A.*1 Every result is above 2.13, the significant level for "T".

*1 See Appendix Pages 61, 62, 65 and 66.
*2 " " " 80 and 81.
*3 " " Page 82
Owing to the small number of observations, the average values of evening specimens taken throughout the first month and the average of evening specimens taken throughout the second to fifth month were obtained. Separate calculations were made for milk from women lactating well and those lactating badly. The results of the "T" test applied to these averages are well above the significant level of 2.13 (Table 18A). Therefore, the difference between average chloride values of milk taken from mothers with adequate lactation and those from mothers with inadequate lactation is significant throughout the first five months of lactation, irrespective of whether the milk specimens are taken in the early morning, or mid-day, or in the evening.

QUESTION 4(b) Is the Significant Difference between Average Conductivity Value for Milk taken from Mothers with Adequate Supply and those from Mothers with Inadequate Supply Detectable at all Stages of Lactation?

The conductivity measurements used in comparing milk from mothers with adequate lactation and that from mothers with inadequate lactation are shown on Table 17B. They have been compiled from data on

*1 See Appendix Page 82.
*2 See Pages 102 and 103.
Tables IB, 2B, 5B and 6B. These averages result from estimations of electrical conductivity made on these specimens which were used for the chloride estimations in Question 4(a). The data is arranged in a Table similar to that used for comparison of chloride values in Question 4(a), (Table 17B). The average conductivity values in any one group are always greater for milk taken from mothers with inadequate supply than for milk taken from mothers with adequate supply in the same group.

The results of the "T" test applied group by group to averages for both early morning and for mid-day specimens are on Table 18B. The values for "T" are above 2.13 - the significant level - except for one (2.1) obtained from comparison of specimens taken in the fourth month of lactation and one (1.69) from specimens taken in the fifth month of lactation. These two insignificant results are due to the small number of observations in these groups. Proof of this is given by the results of the "T" test applied to the data after it had been combined with data for the second series of observations for the corresponding periods of lactation (Tables 3B and 4B). The "T" values are then 3.5 and 5.5 respectively, which are significant (Table 18B).

*1 See Appendix Pages 83, 84, 87 and 88.
*2 " " " 85 and 86.
*3 " " " 102 and 103.
*4 " " Page 104.
The average values for electrical conductivity of evening milk have been obtained for specimens taken in the first month as a whole and for those taken during the second to fifth month of lactation because there are too few observations to subdivide them into seven groups. The results of the "t" test applied to these averages are significant (Table 18B) i.e. they are over 2.13 - the significant level. Therefore, the findings are similar to those obtained from the chloride analysis in Question 4(a). The difference between the conductivity values of milk taken from mothers with adequate lactation and those of milk taken from women with inadequate lactation is significant throughout the first five months of lactation, irrespective of whether the milk specimens are taken in the early morning, at mid-day or in the evening.

CONCLUSION:

A statistical analysis of the chloride content and electrical conductivity measurements of human milk has been made. The tests were carried out on five hundred and five specimens of milk taken from mothers with adequate lactation and two hundred and ninety-two specimens from mothers lactating inade-
The mothers were investigated during the first five months of their lactation period and were divided into seven groups according to the time which elapsed between parturition and the milk investigation.

It has been shown that the average chloride and conductivity values of milk taken from mothers with an adequate supply are significantly lower than those of milk taken from mothers with an inadequate supply. This is true not only when the comparison is made between average values for the first five months of lactation as a whole, but also when the average values for the second, third and fourth weeks of lactation and the second, third, fourth and fifth months of lactation are compared separately. This conclusion is unaltered whether the averages are based on values for milk taken in the early morning, or on those taken at mid-day, or on those taken in the evening. These results are the most outstanding findings in the statistical analysis.

The observed tendency of average weekly and monthly values for both chloride content and conductivity measurements to fall as lactation advanced has been confirmed statistically. The fall occurs during the first month of lactation, more probably towards the end of that month. It occurs whether the mothers are lactating well or badly, and is uninfluenced by the time of day at which specimens
are taken. Statistically, the degree of significance is more marked for values of milk taken from mothers with an adequate lactation than for those taken from mothers with an inadequate lactation. This is because the number of observations are smaller and the range of values greater for milk taken from mothers with inadequate lactation.

During the second to fifth month of lactation there is no significant fall in average chloride values of milk. There is, however, a significant fall in one out of six comparisons made on the conductivity of milk taken during the second to fifth month of lactation. In this case the specimens were taken in the evening from mothers with adequate lactation.

The weekly and monthly averages for the chloride and the conductivity values of milk are usually lowest for early morning specimens and highest for those taken at mid-day. The values for milk taken in the evening tends to lie between the early morning and mid-day value, but this is not invariably so. The rise in the average chloride and conductivity values throughout the day is statistically significant during the first month of lactation. This applies both to values for milk taken from mothers with adequate supply and from those with inadequate supply. There is usually no significant rise throughout the day in the average chloride
values of milk taken during the second to fifth months of lactation. There is, however, a significant rise throughout the day in the electrical conductivity of milk taken from mothers with adequate lactation during the second to fifth month of lactation, although all other daily alterations in electrical conductivity during this period are statistically insignificant.
During the past fifty years, estimations of the chloride content and electrical conductivity of cows' milk have been used as a means of detecting milk obtained from cattle with udder disease. A review of the literature has shown that these tests have been, and still are, of great value for this purpose. It is strange that such measures have not been utilised for the detection of abnormal women's milk. In considering whether the estimation of chloride content and electrical conductivity of women's milk might, with benefit, be applied to women's milk for the detection of mammary disfunction, a review of these tests, as applied to cattle, will be undertaken. At the same time, the efficiency of the two tests will be compared with others used for the detection of abnormal milk. By so doing, it is hoped that the importance of investigating the application of the chloride test and the conductivity test to women's milk may be appreciated.

The chloride test consists of estimating the chloride content of milk. Should the chloride value obtained lie above a given level the composition of the milk is abnormal. Such abnormality can occur in
cattle under physiological conditions, such as during the first three weeks after calving, when lactation is becoming established and during the terminal phase of lactation. After lactation has been established, pathological conditions of the udder which lead to the secretion of milk of relatively high chloride content frequently occur. Mastitis is found in ten to eighty per cent of cattle and the milk secreted from the affected udders has a high chloride content in fifty to seventy percent of cases. (Munch-Peterson, 1938A). Recently, some authors (Angenjo Cecilia, 1940 and McCulloch, 1947) have stated that all, or nearly all, cattle with mastitis have milk with high chloride content. Other types of udder disease also result in a high chloride value for milk, and such disease is found in sixteen to twenty-two per cent of mastitis-free cattle. (Proscholdt, 1933; Munch-Peterson, 1938(A); Hastings and Petersen, 1940; and Little and Plastridge, 1946).

It is not possible to quote a single figure for the efficiency of the chloride test because the incidence of disease varies from country to country, from place to place, and from herd to herd. Moreover, the criteria accepted in the past for the diagnosis of mastitis have varied considerably. Nevertheless, Munch-Peterson (1938 B) who reviewed the literature up to 1935 and summarised ninety-eight reports on the use of the chloride test in mastitis concluded that
the test was a valuable subsidiary method for the diagnosis of the disease. Since his review, the test has been used repeatedly and continually and found of great value. By the use of the test, the contamination of normal milk and distribution of unsuitable milk may be prevented, and the dairymen is able to trace diseased animals and isolate and treat them.

The electrical conductivity of milk varies directly with milk chloride content (Coste and Shelbourne, 1919; Schweers, 1932; and Davis et al, 1943) and for this reason the electrical conductivity test has the same practical value as has the chloride test. When conductivity values exceed a given level this indicates that the milk has either been obtained from cows in either the first three weeks or terminal stages of lactation, or from cows with udder disease. Therefore, the conductivity test is only of use in detecting udder disease when lactation is fully established.

Punch-Peterson (1938 B) after reviewing twenty-three reports on the use of the test in cases of mastitis concluded that the electrical conductivity test was a convenient, quick and valuable method. Other studies made on milk from cattle with udder disease due to bacterial and other causes and are not mentioned by Punch-Peterson have been reported by Lehmert (1897), Krepp, 1933, Rosell, 1934, Lefèvre, 1935, McCulloch, 1940, 1942, 1944 and 1947, Fredholm, 1943, Malcolm
King and Campbell, 1942, Malcolm King Catherine and Campbell, 1944, Malcolm and Campbell, 1946. These authors, without exception, believe that the determination of electrical conductivity of milk is a valuable test for the detection of milk from mastitis-affected cattle.

Recently, a simple electrical apparatus for measuring the electrical conductivity has been constructed by Kohler and Hartnazel (1935) in Germany, by McCulloch (1940) in America and by Davis (1947) in the British Isles. This apparatus is portable and indicates rapidly the conductivity of milk, thus enhancing the value of the test.

The popularity of the chloride test and the electrical conductivity test for the detection of abnormal milk depends largely on whether they are as efficient and convenient as others used for this purpose. A few such comparisons have been made, but they are unsatisfactory, and until some agreement is reached as to what constitutes mastitis on the one hand and normality on the other, it will be impossible to obtain an accurate evaluation of diagnostic procedures. (Lunch-Peterson, 1938 B). The bacteriological examination of milk is very important because it gives conclusive evidence of the presence or absence of mastitis. It should always be made when milk has been shown to be abnormal by means of any other test. Although the bacteriological examination gives con-
clusive evidence of mastitis, it is not practical to use this method to detect sub-clinical udder disease in herds of cattle because the test is prolonged and time-consuming. It is for this reason that quick and inexpensive, indirect tests for mastitis have been devised. They have been designed to detect abnormal cell content, enzymes, hydrogen ion-concentration, physical properties and chemical composition. A summary of these various techniques and their merits follows. A wealth of information may be obtained from examining the centrifuged deposit of cow milk for organisms and cells. If the cell count is noted to be unusually high, it indicates that the milk has been taken from a cow with mastitis. In this way, half to three-quarters of the cattle with mastitis can be detected. The test, however, has greater limitations than would appear at first sight, because two to thirty-seven per cent of milk specimens taken from mastitis-free cattle exhibit pathological cell counts. This is sometimes due to the milk being taken from cows suffering from udder disease, but at other times the cattle are healthy in all respects. (Munch-Petersen, 1938 B and C).

The enzymes present in abnormal cows' milk are generally higher than that of normal milk. Of the methods advocated to detect this difference, the most popular is to estimate the catalase content.
of the milk partially because it is quick and easy to perform, and partially because it detects milk from mastitis-affected cattle as well, or better than, other indirect tests used for this purpose. The test, however, occasionally gives misleading results, a positive reaction being obtained when mastitis, as judged by bacteriological examination, is absent. The converse also occurs. Thus, the test only detects about half to three-quarters of cases of mastitis. Nevertheless, it has been extensively used in Europe for detecting inflammation of the mammary gland in cattle, (Lund-Peterson, 1938 B and C), though in this country it has not gained the same popularity.

The determination of the hydrogen ion-concentration of milk is frequently adopted for detecting milk from mastitis-affected cattle, because their milk is generally less acid than that of healthy cattle. In most cases, a technique involving the use of an indicator rather than a pH meter has been used. The efficiency of the hydrogen ion-concentration test is similar to that of the cell count test. (Lund-Peterson, 1938 B).

Of the physical properties of milk, three in particular have been studied in an effort to discover an efficient test for the detection of abnormal milk. These are the electrical conductivity, refractive index, and freezing point of milk. The only one which has proved of practical value and upon which a useful
test is based is the conductivity. (Lunch-Peterson, 1938 B). This test is as efficient in detecting milk from mastitis-affected cattle as are the tests which depend on abnormal cell counts (Malcolm King and Campbell, 1942), abnormal hydrogen ion-concentration (Munchenberg, 1931) and abnormal chloride content (Strohecker and Beloveschdoff, 1928, and Seelemann, 1931). The conductivity test has the additional advantage that it also detects milk taken from abnormal udders, whose pathology is due to non-bacterial factors. Because of this, the test reveals abnormality in milk specimens which would appear normal if judged by their bacterial content or cell count alone. (Rosell, 1931; Lunch-Peterson, 1938 (a); Malcolm, King and Campbell, 1942 and 1944; Little and Plastridge, 1946; and Malcolm and Campbell, 1946). Moreover, the electrical conductivity test is more informative than the tests for cell count and hydrogen ion-concentration because it not only detects abnormal milk, but milk from cows with sub-normal milk yield. (Dounhofer and Roser, 1930, and Krenn, 1929, 1930 and 1938).

Lunch-Peterson (1938 B) reviewed tests for detecting milk of abnormal chemical composition taken from cows with mastitis and found that of these the chloride test was certainly the one most extensively used. It detects milk from mastitis-affected cattle either as efficiently, or more
efficiently, than tests used for detecting either abnormal chemical composition, (Lunch-Peterson, 1939 C; Rowland and Zein-el-Dine, 1939; and Davis et al, 1939), abnormal cell count (Lunch-Peterson, 1938 C; Jacob, 1938; Rowland and Zein-el-Dine, 1939; Kunicki-Goldfinger, 1939; Hastings and Peterson, 1940; Malcolm King and Campbell, 1942), abnormal enzyme content (Lunch-Peterson, 1938 B and C), abnormal hydrogen ion-concentration (Gloy and Bischoff, 1928; Ehrlich and Bischoff, 1930; Seelemann, 1931; Rosel and Miller, 1933; and Prouty and Ellington, 1934) or abnormal electrical conductivity (Strohecker and Beloverschoff, 1928, and Seelemann, 1931). Since it has already been stated that electrical conductivity varies directly with milk chloride content, it is reasonable to expect the chloride test to be as efficient as electrical conductivity as far as detecting abnormal milk is concerned. Although the efficiency of the various indirect tests for mastitis is approximately the same, the chloride test and conductivity test differ from most other indirect tests by being both quantitative and qualitative, and by detecting milk from cattle with various types of udder disease as well as from cows with mastitis.

The chloride and electric conductivity tests for the detection of milk from the diseased mammary gland is of indisputable value. In the first place, they
are used as an indirect test for milk from mastitis-affected cattle and, in this respect, they are not surpassed by any of the innumerable indirect tests used for this purpose. The fact that they are quantitative, as well as qualitative tests, is a valuable asset. Secondly, the chloride and conductivity tests also detect abnormal milk taken from cattle with udder disease other than mastitis. In such cases, they are indispensable because other suitable tests for this purpose do not exist. It is possible that if the chloride and electrical conductivity tests were applied to women's milk similar results would be obtained. If so, the value of such tests in women would be considerable.
FIGURE XII

EACH GRAPH SHOWS % WOMEN LACTATING 1-5 MONTHS

A
- MILK CHLORIDE BELOW 50 mg
  - WHEN ESTIMATED IN FIRST MONTH
B
- MILK CHLORIDE 51-100 mg
  - WHEN ESTIMATED IN FIRST MONTH
C
- MILK CHLORIDE OVER 100 mg
  - WHEN ESTIMATED IN FIRST MONTH

- MOTHER'S MILK ESTIMATED FOR CHLORIDE
  - IN 2nd WEEK OF LACTATION
- MOTHER'S MILK ESTIMATED FOR CHLORIDE
  - IN 3rd and 4th WEEK OF LACTATION
THE CORRELATION of MILK CHLORIDE VALUES in the FIRST MONTH of LACTATION with the DURATION of LACTATION.

The breast milk of eighty-eight mothers in the second week of lactation and forty mothers in the third and fourth week of lactation was tested for chloride content. Each mother provided 4 to 8 c.c. milk before the 6 a.m. breast feed and a similar amount before the mid-day feed. The mean chloride value, obtained by estimating the chloride content of the two milk specimens, was correlated with the duration of lactation in these mothers.

The findings are recorded in Table 30 (Page 119). The results are divided into three groups; one for mothers with a milk chloride of 0 to 50 mg. per cent, another for mothers with a milk chloride of 51 to 100 mg. per cent and a third for mothers with a milk chloride over 100 mg. per cent. The number of mothers in each group is given. The number of mothers in each group lactating for one, two, three, four and five months is stated as a percentage of the total number of mothers in this group. The results are summarised graphically in Figure XII (Page 117).

The conclusions drawn from the inspection of Table 30 (Page 119) and Figure XII (Page 117) are firstly that the lower the chloride content of the
Table 31.

<table>
<thead>
<tr>
<th>Duration of Lactation</th>
<th>No. Mothers Milk Chloride under 75 mgm%</th>
<th>No. Mothers Milk Chloride over 75 mgm%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2/12</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>2 to 5 months</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

Milk chloride estimations made in second week of lactation.

<table>
<thead>
<tr>
<th>Duration of Lactation</th>
<th>No. Mothers Milk Chloride under 75 mgm%</th>
<th>No. Mothers Milk Chloride over 75 mgm%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2/12</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>2 to 5 months</td>
<td>14</td>
<td>6</td>
</tr>
</tbody>
</table>

Milk chloride estimations made in third and fourth week of lactation.
milk in the first month of life, the more likely is a mother to breast feed her infant for five months, and secondly, that the largest proportion of failures to breast feed occur in the first two months of lactation. To decide whether the former conclusion is statistically significant, the $\chi^2$ test has been applied to the data. The $\chi^2$ test was applied twice, once to figures for women tested for their milk chloride content in the second week of lactation, and once to figures for women tested in the third and fourth week of lactation. The figures were collected in the form of a two by two table, the division was, on the one hand, between mothers who lactated less than two months and those who lactated more than two months, and on the other, between mothers who gave milk with a chloride content of more than 75 mg. per cent and those who gave milk with a chloride content of less than 75 mg. per cent.

The figures used for the statistical analysis are shown in Table 31 (Page 118) and the value of $\chi^2$ obtained by applying the $\chi^2$ formula to the figures is 7.9 for mothers who had their milk tested in the second week of lactation, and 5.1 for mothers who had their milk tested in the third and fourth week of lactation. Both these results substantially exceed the five percent significant level of 3.84. Therefore, the results support the conclusion drawn from Table 30 (Page 119) and Figure XII (Page 117)
<table>
<thead>
<tr>
<th>Milk Chloride mg%</th>
<th>Week of Lactation</th>
<th>Number of Mothers</th>
<th>Percentage Mothers Lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>One month</td>
</tr>
<tr>
<td>0 - 50</td>
<td>2</td>
<td>8</td>
<td>100 (8)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>7</td>
<td>100 (7)</td>
</tr>
<tr>
<td>51 - 100</td>
<td>2</td>
<td>52</td>
<td>71 (37)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>25</td>
<td>100 (25)</td>
</tr>
<tr>
<td>101 - 150</td>
<td>2</td>
<td>28</td>
<td>61 (17)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>8</td>
<td>63 (5)</td>
</tr>
</tbody>
</table>

( ) number of mothers in the group.

Women with a relatively low milk chloride value in the second to fourth week of lactation on the average lactate longer than those mothers with a relatively high milk chloride value in the second to fourth week of lactation.
that women with a long period of lactation tend to have a relatively low milk chloride value in the second to fourth week of lactation, while those with a short period of lactation tend to have a high milk chloride value in the second to fourth week of lactation. The thirty-two mothers who did not need to complement their breast feeds during the first five months of lactation had a milk chloride value of not more than 110 mg. per cent during the second to fourth week of lactation. But women with a milk chloride value of more than 110 mg. per cent in the second to fourth week of lactation found it necessary to give complementary feeds to their infants if they breast fed them for five months.

There are certain abnormalities in the health of the mothers which may occur during the first month of lactation and which give rise to high milk chloride results, (i.e. over 120 mg. per cent), but in these cases if the abnormal condition subsides lactation may proceed normally for five months. The first type of abnormality occurred in two mothers who had undergone Caesarean Section for the birth of their infant and the establishment of breast feeding in these cases was delayed, partly due to the operation and partly to the fact that the babies were not put to the breast until they were five days old. The second type of abnormality was one involving the breasts. Here, breast
feeding was interrupted in order to rest the breast for forty-eight hours in one case, because the mother had a cracked nipple and in two other instances because of mastitis. In these patients the milk chloride value during the resting phase was high, but eventually lactation progressed normally. A third maternal condition, pyrexia, was associated with milk chloride values of over 110 mg. per cent in the second to fourth week of lactation, yet the mother so affected lactated satisfactorily on recovering normal health.

Although the milk chloride values in the second, third and fourth week of lactation has been shown to be an aid in assessing the duration a woman may lactate, the same standards cannot be used when attempting to assess the prognosis of lactation from specimens of milk in the first week of lactation.
TABLE 32.

FOR THE CORRELATION OF ELECTRICAL CONDUCTIVITY OF MILK TAKEN IN THE FIRST MONTH OF LACTATION WITH THE DURATION OF LACTATION.

<table>
<thead>
<tr>
<th>Milk Conductivity $\times 10^{-5}$</th>
<th>Week of Lactation</th>
<th>Number of Mothers</th>
<th>Percentage mothers lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>One month</td>
</tr>
<tr>
<td>150 - 200</td>
<td>2</td>
<td>9</td>
<td>100 (9)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>10</td>
<td>100 (10)</td>
</tr>
<tr>
<td>201 - 250</td>
<td>2</td>
<td>42</td>
<td>74 (31)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>24</td>
<td>96 (23)</td>
</tr>
<tr>
<td>251 - 300</td>
<td>2</td>
<td>38</td>
<td>58 (22)</td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>5</td>
<td>80 (4)</td>
</tr>
</tbody>
</table>

( ) number mothers in the group

Women with a relatively low milk conductivity value in the second to fourth week of lactation on the average lactate longer than those mothers with a relatively high milk conductivity value in the second to fourth week of lactation.
THE CORRELATION OF MILK ELECTRICAL CONDUCTIVITY VALUES IN THE FIRST MONTH OF LACTATION WITH THE DURATION OF LACTATION.

The breast milk of eighty-eight mothers in the second week of lactation and forty mothers in the third and fourth week of lactation was tested for electrical conductivity. Each mother provided four to eight c.c. milk before the 6 a.m. breast feed and a similar amount before the mid-day feed. The mean electrical conductivity value, obtained by estimating the electrical conductivity of the two milk specimens, was correlated with the duration of lactation in these mothers.

The findings are recorded in Table 32 (Page 121). The results are divided into three groups; one for mothers with milk with a conductivity value $150 \times 10^{-5}$ to $200 \times 10^{-5}$ another for mothers with milk with a conductivity value $201 \times 10^{-5}$ to $250 \times 10^{-5}$ and a third for mothers with milk with a conductivity value $251 \times 10^{-5}$ to $300 \times 10^{-5}$. The number of mothers in each group is given. The number of mothers in each group lactating for one, two, three, four and five months is stated as a percentage of the total number of mothers in the group. The results are summarised graphically in Figure XIII (Page 122).
FIGURE XIII

EACH GRAPH SHOWS % WOMEN LACTATING 1-5 MONTHS

MILK CONDUCTIVITY 150 x 10^{-5} - 200 x 10^{-5}
WHEN ESTIMATED IN FIRST MONTH

MILK CONDUCTIVITY 200 x 10^{-5} - 250 x 10^{-5}
WHEN ESTIMATED IN FIRST MONTH

MILK CONDUCTIVITY OVER 250 x 10^{-5}
MILK ESTIMATED FOR CONDUCTIVITY IN 2ND WEEK OF LACTATION

MILK ESTIMATED FOR CONDUCTIVITY IN 3RD AND 4TH WEEK OF LACTATION
The conclusions drawn from the inspection of Table 32 (Page 121) and Figure XIII (Page 122) are firstly that the lower the electrical conductivity of milk in the first month of life the more likely is a mother to breast feed her infant for five months and secondly, that the largest proportion of failures to breast feed occur in the first two months of lactation. To decide whether the former statement is statistically significant the $x^2$ test has been applied to the data. The method of applying this test to the data was similar to that in the previous section (Pages 117 to 120).

The numerical figures used are shown in two by two Table 33 (Page 123) and the value of $x^2$ obtained from the calculations is 8.2 for mothers who had their milk tested in the second week of lactation and 4.2 for mothers who had their milk tested in the third and fourth week of lactation. Both these results exceed the five per cent significant level of 3.84. Therefore, they support the observation made on Table 32 (Page 121) and Figure XIII (Page 122) that mothers lactating for long periods tend to have a relatively low milk conductivity value in the second to fourth week of lactation, while those who lactate only for a short period have a relatively high milk conductivity value in the second to fourth week of lactation.

The thirty-two mothers who did not need to complement their breast feeds during the first five
### TABLE 33.

<table>
<thead>
<tr>
<th>Duration of Lactation</th>
<th>No. Mothers Milk Conductivity under $200 \times 10^{-5}$</th>
<th>No. Mothers Milk Conductivity over $200 \times 10^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2/12</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>2 to 5 months</td>
<td>28</td>
<td>10</td>
</tr>
</tbody>
</table>

Milk conductivity measurements made in second week of lactation.

<table>
<thead>
<tr>
<th>Duration of Lactation</th>
<th>No. Mothers Milk Conductivity under $200 \times 10^{-5}$</th>
<th>No. Mothers Milk Conductivity over $200 \times 10^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2/12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>2 to 5 months</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Milk conductivity measurements made in third and fourth week of lactation.
months of lactation had milk with a conductivity value not more than $275 \times 10^{-5}$ during the second to fourth week of lactation, while women with a milk conductivity value above $275 \times 10^{-5}$ in the second to fourth week of lactation found it necessary to give complementary feeds to their infants if they breast fed them for five months.

There are certain abnormalities in the health of the mother which may occur during the first month of lactation and which give rise to high milk conductivity results (i.e. over $250 \times 10^{-5}$) but in these cases if the abnormal condition subsides lactation may proceed normally for five months. The first type of abnormality occurred in two mothers who had undergone Caesarean Section for the birth of their infants and the establishment of breast feeding in these cases was delayed partly due to the operation and partly due to the fact that the babies were not put to the breast until they were five days old. The second type of abnormality was one involving the breasts. Here, breast feeding was interrupted in order to rest the breast for forty-eight hours in one case because the mother had a cracked nipple and in two other instances because of mastitis. In these patients, the milk conductivity value during the resting phase was high, but eventually lactation progressed normally.
A third maternal condition, pyrexia, was associated with milk conductivity values of over $250 \times 10^{-5}$ in the second to fourth week of lactation, yet all the mothers so affected lactated satisfactorily on recovering normal health.

Although the milk conductivity values in the second, third and fourth week of lactation have been shown to be an aid in assessing the duration a woman may lactate, the same standards cannot be used when attempting to assess the prognosis of lactation from milk specimens taken in the first week of lactation.
THE VALUE OF THE CHLORIDE AND ELECTRICAL CONDUCTIVITY TESTS FOR THE DETECTION OF ADULTERATED MILK.

It has long been customary to take milk from mothers who have a greater amount than is needed for their own infants and to use this milk to feed infants in need of breast milk. Under these circumstances, it is important to know whether or not the milk supplied by the donors has been adulterated with water or cows' milk. Attempts have been made during the past fifty years to find methods of detecting such substances. The innumerable procedures used were concerned with the standardisation of the physical properties, hydrogen ion-concentration or chemical constituents of both normal women's milk and cows' milk. Among these methods, both the chloride and the electrical conductivity tests have taken their place. All the tests enabled the type of milk to be diagnosed and any departure from the range of values normal for that test suggested that the specimen under examination was abnormal and might have been adulterated. The tests, however, varied in their efficiency; while those designed for the detection of added water to human milk gave definite information on the presence or absence of water, those devised for the detection of added cows' milk merely gave suggestive evidence that milk other than human was present and
in some instances, indicated the degree of adulteration.

To prove that human milk has been adulterated with cows' or other foreign milk, it is necessary to use a further test involving a specific serological reaction which distinguishes between various types of milk.

In assessing the relative efficiency of the chloride and conductivity tests in the detection of adulterated milk, it is necessary to compare them with other tests used for the same purpose, both as regards accuracy and practicability. Such a review and comparison of methods will be found in the following pages.

The estimations of the chloride content of women's milk can give information regarding gross adulteration with either water or cows' milk. If the chloride estimation reveals abnormally low values for the milk tested, this indicates that at least ten per cent of water has been added to the milk specimen (Beckel, 1932, and Schuler, 1938). If both the chloride and lactose content of the milk are estimated, an examination of the results makes it possible to state whether or not the milk has been adulterated with cows' or other foreign milk. (Munchen and Kellar, 1930; Mai and Keller, 1930; Moruzzi and Tanzi, 1931; Dodds and Cowan, 1943). The calculation required for this will be given using the average value for lactose in human milk 7.48 grams per cent, and that of chloride 34.5 mgm. per cent. If the chloride is divided by the lactose
value and the result multiplied by 100, then the value of $0.44 \pm 0.015$ is obtained. A similar determination on cows' milk gives a figure of $2.23 \pm 0.082$ which is considerably higher than that for women's milk. (Mei and Kellar, 1930). Thus, if cows' milk is added to women's milk, the chloride-lactose ratio will be higher than that for undiluted women's milk. The alteration in the ratio becomes apparent when more than ten per cent of cows' milk has been added to the human milk.

Literature concerning the electrical conductivity test for the detection of adulterated milk applies to milk obtained from cows and not to that obtained from women. Therefore, the value of the test can only be judged from its use in the detection of adulterated cows' milk. At least twelve reports advocating the test for this work have been noted. (Thorner, 1891; Koenre, 1898; Binaghi, 1910; Fohil, 1911; Coste and Shelbourne, 1919; Plucker and Steinbuck, 1930; Schweers, 1932; Kreun, 1932 and 1933; Davies, 1931; Raffaeli, 1934; Davies, 1935; Montefrodine, 1942, and Davies, 1947) but the authors of these reports were concerned with the detection of water in cows' milk and did not consider the conductivity test as a method of detecting a mixture of two different kinds of milk. The efficiency of the test varies because it is necessary to add from five to twenty per cent of water to the milk (Fohil, 1911, and Tormans-Uzkains, 1938) before
the electrical conductivity value of the milk mixture falls below the minimum normal value for unadulterated cows' milk which is about $420 \times 10^{-5}$ at $25^\circ C$. (Krenn, 1938). As the conductivity of milk is usually $440 \times 10^{-5}$ at $25^\circ C$. (Krenn, 1938), it is probably necessary to add twenty-five per cent of water before adulteration can be detected. This figure is based on the knowledge that the electrical conductivity of cows' milk falls by 3.6 per cent for every five per cent water added (Taylor, 1913).

Watering of milk in dairies and large institutions may be readily detected and the degree of adulteration determined by finding electrical conductivity of the milk, provided that the test is applied to milk of known conductivity. (Coste and Shelbourne, 1913). Under these circumstances, a one per cent fall in conductivity indicates the addition of 1.25 per cent water. (Taylor, 1913).

The popularity of the chloride and electrical conductivity tests as methods for detecting adulteration of women's milk depends chiefly on their efficiency as compared with that of other tests recommended for this purpose. It is necessary, therefore, to compare the efficiency of the innumerable tests used for detecting adulteration. Some of these tests have never been used to detect adulterated women's milk. In these cases, reference will be made to findings which apply to cows' milk. Such findings
while not directly applicable to human milk, may suggest the possibility of further research. The test will be mentioned in the following order:—those involving the estimation of physical properties, those involving estimations of hydrogen ion-concentration, and those depending on the chemical properties of milk.

The physical properties of milk which have been used most frequently for the adulteration of milk are the freezing point, refractive index, electrical conductivity, vapour pressure and luminescence. The determination of the freezing point is used to detect added water since the addition of water to milk depresses the freezing point. This method has been practised on cows' milk for at least fifty years, tested by innumerable investigators, and widely used in this country and in Europe. It has been found to be both reliable and accurate. As little as two to three per cent added water can be detected by this procedure. (Kramer, 1928; Filippo, 1928; Andrew, 1929; Buchanan and Lowman, 1928; Elsdon and Stubbs, 1930, 1931 and 1936; Leyko, 1935; Blackie, 1936; Beckel, 1937; Dodds and Cowan, 1945; Dept. Health Scotland, 1946). The results may be affected by altering the composition or by processing the milk. For instance, slight inaccuracies in the results occur when milk is stale or sour (Elsdon and Stubbs, 1930) or if sodium carbonate has been added (Leyko, 1935). Churning or pasturisation of milk results in a slight increase in
the depression of the freezing point. (Stewart and Banerjea, 1930). Nevertheless, this method is not surpassed by any other in efficiency and is generally accepted as the method of choice for the detection of added water to milk. While these findings are based on facts derived from observations on cow milk, it is very probable that the same conclusions would be drawn if the test were applied to women's milk.

The refractive index and electrical conductivity of milk have been utilised for the detection of mixtures of cow milk and water, but have not been as popular in this country as in Europe, the reason probably being that in England in the estimation of fat or solids, non-fat is legally accepted as the method of detecting adulteration, whereas on the Continent this is not the only recognised method. The majority of cow milk samples have a refractive index which differs little from one another and, in these specimens, ten per cent or more of added water can be detected. Occasionally, however, milk had a refractive index outside the usual range of values and in such cases the results can be misleading. (Hanley, 1928; Elsden and Stubbs, 1928; Solomon and Shohl, 1929; Davies, 1936; Schuler, 1938 and 1942). The electrical conductivity test for watered cow milk has already been discussed (Pages 127 & 128) and shown to be only reliable for detecting twenty per cent or more of added
water to the milk. Both these tests fail to give accurate results because cow milk has a marked daily and seasonal variation in the values for these physical qualities. A similar wide range for refractive index and electrical conductivity of women's milk has been noted. Comparable results have been obtained when applying the refractometric test to watered milk from women (Zaribnický, 1942) and it is likely that the same will be true for results of the electrical conductivity test when used for the same purpose, but proof of its efficiency is awaited.

Mention of yet another test involving a physical property for the detection of watered milk is necessary because it may become of practical importance after it has been given a trial. This is the measurement of the vapour pressure of milk or milk mixed with water. It is claimed that as little as two to three per cent of added water may be diluted by this method. (Scott-Blair, Dixon and Wagstaffe, 1941). So far, this method has been limited to the estimation of cows' milk, but there seems no reason why it should not be applied to human milk.

Tests for physical properties of milk have also been used for detecting mixtures of women's milk with cow or animal milk. Those of practical importance are the refractometric test which detects the addition of five per cent of cows' milk to women's milk, (Kappeller and Gottfried, 1920, and Zaribnický, 1942) and the
luminescent phenomena of milk, which can be used for
detecting small amounts of cows' milk. The latter test
depends on examination of the milk specimen micro-
scopically under dark ground illumination; human milk
does not appear luminescent, while milk from cows or
other animals is luminescent. Thus, the presence of
luminescence in a specimen of human milk proves that
the specimen has been adulterated. (Kreidl and Neumann,
1906; Bayle and Fabre, 1924; Popp, 1927; Volmer, 1927;
Litterscheid, 1927; Griebel, 1936, and Kayser, 1938).

A second group of tests which depends on the
determination of the hydrogen ion-concentration has
been used for fifty years, but is now largely replaced
by more accurate procedures. These tests have been used
repeatedly to determine the adulteration of women's milk
with cows' milk since Umboff (1891) introduced the
method. The technique involves the use of an indicator
whose reaction is not only dependent on the pH of milk,
but also on the presence of citric acid, chalk, iron
(Sieber, 1900) and lactose (Grimmer, 1907). Of the
many modifications of this method, Moro's (1912) is
as satisfactory as any. He used neutral red as an
indicator and noted that adulteration of human milk
by ten per cent or more of cows' milk could be detected.

The third group involves the quantitative analysis
of different constituents of milk. Those which are of
practical value necessitate the estimation of either
the fat, or solid not-fat, or chloride, or lactose
content of milk. Estimations of either the fat or the solid not-fat content are used to detect watered cows' milk, and these are the only tests at the present time legally accepted for this purpose. A value of less than three per cent fat or 8.5 per cent solid not-fat is highly suggestive of watered milk (Davis, 1947).

Genuine unadulterated milk, however, may have such a fat or solid not-fat content (Toc Jer, 1925; Cranfield et al., 1927; Lesser, 1932; Rowland, 1942, and Smillie, 1940 and 1941), so that when such a value is found it is necessary to repeat the tests on further samples of milk from the same source before making a final decision on the presence or absence of added water. The verdict of watered milk is only permissible when a low result is obtained for the first test and normal ones are obtained at subsequent tests. No references to the use of these tests to determine adulteration of women's milk have been found. The estimation of the chloride content of women's milk for detecting gross adulteration with water and the chloride-lactose test for the detection of women's milk with at least ten per cent of added cows' milk and the importance of such tests have been discussed on a previous page. (Pages 126 and 127).

Analytical methods which have proved of no practical value for detecting adulterated women's milk include the estimation of iron (Edelstein and
Csonka, 1921), copper (Zondek and Bandmann, 1931), phosphorus (Henrici, 1929) and dicalcium phosphate, (Bosworth and Van Slyke, 1916). These estimations enabled one to differentiate between milk from different animals, but owing to the use of simple or more accurate procedures, they are now obsolete.

A comparison of the various tests used for the detection of added water to cows' milk shows that there are six which have been of practical value. These are the determination of freezing point, refractive index, electrical conductivity, and the fat, solid not-fat and chloride content of milk. Of these six, the only one which is sufficiently accurate for practical purposes is the determination of the depression of the freezing point, and by this method it is possible to judge the degree of adulteration by comparing the values obtained for the specimen with that of normal milk. (Dept. Health Scotland, 1946). Nevertheless, the estimation of either the fat or solids not-fat content is the method generally adopted, because these tests are the only methods legally accepted. (Davis, 1947). Other tests used to detect watered milk are less popular and can only be relied upon to detect gross adulteration. Of the above procedures, only the refractive index and the milk chloride value have been used to detect women's milk which has been adulterated with water; it is highly desirable that the depression of the freezing point of human milk should be investiga-
The adulteration of women's milk with cows' milk can be detected by measuring the refractive index, luminescence, hydrogen ion-concentration, or chloride-lactose values of milk. The luminescence phenomenon is reliable, easily determined, and detects the presence of very small amounts of cows' milk. The other methods are only reliable for the detection of gross adulteration. None of the tests, however, gives conclusive evidence of the type of animal milk which has been added to a given specimen of women's milk. Thus, when adulterated milk has been discovered by one of the above tests, it is generally desirable to resort to a specific serological test to determine the animal source of the adulterant. Such a test may also be necessary if the cruder methods of detecting adulteration have failed to reveal any abnormality.

The advantages of the serological test are that it can be performed quickly and easily, and the results are reliable and more informative than those obtained from any other test for this purpose. The test is carried out with a drop of fat-free milk placed on a slide. The drop is mixed with three or four drops of specific serum. After allowing the mixture to stand for a few minutes, it is examined microscopically. If a precipitate is seen, foreign milk is present, and the type of the milk is indicated by the type of specific serum used for the test. The presence of as little as...
two to five per cent of foreign milk can be detected in this way. Moreover, the test is equally reliable when the milk has been boiled, because the antigen in milk is thermostabile and reacts normally with precipitin in the specific serum. (Schlossmann and Moro, 1903, Uhlenhuth and Weidanz, 1908, and Urbach, 1938). The serological test, therefore, is the one and only test recommended for routine use in the detection of women's milk to which foreign milk has been added. (Jordanhoff, 1932; Urbach, 1938; Kayer, 1938; Raettig, 1940; Roszler, 1942).
FIGURE XIV

ADULTERATION OF NORMAL WOMEN'S MILK WITH WATER

-- EXPERIMENTAL VALUES

--- THEORETICAL VALUES

% ADDED WATER

0  20  40  60  80
THE DETECTION OF ADULTERATED WOMEN'S MILK BY MEANS OF

THE ELECTRICAL CONDUCTIVITY TEST.

It is of practical importance when accepting human milk for use in milk banks to be able to detect whether such milk has been adulterated either with water or foreign milk. Various methods have been recommended for this purpose, but a review of the subject shows that electrical conductivity estimations have not been used in women although the method is a popular means of detecting gross adulteration of cows' milk with water. Theoretically, the measurement of electrical conductivity might be of value in detecting women's milk to which either water or cows' milk had been added. To determine whether this is so, the following investigations have been made.

I. A specimen of woman's milk was taken and its electrical conductivity estimated. Equal fractions of the sample were then diluted with water, the various dilutions ranging from ten to one hundred per cent, and the electrical conductivity of each diluted specimen was estimated. The results are recorded in Figure XIV (Page 137) where the electrical conductivity has been plotted against percentage dilution of the milk and the resultant points marked by crosses which are connected to one another by an interrupted line. They
show there is a decrease in the electrical conductivity of adulterated milk which is almost proportional to the amount of water which has been added. In Figure XIV a continuous line has been drawn which joins the conductivity value for the pure milk used in the investigation with the conductivity of distilled water. From this continuous line, it is theoretically possible to calculate the electrical conductivity of milk which will result from the addition of water to this specimen of milk. The experimental values, however, lie above those obtained theoretically. This shows that, in practice, the dilution of women's milk with water results in a smaller reduction in the conductivity of the milk than would be expected on a theoretical basis. The extent to which the experimental and theoretical values differ varies. The difference becomes gradually more marked as the dilutions with water increase up to fifty per cent; thereafter, the difference between the experimental and theoretical values becomes progressively less with greater degrees of adulteration. For example, for milk diluted with twenty-five per cent water, the experimental value for electrical conductivity differs from the theoretical by about 4.5 per cent, while for fifty per cent dilution, the difference between the two values is nine per cent. This difference is presumably due to the experimental values being partially influenced by the reduced viscosity of the milk and by the increased dissociation of certain
Figure XV

To show the maximum amount of water which can be added to women's milk without reducing the conductivity of the milk below normal.
electrolytes in the milk.

With the knowledge of the range of conductivity values for milk taken from women lactating adequately in the second to fifth month of lactation and of the conductivity of distilled water, it is possible to construct a graph to show the maximum amount of water which must be added to such milk in order to make the conductivity of the mixture fall below the minimum value for normal human milk. Figure XV (Page 139) shows that the maximum electrical conductivity value for such milk is $210 \times 10^{-5}$. The continuous line on the graph joins $210 \times 10^{-5}$ the value for pure milk to the conductivity value for distilled water which is 0. Values on this line indicate the percentage of added water which is theoretically necessary to reduce the conductivity of milk with an electrical conductivity of $210 \times 10^{-5}$. Thus, twenty-nine percent of water must be added to milk of such a conductivity before the conductivity of the mixture falls below $150 \times 10^{-5}$, the minimum value for normal milk. This theoretical value will, however, differ from that obtained experimentally. It has been shown that, in practice, about 4.5 percent more water than has been estimated is necessary to reduce the conductivity of this milk below the minimum level of $150 \times 10^{-5}$, (Figure XV Page 137). Therefore, thirty-three percent or more of water would be
necessary to add to lower the conductivity below the normal minimum value of human milk. It is apparent, therefore, that the conductivity test can detect thirty-three per cent or more of water added to milk taken from mothers lactating adequately in the second to fifth month of lactation.

It would, however, be necessary to add thirty-three per cent water only to those milk specimens with a maximum normal electrical conductivity. In practice, the majority of normal milk specimens have a conductivity of less than $210 \times 10^{-5}$ the average being $180 \times 10^{-5}$ (Appendix Page 91). When women are lactating adequately in the second to fifth month of lactation, this value lies mid-way between the maximum and minimum values for milk taken from this type of mother and, therefore, in such cases the electrical conductivity test will detect approximately sixteen per cent or more of added water. Although electrical conductivity values for human milk which lie below $150 \times 10^{-5}$ are regarded as abnormal, any value below $160 \times 10^{-5}$ must be considered with suspicion because out of three hundred and seven specimens of milk with conductivities ranging from $150$ to $210 \times 10^{-5}$ only four per cent had a value below $160 \times 10^{-5}$. To decide whether such milk had been adulterated, it could be subjected either to a more delicate test for adulteration with water or the conductivity test should
ADULTERATION OF NORMAL WOMEN'S MILK WITH COW'S MILK

CONDUCTIVITY RACE OF NORMAL UNADULTERATED WOMEN'S MILK

CONDUCTIVITY

% ADDED COW MILK
be repeated on three specimens of milk obtained from the same women by a nurse, who would observe the following: one specimen must be taken at each of the following times - early morning, noon and night - and each specimen must consist of fore-milk. If the conductivity of any of these specimens were similar to that obtained for the initial test, then the first milk specimen should be considered as genuine unadulterated milk. If, however, none of the conductivity values of the second series of milk specimens was as low as that for the original specimen, the first sample of milk should be considered adulterated with water and more accurate tests for adulteration of human milk used to confirm or refute the result.

The adulteration of women's milk with cows' milk was studied in a similar manner. The electrical conductivity of a sample of human milk was estimated. Fractions of the sample were then adulterated with different quantities of cows' milk, the percentage adulteration varying from ten to one hundred percent. The electrical conductivity of each adulterated specimen was recorded and the results are to be found in Figure XVI (Page 141). Figure XVI shows that the conductivity of women's milk rises with the addition of cows' milk and that the rise is proportional to the degree of adulteration. It is possible to
FIGURE XVII

To show the effect on electrical conductivity of women's milk by adulteration with various percentages of cow's milk.

CONDUCTIVITY $\times 10^{-5}$

% ADDED COW MILK

A
B
C
D
E
F

200
300
400
500
calculate the theoretical values for the conductivity of human milk with similar percentages of cows' milk. When these values are compared with the experimental values for the same dilutions, a difference of 0 to 5 per cent is found between the two. The difference can probably be accounted for by experimental error.

Figure XVII (Page 142) has been constructed to show the maximum amount of cow's milk which may be required to raise the conductivity of human milk above normal. The graph shows the relationship between the electrical conductivity of human milk diluted with cow's milk and the percentage adulteration of the human milk which varies from 0 to 100 per cent. The minimum normal electrical conductivity value for women's milk (150 x 10^{-6}) is represented in the graph by a point "C" and the minimum value for pure cows' milk (Krenn, 1933), 420 x 10^{-6} is represented by a point "D". These two points have been joined by a straight line which shows the theoretical values of electrical conductivity which will be obtained by adulterating human milk of 150 x 10^{-6} conductivity with various percentages of cows' milk of conductivity value 420 x 10^{-6}. This line intersects an interrupted line, EF, which indicates the maximum normal conductivity value (210 x 10^{-6}) obtained for milk taken from women lactating adequately in the second to fifth month of lactation. The point of
intersection of these two lines shows the maximum amount of cows' milk of minimum conductivity that can be added to human milk of minimum conductivity without the conductivity value of the resulting mixture exceeding the maximum normal conductivity value for women's milk. The amount of cows' milk of the type which can be added is twenty-two percent. Thus, the addition to human milk of more than twenty-two percent cows' milk of minimum conductivity will cause the electrical conductivity of the adulterated specimen to be abnormally high.

In a similar manner, a second line "AB" has been constructed in Figure XVII (Page 142) to determine the amount of cows' milk of average conductivity (Kreiner, 1938) \(440 \times 10^{-5}\) which must be added to human milk of average conductivity, \(180 \times 10^{-5}\) (Page 91 Appendix) in order to raise the conductivity of the adulterated human milk above the normal maximum for human milk \(210 \times 10^{-5}\) (Line "EF"). Point "A" represents the value of pure human milk of average electrical conductivity and Point "B" represents the value for pure cows' milk of average conductivity. The point of intersection of lines "AB" and "EF" shows the maximum amount of cows' milk of average conductivity that can be added to human milk of average conductivity without raising the conductivity of the resultant mixture above the normal maximum level for human
milk. This figure is eleven per cent. Therefore, the addition of more than eleven per cent of cows' milk of average conductivity to human milk of average conductivity will cause the conductivity of the resultant mixture to be abnormally high.

The electrical conductivity test will, in every case, detect adulteration of women's milk to which twenty-three per cent or more of cows' milk has been added. In fact, it may detect a considerably lower per cent of adulteration if either the conductivity of the human milk, or of the added cows' milk, is average or above the average.
SUMMARY of RESULTS into the CHLORIDE CONTENT and ELECTRICAL CONDUCTIVITY of WOMEN'S MILK.

The variations in both the chloride content and the electrical conductivity of milk obtained from women with adequate and inadequate lactation have been determined and the practical value of such estimations has been investigated.

The statistical analysis of the chloride and electrical conductivity values for milk specimens taken from women throughout the day and during the first five months of lactation has revealed the following facts: - The average values for chloride content and conductivity of milk falls during the first month of lactation. This fall probably occurs towards the end of that month, irrespective of whether the mothers are lactating well or badly and is uninfluenced by the time of day at which specimens are taken. During the second to fifth month of lactation, there is no significant variation in the average chloride values of milk. This is also true for five similar analysis utilising average values for conductivity. There is one analysis, however, made with conductivity values which did show that there was a significant fall in the conductivity values for milk taken from women in the second to fifth month of lactation. A comparison of the chloride values for milk taken in the early morning...
with those for specimens taken at mid-day shows that the values are usually lowest in the morning and highest at mid-day. Values for specimens taken in the evening lie generally between those for morning and mid-day specimen. The conductivity values for morning, mid-day and evening specimen showed the same relationship to each other as did the chloride values. The rise noted for both the chloride content and the conductivity of milk during the morning is statistically significant. This rise, however, was only noted for specimens taken in the first month of lactation and for one set of average values for specimens taken in the second to fifth month, while three sets of average values for milk taken in the second to fifth month did not differ significantly throughout the day.

Chloride and conductivity estimations of women's milk aid the determination of adequate lactation. This has been shown by comparing the milk values for women with adequate lactation with those for women with inadequate lactation. A statistical analysis of the results has revealed that both the average chloride and average electrical conductivity values for milk from women with adequate lactation are significantly lower than those for women with inadequate lactation. The importance of testing milk from both breasts when investigating the
adequacy of lactation has been demonstrated by a presentation of a series of cases, some of which exhibit unilateral breast dysfunction. When such cases are discovered by the chloride or the conductivity test, further investigation is necessary before a decision on the woman’s ability to breastfeed her infant satisfactorily can be made. The occasional occurrence of a fallacious result has been emphasised. This could not be eliminated and was thought to be due to hypogalactia occurring as a result of hypoplastic breasts.

Because it is generally possible to determine adequacy of lactation by estimating either the chloride content or the electrical conductivity of milk, it follows that there must be a correlation between milk yield and both the chloride content and the electrical conductivity of women's milk. This deduction has been confirmed by comparing the daily milk yield from one breast with the chloride content of the milk and with the electrical conductivity of the milk, the values both for chloride and conductivity being greater as the milk yield falls. This method can be used to estimate a woman’s milk yield. Since, however, these investigations have also shown that the higher the chloride and conductivity, the closer is the relationship between them and milk yield, it is evident that the milk yield can be more accurately
assessed when the chloride or conductivity value of the milk is high.

The estimation of the chloride content or the electrical conductivity of women's milk is of value in determining whether milk has been obtained from a normal or a diseased breast, since milk obtained from women with mastitis has a higher chloride content and electrical conductivity than normal. These investigations may also assist in the detection of women's milk to which water or cow's milk has been added, because such results show an exceptionally low chloride and electrical conductivity value. Evidence in support of these statements has been obtained partly from the literature and partly by personal observations.

Yet another indication for estimating both the chloride and the electrical conductivity of milk is shown by the fact that when these estimations are made on specimens of milk taken from women in the second, third and fourth week of lactation, both the chloride and the electrical conductivity values obtained will give an indication as to how long the women can be expected to lactate. This statement is founded on the results of an investigation which
showed that the lower the chloride or electrical conductivity of women's milk during the second to fourth week of lactation, the more likely is it that she will lactate for at least five months.
The relationship of blood sugar to both the lactose content of milk and milk yield in various animals has been reviewed, because a search is being made for tests for adequacy of lactation. It is possible that the level of blood sugar in a lactating woman might vary with her ability to lactate, because blood glucose is the main precursor of milk lactose (Petersen, 1944, and Espe, 1946). Unfortunately, this relationship has been studied almost entirely in animals and only a few similar observations have been made on women. Nevertheless, the results of the observations in women are in agreement with those obtained for domestic animals.

The correlation of blood sugar with both lactose content of milk and milk yield in animals has been made on numerous occasions and under varied conditions. The results of these observations show that under normal circumstances the blood sugar level in lactating animals does not influence the lactose content (Gowen and Tobey, 1931 and 1932) nor alter the milk yield (Brown Peterson and Gartner, 1936) of these animals. Even alimentary hyperglycaemia has no appreciable effect. Under experimental conditions which produce either abnormally high or exceptionally low blood sugar, the quantity and quality of the milk
is affected. An increase in the blood sugar by an intravenous injection of small amounts of mono- and di-saccharides on goats increases their milk yield, while large quantities injected daily for several weeks reduce their milk yield (Piantoni, 1908). If the sugar was given intravenously, no alteration in milk yield is observed (Nitzescu, 1925, and Macchiariulo, 1928). Subcutaneous injection of sugar in cattle, however, can increase their milk yield (Monaco et al., 1925). Sub-normal blood sugar levels resulting from insulin therapy (Guiste and Rietti, 1923, Nitzescu and Nicoleau, 1924, Macchiariulo, 1928, and Bucciardi, 1928) or phloridzin administration (Patton and Catheart, 1911) in animals lowers both the lactose content of their milk and milk yield. Therefore, it can be said that there is no relationship between the blood sugar of domestic animals living under normal conditions and either the lactose content of their milk or their milk yield. Under experimental conditions, however, hyperglycaemia had no constant effect on the milk yield (Bottomley, Folley and Walker and Watson, 1939), and hypoglycaemia reduced not only the milk yield, but also the sugar content of their milk.

Observations on the glucose content of the blood of lactating women have been made in the past, but only one worker has correlated his findings with the mother's milk yield. The relationship of women's blood sugar to the lactose content of their milk has not been studied. Evidence obtained from
the literature shows that the concentration of glucose in women's blood during pregnancy and the puerperium does not differ from that found in non-lactating women (Sanders, 1941). During pregnancy, however, eighty per cent of women develop a low sugar tolerance similar to that found in diabetic patients, while twenty per cent of pregnant women have an increased sugar tolerance (Richardson and Bitter, 1932). The rate at which the blood sugar returns to the fasting level is normal (Wolf, 1939). In the puerperium, the sugar tolerance of those women who had a low tolerance during pregnancy returns to normal within a few weeks of delivery, while those women with high sugar tolerance curve in pregnancy continue to exhibit this type of curve after parturition (Williams and Wills, 1929). Rowe, (1927), stresses the fact that the sugar tolerance of women who have been lactating for six months is the same as that of women who have not lactated. Although the blood sugar levels differ from mother to mother, the lactose content of women's milk remains fairly constant throughout lactation (Wolf, 1939). It is apparent, therefore, that there is no evidence to suggest any correlation between blood sugar and either milk yield or milk sugar levels in lactating women and, therefore, it is extremely unlikely that the estimation of a woman's blood sugar or sugar tolerance will help to assess her ability to lactate satisfactorily. Further observations on women with a
high sugar tolerance might, however, indicate whether or not this type of mother will lactate adequately.
The correlation of blood protein with milk yield in lactating women has been chosen for investigation because blood protein is the main precursor of milk protein, while the amino-acids and possibly the non-protein nitrogen in the circulating blood play only a minor role in milk protein synthesis (Petersen, 1944, and Esne, 1946). Moreover, the percentage of proteins and their relative proportions in the blood of lactating women differ in some cases from the values obtained for blood from non-pregnant and non-lactating women. The amino-acid content of the systemic circulation is, on the other hand, unaffected by milk production in lactating women and is the same as that found in women who are not pregnant, though experiments show that some amino-acids are in fact removed from the blood as they circulate through the mammary glands (Harding and Downs, 1929). Although blood protein estimations have been made during pregnancy and lactation, the results have only been correlated with the mother's milk yield by Lidneboon (1946). He compared the blood protein of three women with high milk yield with that of then women who were not lactating because they had still-born infants and he concluded that the blood protein did not bear any relation to milk
yield. Before giving details and results of the investigation into the possible relationship between blood protein and milk yield, a review of the literature relating to serum and plasma proteins during pregnancy and lactation will be given.

When women become pregnant, the percentage of their plasma and serum proteins diminishes (Zange-
meister, 1903, Plass and Bogert, 1924, Plass and Matthews, 1926, Halban et al, 1926, Strauss, 1935, Walf, 1939, Stander, 1941, and Snapper and Bandien cited by Lindeboon, 1946). This change is generally first detected in the fourth month of pregnancy and becomes more marked as pregnancy advances. The lowest average value for plasma protein is obtained for women who are in the ninth month of pregnancy, the average value being 9.3 per cent less than those for non-pregnant women (Plass and Matthews, 1926, and Wolf, 1939). Whilst in pregnancy, the lowest serum protein values are obtained in the tenth month; a few days after parturition, however, the values obtained are, on the average, slightly lower. These values ranged from 5.0 to 7.4 grams per cent or averaging 6.13 grams for twenty-five women after parturition (Defjckmann and Wagner, 1934).

After the lowest average values are recorded for the groups of cases under investigation, the averages for both the plasma and serum proteins rise throughout the puerperium. The values for plasma
protein reach the level found in non-pregnant and non-lactating women between the first and third week of the puerperium (Plas and Bogert, 1924, and Plas and Matthews, 1926), while those for serum proteins return to the values found in women who have not been pregnant by about the eighth week of the puerperium (Dieckmann and Wagner, 1934 and Lindeboon, 1946). The rise in the nineteen women investigated is relatively rapid during the first ten days of the puerperium and amounts to a ten per cent increase in the serum protein, or a daily increase of 0.94 mg. per cent; thereafter, the increase is slow, the weekly increase for two to six weeks being 1.33 per cent (Lindeboon, 1946). Dieckmann and Wagner (1934) add that the average blood protein values for lactating women only indicate the tendency of such values to rise to the values obtained in non-pregnant and non-lactating women and are not significant, because serum protein in lactating women may, in a proportion of cases, diminish during the first seventeen weeks of lactation.

The observed diminution in serum and plasma proteins during pregnancy and lactation is not due to a proportional reduction of all the protein constituents. Only the albumin fraction diminishes, while the percentage of both globulin and fibrin actually increases (Dieckmann and Wagner, 1934).
Whether this increase in globulin is always observed is doubtful, because Lindeboon (1946), who examined nineteen lactating women found that the low serum protein returned to the "pre-pregnant" level during lactation, by not only the albumin fraction of the blood increasing, but also the globulin fraction. At all events, the relatively low albumin-globulin ratio found in pregnancy returns to that found in the pre-pregnant mother by the eighth week of lactation. The degree to which this change may occur is shown by the albumin-globulin ratio falling from 3 in 1 to 2 in 1, or even 1½ in 1 during pregnancy (Wolf, 1939).

Four explanations for the decrease in blood protein in pregnancy, and two for the low values in lactation, have been offered. Those applicable to pregnancy are: - (1) an increase in plasma protein volume, without a proportional increase in the total serum protein (Dieckmann and Wagner, 1934) or a disturbance in protein metabolism (Lindeboon, 1946); 
(2) utilisation of protein by the embryo; (3) renal disease in some cases; and (4) inadequate diet (Lindeboon, 1946). In no case has any given theory been proven to be entirely responsible for the anomalous blood protein value in the puerperium.

The two explanations given for low blood protein in lactating women are: - (1) disturbance in protein metabolism similar to that found in
pregnant women and this alteration in protein metabolism rapidly disappears after parturition (Lindeboon, 1946); and (2) a less tenable theory is that large amounts of protein are lost in milk (Peacock and Hinman, 1935): this conclusion was reached by Peacock and Heinman (1935) who took specimens of blood from six women immediately before breast feeding their infants and again two hours later. The albumin and globulin content of the two specimens taken from any given woman did not appreciably differ and thus the authors concluded that the loss of protein in milk has no recognisable effect on the blood protein in the systemic circulation. This deduction, although not conclusive, is probably correct, because milk secretion usually takes place fairly continuously over the whole twenty-four hours of the day (Kay, 1947). Moreover, such a theory is unlikely, because during the major part of their lactation period, the blood protein is not low.
OBSERVATIONS on the PLASMA PROTEIN of LACTATING and NON-LACTATING WOMEN.

Investigations have been made into the relationship between plasma protein and adequacy of lactation in women in the hope of obtaining more information about mothers who have insufficient milk for their infants. No evidence favouring the existence of such a relationship has been found in the literature. It has been stated, however, that the plasma proteins in women do vary during the first two weeks of the puerperium, the values being at first relatively low and becoming normal by the end of the second week of lactation. This alteration in the composition of the blood has not been correlated with the mother's milk yield.

A series of eighty women have been investigated. Of these women, thirty-seven had neither been pregnant nor lactated for at least a year prior to the investigation and their ages varied between fourteen and forty years. The remaining forty-three women were lactating, twenty-three adequately and twenty inadequately. At the time of investigation, the women had been lactating for from three to eight weeks. From each of the eighty women 5 c.c. venous blood was obtained and placed in a test tube containing sufficient oxalate to prevent it from clotting.
Each specimen of oxalated blood was examined for plasma protein and albumin by the Kjeldahl method as described by King (1946). Having ascertained the amount of albumin and protein in the plasma, the percentage of globulin was calculated by obtaining the difference between the amount of protein and albumin. The results of the investigation have been recorded in the Appendix, (Pages 195 to 200).

To determine whether there was a relationship between plasma protein and adequacy of lactation, the plasma protein, albumin and globulin values have been used for three series of calculations. The first was made to decide whether the plasma protein value for women in the first month after parturition was the same as that obtained during the second month. The second series of calculations was made to decide whether the plasma protein level of lactating women was the same, or different, from that of non-lactating women. If a difference did exist, then a third series of calculations had to be made to decide whether the plasma protein value for women varied with the mother's ability to breast-feed her infant.

The Comparison of the plasma protein values for women in the first month of lactation with those for women in the second month of lactation:

The first series of calculations has been made
to decide whether the value of plasma protein for women in the latter half of the first month of lactation was the same as that for women in the second month of lactation. The data used for the comparison consists of twenty plasma protein values obtained from women in the third and fourth week of lactation, ten being for women with adequate lactation and ten for women with inadequate lactation. These values have been compared with twenty-three plasma protein values obtained from women in the second month of lactation, thirteen being obtained from women with adequate lactation and ten from women with inadequate lactation. (Appendix Pages 197 to 200).

The range of plasma values for the two groups of women was similar. The values for women in the first month of lactation ranged from 6.02 to 7.73 grams per cent, and those for women in the second month of lactation ranged from 6.18 to 7.49 grams per cent. Similarly, the mean value for the two groups of women differ very little indeed. The mean value for women in the first month of lactation was 6.68 grams per cent and that for women in the second month of lactation was 6.82 grams per cent. To decide whether the difference between these two means was significant, the "t" test was applied to the data. The calculations were made in the usual way (Appendix Pages 201 and 202), and the value obtained for "t" was 1.0. The result showed that
although the mean plasma protein value was higher for women in the second month of lactation than for those in the first month of lactation, the difference was not of statistical significance (when $P = 0.1$ and "$t" = 1.64).

The comparison of the plasma protein values obtained from lactating women with those for non-lactating women:

Data for the comparison of plasma protein values for lactating women with those for non-lactating women will be found in the Appendix (Pages 195 to 200). Forty plasma protein values, with a corresponding number of plasma, albumin and globulin values for lactating women, have been recorded. Because the values were obtained from women between the third and eighth week of lactation, they have been regarded as a homogeneous group, and, therefore, could be compared with thirty-seven plasma protein values obtained from non-lactating women. The values for the group of lactating women have, however, been sub-divided into those from cases lactating adequately (twenty-three), and those from women lactating inadequately (twenty).

The range of plasma protein values for each of the two groups of lactating women has been compared with that for non-lactating women. The range for
women with adequate lactation was from 6.01 to 7.54 grams per cent, and that for women with inadequate lactation was 6.05 to 7.74 grams per cent. That for non-lactating women was from 5.40 to 7.84 grams per cent. Thus, the range of values for lactating women, whether lactating adequately or inadequately, was similar to the range obtained from women who were not lactating.

However, when the mean of the plasma protein values, obtained either for women with adequate lactation or for women with inadequate lactation, was compared with the mean plasma protein value obtained for non-lactating women, these values differed; the mean value for those who lactate adequately, being 6.58 grams per cent, that for women with inadequate lactation being 6.97 grams per cent, and that for women who were not breast-feeding being 6.32 grams per cent. To decide whether the observed differences of 0.26 and 0.65 grams per cent respectively were statistically significant, the "t" test was applied to the data in the usual way (Appendix Pages 204 to 206). When the mean plasma protein value from women with adequate lactation was compared in this way with the mean for non-lactating women, a "t" value = 1.8 was obtained, which indicated a significant difference between the two mean values (when P = 0.1 and "t" = 1.64). Using the "t" test to determine the
significance of the difference between the mean protein value for women with inadequate lactation and that for women who were not lactating, the value obtained for "t" was 4.3. Therefore, the difference between the two mean values was highly significant. Thus, the average plasma protein values for women with both adequate and inadequate lactation were significantly higher than that for non-lactating women.

To decide whether the relatively high plasma protein in lactating women was due to an increase of albumin or globulin, or of both these factors, a series of calculations using first the albumin values and then the globulin values was made.

The mean plasma albumin value for women with adequate lactation was compared with that for non-lactating women, as was that for women with inadequate lactation. The mean plasma albumin value for women with adequate lactation was 4.35 grams per cent, that for women with inadequate lactation was 4.48 grams per cent, and that for non-lactating women was 4.01 grams per cent; the two values for lactating women being greater than that for non-lactating women. To determine whether the difference between these mean values was significant, the "t" test was applied to the data in the usual way (Appendix Pages 206 and 207). When the significance of the difference between the mean
value for women with adequate lactation and that for non-lactating women was calculated, the value obtained for "t" was 3.4, showing that a significant difference between the mean albumin values for the two groups of women existed (P = 0.1 and "t" = 1.64). Similar calculations using the values for women with inadequate lactation and those for non-lactating women gave a "t" value of 3.9, proving that the difference between the two mean albumin values was significant.

A study of the mean plasma globulin value for both lactating and non-lactating women was necessary to decide whether the globulin fraction accounted for plasma protein being relatively higher when a woman breast-fed her infant than when she was not lactating. The mean plasma globulin value for the non-lactating group was 2.31 grams per cent, that for women with adequate lactation was 2.23 grams per cent, and that for women with inadequate lactation was 2.49 grams per cent. Therefore, the average globulin value for lactating women was more and the other was less than that obtained for non-lactating women. When the "t" test was used to determine the significance of the difference between the average globulin value for non-lactating women and that for women with adequate lactation, the value obtained for "t" was 0.8 (Appendix Pages 207 & 208) which indicated an insignificant difference between the mean values for the two groups.
of women (when $P = 0.1$ and "$t" = 1.64). A statistical analysis to determine the significance of the difference between the mean globulin value for non-lactating women and that for women with inadequate lactation was also made (Appendix Pages 207 and 208). The result was "$t" = 1.5$, showing that the difference between the two mean globulin values was insignificant.

Therefore, the significant difference which existed between the average plasma protein of non-lactating women and that of lactating women was due to a significant increase in the albumin fraction of the blood of the latter, the difference between the globulin fractions of the two groups being insignificant.

The Comparison of the plasma protein values for women with adequate lactation with those for women with inadequate lactation:

A third series of calculations was made to decide whether the plasma protein values of women with adequate lactation differed from those of women with inadequate lactation. The women from whom the data was obtained were those included in the previous investigation, namely the twenty-three women with adequate lactation and the twenty women with inadequate lactation. The values of their plasma protein, albumin and globulin were recorded.
in the Appendix (Pages 195 to 200). While the range of plasma protein values for the two groups did not differ appreciably, the mean plasma protein value of 6.97 grams per cent for women with inadequate lactation was relatively higher than the mean value of 6.58 grams per cent for women with adequate lactation. To determine whether the difference between the mean values was statistically significant, the "t" test was applied to the data (Appendix Pages 209 and 210). The value thus obtained for "t" was 3.0 which showed that the difference between the average values for the two groups was significant (when P = 0.1 and "t" = 1.64).

Calculations have also been made comparing the albumin fraction of the plasma protein from women with adequate lactation with that from women with inadequate lactation and a similar comparison has been made between the globulin fractions in the two groups to ascertain whether one or both these fractions were responsible for the difference between average plasma protein values in the two types of mother. From women with adequate lactation, the mean albumin value was 4.35 grams per cent, while that for women with inadequate lactation it was 4.48 grams per cent. The "t" test was applied to the data to determine the significance of the difference between the two mean values (Appendix Page 209). The results of the analysis gave a "t" value = 1.3, indicating
no significant difference between the two means (when $P = 0.1$ and "$t" = 1.64). The mean blood globulin value for women with adequate lactation was 2.23 grams per cent and that for women with inadequate lactation was 2.49 grams per cent. The "$t" test was then used to determine the significance of the difference between the two averages. According to this analysis, "$t" = 2.6$ (Appendix Page 210) which showed that the difference between the two globulin values was significant (when $P = 0.1$ and "$t" = 1.64). Thus, the significant difference between the mean plasma protein value for women with adequate lactation and that for women with inadequate lactation was due to the globulin being less for women with adequate lactation than it was for women with inadequate lactation.

CONCLUSION DRAWN FROM OBSERVATIONS ON PLASMA PROTEINS:

From the investigations on women's plasma protein, albumin and globulin, it has been concluded that there is a relationship between plasma protein and adequacy of lactation in women. The mean plasma protein value for women with inadequate lactation is significantly higher than that for women with adequate lactation. This difference between the two values is due to the globulin of the plasma being relatively higher in women with inadequate lactation.
while the albumin fraction in these women is not significantly different from that obtained for women with adequate lactation.

Additional information regarding the plasma protein in lactating women has been obtained in the course of the above investigation. Although plasma protein is relatively low during pregnancy and does not return to a normal level until the end of the second week of lactation, it has been shown to be relatively high during the third to eighth week of lactation. The increase is due to a rise in the albumin content of the blood while there did not appear to be a significant alteration in the globulin content. The change in albumin is small, but it is statistically significant when the mean values for lactating women was compared with the mean value for non-lactating women. This applies when either values for women with adequate lactation or those for women with inadequate lactation are compared with values obtained for non-lactating women. How long the plasma proteins remain relatively high in lactating mothers is a matter for investigation. It can be said, however, that there is no tendency for the mean protein value to be lower in the second month of lactation than it was in the third and fourth week of lactation. On the contrary, the mean value is greater in the second month than in
the preceding month. The difference, however, is statistically insignificant.

Single observations on plasma protein in lactating women are of no value in assessing adequacy or lactation because the range of values for women lactating well does not differ appreciably from that for women with inadequate lactation. Nevertheless, repeated observations on the same woman throughout lactation might be of clinical value. To ascertain a series of observations correlating plasma protein with adequacy of lactation in the same woman would be necessary.

The cause of the relatively high plasma protein in lactating women is a matter for conjecture. Since the control of lactation is largely hormonal, it is probable that activity of the endocrine glands may be chiefly responsible for the alteration in the protein content of the blood. The mode of action of hormones upon protein metabolism, however, is not understood. The hormones which may be involved in the alteration in the composition of the blood are those secreted by the adrenal gland, the thyroid and the ovary. Secretions from any one of these structures, or from a combination of them, may play a part because there is evidence to show that the adrenal gland and, possibly, the thyroid gland influence protein metabolism (Turner, 1948), while administration of oestrogens has also been shown to
increase serum protein (Editorial, B.M.J., 1949). Until more is known of the biochemical changes brought about by circulating hormones, the cause of the raised plasma protein in lactating women is unlikely to be fully understood.
THE RELATIONSHIP OF BLOOD LIPIDS AND MILK YIELD IN WOMEN.

An increase in all blood lipids occurs in women at the conclusion of labour (Slemons and Stander, 1923). The cause and purpose of this change is obscure, but it is possible that it may be related to the adequacy of lactation. The literature shows that only one fraction of the blood lipids (the phospholipids) has been correlated with the milk yield (Harding and Downs, 1929). These workers estimated the lipid phosphorus in the blood of women with little milk, with normal milk yield, and with large amounts of milk and found that the average phosphorus values for each of the three groups did not differ appreciably. Therefore, the estimation of lipid phosphorus in the blood is valueless as a method of determining adequacy of lactation in women.

The second form of blood lipid which might alter with a woman's ability to lactate is neutral fat, particularly since neutral fat is the main precursor of milk fat (Peterson, 1944, and Espe, 1946). No observations relating to the neutral fat content to the milk output have been found in the literature and, unfortunately, such an investigation was found impracticable because of a complicated technique, involving the estimation of total fatty acids, phospholipids and cholesterol esters (Hawk et al., 1947). Even if this procedure did reveal a
relationship between the neutral fat in the blood and the milk yield, such a method would be too complicated and time-consuming to be of practical value.

The remaining lipid constituent of the blood is cholesterol. It is known that the level of cholesterol in the blood is altered during lactation, but only one reference dealing with the relationship between this change and milk yield has been found, and this showed that a woman with a normal blood cholesterol on the fifth day of lactation did not experience any difficulty in breast-feeding her infant (Pribram, 1923). It is, therefore, apparent that these two factors have not been satisfactorily correlated. It has been decided to carry out such an investigation, the details of which, together with the results obtained, follow a review of the literature on blood cholesterol in pregnant and lactating women.

HISTORICAL REVIEW:

The blood cholesterol in pregnant women generally begins to increase during the third or fourth month of gestation and, as pregnancy advances, the cholesterolaemia becomes progressively more marked and attains its maximum at term. This increase is probably always detectable by the eighth month of pregnancy. (Hermann and Newmann, 1911 and 1912,
Chauffard, Laroche and Grigant, 1911, Bloor, 1921, Plass and Tompkins, 1923, Slemons and Stander, 1923, Tyler and Underhill, 1925, and Palacios-Costa and Falsia, 1930). The time taken for the blood cholesterol to return after parturition to the level found in women who are not pregnant is variable. Chauffard and his co-workers (1911) noted that blood cholesterol in women falls to the "pre-pregnant" level of 165 mg. per cent within twenty-four hours of parturition in the majority of cases. According to Palacios-Costa and Falsia (1930) a somewhat similar level of 172 mg. per cent was obtained for the thirty women they examined on the seventh day of lactation. In the second week of lactation, however, the women again develop hypercholesterolaemia, the blood cholesterol level being about 260 to 270 mg. per cent (Chauffard et al., 1911). Tyler and Underhill (1925) who examined eleven women on the tenth to twelfth day of their lactation obtained blood cholesterol values for these women ranging from 230 to 385 mg. per cent, and averaging 280 mg. per cent. These values were considerably higher than those obtained for ten women who had not been pregnant, whose values ranged from 180 to 249 mg. per cent with an average of 207 mg. per cent. These high values persisted in seven out of nine mothers between the fifteenth and fortieth day of lactation, but by the end of the second month of lactation the
Chauffard, Laroche and Grigant, 1911, Bloor, 1921, Plass and Tompkins, 1923, Slemons and Stander, 1923, Tyler and Underhill, 1925, and Palacios-Costa and Falsia, 1930). The time taken for the blood cholesterol to return after parturition to the level found in women who are not pregnant is variable. Chauffard and his co-workers (1911) noted that blood cholesterol in women falls to the "pre-pregnant" level of 165 mg. per cent within twenty-four hours of parturition in the majority of cases. According to Palacios-Costa and Falsia (1930) a somewhat similar level of 172 mg. per cent was obtained for the thirty women they examined on the seventh day of lactation. In the second week of lactation, however, the women again develop hypercholesterolaemia, the blood cholesterol level being about 260 to 270 mg. per cent (Chauffard et al., 1911). Tyler and Underhill (1925) who examined eleven women on the tenth to twelfth day of their lactation obtained blood cholesterol values for these women ranging from 230 to 385 mg. per cent, and averaging 280 mg. per cent. These values were considerably higher than those obtained for ten women who had not been pregnant, whose values ranged from 180 to 249 mg. per cent with an average of 207 mg. per cent. These high values persisted in seven out of nine mothers between the fifteenth and fortieth day of lactation, but by the end of the second month of lactation the
values were almost the same as those obtained for women who had not been pregnant, their average value being 230 mg. per cent (Chauffard et al., 1911).

Suggestions as to the purpose of the relatively high blood cholesterol values in pregnant and lactating women have been made, but proof of their validity has not been obtained. Selmons and Stander (1923) suggest that the rise in blood cholesterol in pregnancy is possibly to prepare the mother for successful lactation. Evidence supporting this theory is that the mother has a lowered fat tolerance during pregnancy and stores fat during this period. After parturition, the production of milk requires an appreciable supply of fat which is derived from the mother's body if her diet is adequate. It is reasonable to suppose, then, that the unusual quantity of cholesterol in the blood during the latter part of pregnancy is explained by the transportation of this material to storage centres whence it may be utilised when the breasts become active.

If a high blood cholesterol predisposes to successful lactation, it is likely that either a direct or indirect relationship exists between the two. Evidence shows that the latter is present because in the first few weeks of lactation the blood cholesterol of the mother is high and her milk yield is relatively low, while in the later weeks of
lactation the value of her blood cholesterol is lower and her milk yield greater. Caution, however, must be exercised in accepting this as significant because in cows there is only rarely any correlation between the cholesterol content of the blood and total milk production (Doulkin and Helman, 1934). Even by varying the percentage of fats in a mother's diet (Ruzicic, 1938) which influences the amount of fat in the blood (Duncan, 1942) or by altering the percentage of phospholipids in women's blood (Harding and Downs, 1929) it is not possible to produce an appreciable effect on the milk yield. Therefore, it is doubtful whether high blood cholesterol does predispose to adequate lactation.

Another possible effect of high blood cholesterol in lactating women is to raise the percentage of this constituent in the milk. This, in fact, is believed to occur (Fox and Gardiner, 1924) since the cholesterol content of colostrum and of milk taken during the first twelve days of lactation has an average of 440 mg. per cent and that for milk taken after this period is 210 mg. per cent (Dorlencourt and Palfy, 1925). This reduction in value coincides with the fall in the cholesterol level in the blood. Although the relatively high blood cholesterol in women may explain the high cholesterol content of their milk, it may also influence their milk yield.
OBSERVATIONS ON THE PLASMA CHOLESTEROL OF LACTATING AND NON-LACTATING WOMEN.

In the past, no satisfactory explanation has been offered for the cholesterolaemia present in lactating women. It has been suggested, however, that it may be related to successful lactation, but insufficient evidence to confirm, or refute, this suggestion has been obtained. Investigations have, therefore, been made to clarify the position, the details of which, together with the results and conclusions, are given below.

The problem has been approached along the same lines as were adopted for the investigation of plasma protein in lactating women. The eighty women studied during the enquiry into plasma protein levels were also examined to obtain data for the plasma cholesterol investigation. An estimation of the blood cholesterol was carried out, in addition to the plasma protein observations already mentioned, on the blood specimen obtained from each woman. The Sackett method described by King (1946) was used to estimate the cholesterol in the blood plasma. The results of the tests are recorded in the Appendix (Pages 195 to 200).

To determine whether there was a relationship between the plasma cholesterol and adequacy of...
lactation in women, the data obtained from the investigation was analysed by subjecting it to three series of calculations. In the first series, the plasma cholesterol for women in the third and fourth week of lactation was compared with that for women in the fifth to eighth week, in order to ascertain whether these values differed significantly. In the second series, the cholesterol values for lactating women were compared with those for non-lactating women to confirm, or refute, the statement that the plasma cholesterol values obtained from lactating women were greater than those obtained from non-lactating women. The third series was made to determine whether the plasma cholesterol value bore a relationship to adequacy of lactation in women.

The Comparison of the plasma cholesterol values for women in the first month of lactation with those for women in the second month of lactation:

To decide whether the plasma cholesterol values obtained for women in the first month of lactation differed from those obtained for women in the second month of lactation, twenty values for women in the third and fourth week were compared with twenty-three values for women in the fifth to eighth week of lactation. (Appendix Pages 197 to 200).
The range of plasma cholesterol values for women in the third and fourth week of lactation lay between 165 to 310 mg. per cent and that for women in the second month was very similar, namely between 125 to 300 mg. per cent. The average value for plasma cholesterol obtained for women in the first month was 224.5 mg. per cent and that obtained for women in the second month was 214 mg. per cent. It was necessary to apply the "t" test to the two mean values and relevant data to determine the significance of the difference between the two means. The calculation was made in the usual way (Appendix Page 203), and the value obtained for "t" was 0.79, indicating that the difference between the two means was not significant (when $P = 0.1$ and "t" = 1.64). Therefore, although the average plasma cholesterol value became lower as lactation advanced, the difference was statistically insignificant.

The comparison of the plasma cholesterol values obtained from lactating women with those for non-lactating women:

The second series of calculations was made to determine whether a difference did exist between the plasma cholesterol values for lactating women and those for non-lactating women as stated by some
authors. The data consisted of forty-three values obtained from lactating women and thirty-seven from women who were not lactating (Appendix Pages 195 to 200). The values for the group of forty-three lactating women were sub-divided into those from cases lactating adequately (twenty-three) and those from women lactating inadequately (twenty). The range of plasma cholesterol values for women with adequate lactation was from 125 to 290 mg. per cent, that for women with inadequate lactation was 130 to 310 mg. per cent, and for non-lactating women from 87 to 235 mg. per cent. Thus, though the individual ranges overlapped to a considerable degree, the highest values were obtained from women who were lactating.

The tendency for the plasma cholesterol values to be higher from lactating women than from non-lactating women was also observed when the mean values for each group were compared. The mean plasma cholesterol values obtained for the non-lactating group was 150 mg. per cent, that for women lactating well was 209.3 mg. per cent, and that for women lactating badly was 230 mg. per cent. To decide whether the difference between the mean value for non-lactating women and that for women with adequate lactation was significant, the "t" test was applied for the data in the usual way.
(Appendix, Pages 211 and 212). The value obtained for \( t \) was 6.6 which indicated that the difference between the mean values for the two groups was highly significant (when \( P = 0.10 \) and \( t = 1.64 \)). When using the \( t \) test to determine the significance of the difference between the plasma cholesterol value for non-lactating women and that for women with inadequate lactation, the value for \( t \) was 9.1, a highly significant value indicating that the difference between these two mean values was statistically significant. Therefore, the mean cholesterol value for both women with adequate and inadequate lactation was significantly higher than that for non-lactating women.

The Comparison of the plasma cholesterol values for women with adequate lactation with those for women with inadequate lactation:

A third series of calculations was made to decide whether the plasma cholesterol values for women with adequate lactation differed significantly from those for women with inadequate lactation. Twenty-three women with adequate lactation and twenty with inadequate lactation were studied to obtain the data for this investigation. The plasma cholesterol values for these women were recorded in the Appendix (Pages 197 to 200). The cholesterol
values for women with adequate lactation ranged from 125 to 290 mg. per cent, and those for women with inadequate lactation ranged from 130 to 310 mg. per cent, the difference between the two ranges being slight. The mean of the values obtained for women lactating well was, however, 209.3 mg. per cent, and that for women lactating badly was 230 mg. per cent, a difference of 20.7 mg. per cent. To determine whether the difference was significant, the "t" test was applied to the data (Appendix, Page 213.) The value obtained for "t" was 1.7, a value which is just significant (when \( P = 0.1 \) and "t" = 1.64). It was, therefore, concluded that the mean cholesterol value was significantly higher in women with inadequate lactation than in women with adequate lactation.

Conclusions drawn from observations on plasma cholesterol:

It has been shown that women with inadequate lactation have a significantly higher blood cholesterol than women with adequate lactation. Therefore, there is an inverse relationship between adequacy of lactation and plasma cholesterol in the series of women studied during the first two months following parturition.

It has also been shown that the mean plasma
cholesterol values of both adequately lactating and inadequately lactating women are significantly higher than that for non-lactating women. Moreover, the relatively high value found in lactating women persists throughout the first two months of lactation, no significant difference being noted between values obtained for mothers in the first month and those obtained for mothers in the second month of lactation. These facts confirm reports in the literature, in so far as it is stated that the blood cholesterol may be raised during the first two months following parturition, but do not support the view that the blood cholesterol returns to a "normal" level either before, or by about the end of, the second month of lactation.

Single estimations on the plasma cholesterol of lactating women are of no practical importance in assessing the adequacy of lactation, because the range of values for women lactating well does not differ appreciably from that for women lactating badly. It is possible, however, that if a series of observations were made correlating the plasma cholesterol values with milk yield in the same woman, information might be obtained which would be helpful in understanding the physiology of lactation more fully.
Of the many factors which influence cholesterol metabolism, hormonal activity is the most likely explanation of the relatively high plasma cholesterol in women during the first two months of their lactation. In the series of cases under review, the factors which might influence lipid metabolism, but which played little or no part in raising the plasma cholesterol in the lactating women, include the change of climate and temperature (Doulbin and Helman, 1934, Allen, 1938, and Scott, 1940), but climatic conditions did not differ markedly for the various women under investigation. The effect of the varied composition of the women's diets could account for some alteration in the plasma cholesterol (Jordon, Hart and Patton, 1906, Maynard et al, 1931, and Duncan, 1942), but this effect is unlikely to be great in the women since the amount of fat available for each member of the population is strictly limited. Moreover, the women were members of an urban population who had not free access to farm products rich in fat. Disease, no doubt, can account for considerable variation in the cholesterol level of the blood, but constant care to eliminate this possibility was exercised.

Perhaps the most convincing evidence to show that some factor or factors directly concerned in the control of lactation must play a major role in
explaining the cholesterolaemia present in lactating animals has been supplied by Maynard and his associates (1931). These workers studied the blood cholesterol of cattle during pregnancy and lactation, under carefully controlled conditions. Evidence supporting the theory that different hormones are responsible for the relatively high plasma cholesterol level in lactating women has been obtained from both clinical and experimental observations. For example, blood cholesterol is usually raised in patients with hypothyroidism and in experimental rabbits, rats and hens to which oestrin has been administered (Lorenz, Chaikoff and Enterman, 1938, Loeb, 1942, and Pfeiffer, 1943). A reduction in blood cholesterol is sometimes observed in people with thyrotoxicosis and in cattle to whom thyroxin has been administered (Dastur and Smith, 1939, and Smith and Dastur, 1940). A decrease in the blood cholesterol has been observed in a woman after oophorectomy (Pribram, 1923). Whether one hormone, or a combination of several, is responsible for the relatively high plasma cholesterol obtained in women during the first two months of lactation remains a problem for investigation.
SUMMARY OF RESULTS OF THE INVESTIGATIONS INTO BOTH
THE PLASMA PROTEIN AND THE PLASMA CHOLESTEROL CONTENT
OF THE BLOOD IN LACTATING AND NON-LACTATING WOMEN.

Estimations have been made of both the plasma protein and the plasma cholesterol of women's blood, to determine whether such estimations are of practical value in assessing adequacy of lactation in women. The results have been analysed and are given below.

From the investigations on plasma protein, it was concluded that the mean plasma protein value for women with inadequate lactation is significantly higher than that for women with adequate lactation. This difference between the two values is due to the plasma globulin being relatively higher in women with inadequate lactation, while the albumin fraction in these women is not significantly different from that obtained for women with adequate lactation. Single observations on plasma protein in lactating women, however, are of no value in assessing adequacy of lactation because the range of values for women lactating well does not differ appreciably from that for women with inadequate lactation.

A comparison of the average plasma protein value of lactating women with that of non-lactating women shows that the average plasma protein value for women in the third to eighth week of lactation is
significantly higher than that obtained for non-lactating women. The relatively high value for lactating women is due to an increase in the albumin fraction of the blood, while there is no significant alteration in the globulin content. This increase in plasma protein in lactating women is maintained throughout the first two months of lactation since there is no statistically significant difference between the value obtained for mothers in the first month of lactation and that for mothers in the second month of lactation.

The results of the investigation on the plasma cholesterol showed that women with inadequate lactation have a significantly higher plasma cholesterol than women with adequate lactation, when the mean value for each group is compared. Single estimations on plasma cholesterol of lactating women are of no practical importance in assessing adequacy of lactation, because the range of values for women lactating well does not differ appreciably from that for women lactating badly.

It has been shown that the mean plasma cholesterol values for both adequately lactating and inadequately lactating women are significantly higher than that for non-lactating women. Moreover, the relatively high value obtained for lactating women
persists throughout the first two months of lactation, no significant difference being noted between mean values obtained for mothers in the first month and those obtained for mothers in the second month of lactation.

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