FACTORs AFFECTING THE COMPOSITION OF COW'S MILK

by

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INTRODUCTION
INTRODUCTION

Extensive studies have been made of factors causing variation in the total yield and butterfat content of cows' milk. This has been possible because of the spread of organised milk recording and the simple and rapid methods available for determining fat percentage. Herd Book Societies and Cow Testing Associations offer incentives of various kinds, including special awards for bulls whose daughters are outstanding producers and similar recognition for outstanding lactation performances by dairy females. The net result has been a marked improvement in yield among dairy cattle. Part of this gain is due to a better knowledge of nutrition, coupled with better management. Some of the gain must, however, be regarded as genetic and the result of selection.

The economic incentive to breed cows giving more milk of a higher fat content has been paralleled by an equal neglect of solids-not-fat. Actual data on solids-not-fat is surprisingly scarce. No study so far made on factors causing variation in solids-not-fat and its relationship to fat percentage has sorted out the effect of those factors in a satisfactory way. Considerable data on milk composition has been collected but it has nearly always been the result of research carried out for reasons other than livestock improvement. Results have been presented in an extremely uncritical fashion and the aim seems to have been to establish the statistical relationship between different milk constituents. The study
has often stopped at this stage. No attempt has been made to get at the causes behind a given correlation or the factors affecting it, either temporarily or permanently.

The large amount of data available from Milk Recording Societies has enabled population studies to be made which have given us a fairly concrete idea of the relative importance of heredity and environment on total yield and fat percentage. Lush and his co-workers ascribe a heritability of 0.25 to total yield and 0.5 to fat percentage. Similar estimates have never been made for constituents other than fat, because no adequate body of data has been available on which to work. This is understandable, as there has been no economic incentive nor are simple analytic methods available. What data does exist is too limited in amount to give reliable estimates of heritability in Lush's sense.

The relationship between different milk constituents and the extent to which it is genetic is important. With continuous selection for increased butterfat percentage, we have no idea what are the effects on solids-not-fat. Most countries have legal minima for fat and solids-not-fat. In Great Britain this is 3.0% for fat and 8.5% for solids-not-fat. This reflects the current confusion of thought in the matter. Solids-not-fat embraces a series of substances related in various degrees to fat, and in the case of protein and lactose in opposite ways - at least physiologically! We shall see that a "within cow" relationship is by no means the same as a
"between cows" ones. The first is physiological and characteristic of all cows as far as we know. The second is partly hereditary and partly environmental and the relationships for each and every cow, with the possible exception of identical twins, are different.

The relationships between different milk constituents are now well known and are backed up with a good deal of analytical data. But the factors operating on these relationships are practically unknown, as is the extent to which significantly correlated substances like fat and protein can vary independently of each other. Until enough data is available for a reliable estimate of heritability, we must be content with a more thorough analysis of environmental effect, coupled with a reserved appraisal of the extent to which bulls in a given herd affect the total variance of the constituents in the milk of their daughters. Some of these factors are dealt with in the following pages. In each case an attempt has been made to sort out the effect of these factors which form the environment, whilst holding the effect of other factors constant, or eliminating them by suitable corrections.
STUDIES IN MILK COMPOSITION

A review of the literature.
STUDIES ON MILK COMPOSITION

Early studies of milk composition were confined to butterfat percentage on one hand and solids-not-fat percentage on the other. No attempt was made to study the variation of the components of the solids-not-fat fraction. Thus, an early paper by Gowan (1919) deals with the effect of age on butterfat percentage and solids-not-fat percentage. Tocher (1925) was one of the first workers to make an exhaustive analysis of the milk of a considerable number of cattle. He calculated a series of regression equations between the different substances and of each substance on age. He also records the variation in milk composition with advance in lactation. These, and many other studies, give a fair idea of the range of variation to be expected. Despite this, no paper provides a satisfactory picture of those factors tending to modify composition. This has been principally due to inadequate statistical techniques. The analysis of variance has been little used and regressions and correlations have been carried out with no distinction between intra- and inter-cow relationships. Thus, the generally accepted relationship between butterfat, protein and lactose which is the same for all cows, individually, is not so between cows. In the present investigation a significant positive correlation between fat, protein and lactose was found between, as distinct from within cows. A fact no other author has reported, apart from Overman, in a study of the same breed - Ayrshires. This may be a peculiarity of Ayrshires, but is
more likely to be due to faulty treatment of data by other workers. Until Fisher's analysis of variance (developed by Yates, Snedecor, Lush and others, to suit particular problems) there was no satisfactory method of distinguishing between causes. As far as we are aware, only Becker and Arnold (1935) have exploited the two-way table and the analysis of variance, as a means of measuring the separate effects of season of the year and stage of lactation on butterfat percentage. Similar studies on crude protein, lactose, chloride and density have been made in the present investigation.

The Relationship of different Milk Constituents to each other

Tocher (1925) whose investigation for many years provided the standard of comparison for investigations carried out in this country, noted the following relationships between the principal constituents of milk:

(i) A positive linear relation between butterfat percentage, solids-not-fat percentage and casein percentage.

(ii) The greater solids-not-fat percentage, the greater was lactose.

(iii) The greater percentage lactose the smaller was butterfat percentage.

(iv) There was practically no relation between percentage lactose and casein nitrogen.

(v) The regression, ash on lactose percentage was slightly curvilinear.
Tocher makes some interesting comparisons between lactose content and total yield. 3.3% lactose was associated with 8 lb. total yield and 5.5% with 16 lb. The higher the yield, the greater the percentage lactose. The relationship was not a straight line one, however. Gaines (1936) classified cows by fat percentage and found that within fat groups 1 lb. lactose was accompanied by 18 lb. milk water. Lactose and other constituents in true solution in milk undoubtedly have an important role in water movement, from blood to milk. They are responsible for maintaining osmotic pressure at a time when other substances in solution are being converted into more complex ones which are not, e.g. amino-acids to casein; acetalde and glucose to fat. Work to date has not given an adequate explanation of the part osmotic phenomena play in milk secretion. This subject is returned to in a subsequent section when the osmotic effects of lactose and chloride are discussed in relation to the present investigation.

Black and Veris (1934) in a study of the statistical relationships between the constituents of milk from 12 Holstein Friesians in the U.S. report as follows:-

Fat is regarded by many as the most variable constituent of milk but results show that sodium, chlorine and magnesium vary more and protein as much. Solids-not-fat percentage show least variability. Of its components, ash varies least, followed in ascending order of variability by lactose, phosphorus, calcium, chlorine, magnesium and sodium.
Thus:

(i) As fat percentage increases, the energy value, protein, calcium and magnesium increase.

(ii) As energy values increase, protein, calcium, magnesium, phosphorus and solids as a whole increase.

(iii) As protein percentage increases, calcium and total ash increase and potassium decreases, whilst there is an increase in total solids percentage.

(iv) As lactose percentage increases, it causes a rise in solids-not-fat percentage as a whole, whilst sodium and chlorine decrease.

(v) Sodium and chlorine are closely and positively correlated.

From these studies we gain a good idea of the intra-cow relationship of the various milk constituents. Various other workers, who have worked on a smaller number of substances but using a greater number of animals agree, in general, with these findings, although the relationships that Tocher finds between percentage lactose and total yield are not borne out by the results from other investigations.

The effect of age.

The effect of age on butterfat yield has been dealt with exhaustively by Johansson and Hansson (1940) in a study of 8,000 Swedish cows in 23 herds, between the years 1925-39.
The effect of age on butterfat percentage is found to be much slighter than for butterfat yield and in their data, Johannson and Hansson ignore it. A slight decrease in fat content did occur with increasing age but this has been found to be so slight as to be unimportant by Langmack (1921), Gowen (1920 and 1924) and Tuff (1931). For other constituents, Tocher finds little correlation. The highest values were obtained for the positive correlation with butterfat and the negative ones between age and lactose, refractive index, solids-not-fat and casein. Butterfat percentage and albumin percentage showed a non-linear relationship with age. In Tocher's data the following is shown:—

(i) Butterfat percentage increased to five years and then gradually decreased.

(ii) Total yield rose to 7½ years and gradually declined.

(iii) Albumin percentage gradually rose to 10 years, and then gradually fell.

(iv) Lactose percentage was highest in young cows and uniformly fell as the animals grew older.

(v) Total ash remained practically constant.

(vi) Total nitrogen remained constant, on the whole, for all ages.

The effect of age on the yield of the various constituents would, however, appear to be more pronounced, if it can be inferred from its effect on butterfat yield.
Johansson and Hansson correct for length of calving interval, age and season. No correction was made for the influence of the previous dry period on butterfat yield. They found a significant correlation between successive dry periods of the same cow. Thus, in the case of a single record, it is practically impossible to decide if the dry period is short or long due to genetic or environmental causes. A correction for length of dry period may eliminate genetic differences.

**The Effect of Advance in Lactation.**

Fat and protein content decrease at the beginning of a lactation but increase later on. The lactose content is quite stable during the first part of the lactation but decreases as lactation proceeds. Tocher reports fat at a minimum 14-16 weeks after calving, which is later than in most investigations. The present findings would indicate that this is due to seasonal effects which Tocher did not eliminate. Lactose percentage was at a maximum 14 days after calving - an observation in agreement with the present study - but Tocher reports that random variation does not occur to any marked extent between correlated substances. This is not borne out by the present investigation. Azarn (1939) adopted the novel method of taking groups of animals at different stages of lactation, testing each animal over a period of 4 months. In this way he constructed a composite lactation curve in order to study the variation of total protein, casein and albumin plus globulin nitrogen. Azarn's method is satisfactory for a study of one character such as protein and its components.
The confining of tests for all animals to four months also has
the merit of creating a common seasonal effect. However,
Azarns tacitly assumes that there is no interaction between
stage of lactation and season of year, so that the effect of
season is the same for all cows. This introduces a probable
source of error into his calculations. Nevertheless, the fall
in crude protein at the beginning of lactation is in good
agreement with the present findings, falling to a minimum four
weeks after calving. A slow rise then ensued becoming more
pronounced towards the end of the lactation. The same was
ture of casein and albumin plus globulin nitrogen, but with
the latter only the decrease at the beginning and increase at
the end of lactation were sharp. Azarns found a high correla-
tion between total yield and protein yield.

The Effect of Season of the Year

The authority most often quoted is Overman, who has
published three successive studies of Bowler Herd data (1929,
1939, 1945). No attempt is made to separate seasonal and
stage of lactation effects. Overman instead takes animals at
various stages of lactation and tests them throughout a year
and assumes random distribution of animals throughout a lacta-
tion will cancel out stage effects in any particular calendar
month. There is some justification for this view. Overman,
on the basis of monthly tests, notes a drop of 0.51% in butter-
fat percentage of Ayrshire cows from February to August, and
a fall of 0.24% in protein from January to July. Lactose was
at 4.91% in August and 4.66% in September. This peculiar
jump in lactose occurs in September in the current study, but
it is accompanied by a similar variation in both protein and
fat. Immediately afterwards, the percentage drops again to
approximately the same level as the previous month. Davies
et al. (1946) in a study of factors affecting milk composition
under Arizona conditions found that high summer temperatures
tended to depress total solids. The effect was more than
neutralised among those cows in late lactation. Chloride
values were highest in summer, a fact reported by Herman (1938)
and Davies (1936) under drought conditions in Great Britain.

Becker and Arnold (1935) have made the most satisfactory
analysis of the relative effects of season of the year and
stage of lactation on butterfat percentage. By using a two-
way table, they obtain a series of values for calendar months
and for months of lactation, each free of the effects of the
other. They found that, in general, higher butterfat tests
were associated with cooler months of the year and lower tests
with the warm summer months. As the herd from which the data
came is situated in Florida, Becker and Arnold assume, with
some justification, that seasonal effects are due, in large
part, to wide fluctuations in seasonal temperatures. A spread
of 0.72% fat was found between 57 and 81°F. The regression
appeared strictly linear and points were spread evenly along the
regression line. Further results published for herds in
Missouri, Iowa and Sweden, were treated in the same way. The
general seasonal trend appeared the same and is in good agree-
ment with the present findings.

The range of seasonal variation is considerably greater
for some breeds and for some localities than others. The
following table summarises the position:

**TABLE I**

Range of Variation by breed and temperature
in Butterfat percentage.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Breed</th>
<th>Range of Butterfat percentage</th>
<th>Highest month</th>
<th>Lowest month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida</td>
<td>Jersey</td>
<td>0.73</td>
<td>December</td>
<td>August</td>
</tr>
<tr>
<td>Missouri</td>
<td>Jersey</td>
<td>0.64</td>
<td>Dec/Jan</td>
<td>July/Aug</td>
</tr>
<tr>
<td></td>
<td>Holstein</td>
<td>0.30</td>
<td>November</td>
<td>June</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>Guernsey</td>
<td>0.36</td>
<td>December</td>
<td>August</td>
</tr>
<tr>
<td>Missouri and Iowa</td>
<td>Mixed</td>
<td>0.67</td>
<td>December</td>
<td>August</td>
</tr>
<tr>
<td>Sweden</td>
<td>Ayrshire</td>
<td>0.46</td>
<td>November</td>
<td>June</td>
</tr>
<tr>
<td>Scotland</td>
<td>Ayrshire</td>
<td>0.21</td>
<td>November</td>
<td>January</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17</td>
<td>November</td>
<td>July/Aug</td>
</tr>
</tbody>
</table>

In the current data there was a sudden drop in butterfat
percentage in January but an almost immediate recovery and
then another, more pronounced fall in July and August. From
the table it can be seen that variation is least in Scotland,
where the herd studied is on high ground. There appears to
be a temperature and a breed effect. Breeds giving milk
highest in fat vary most and temperature also plays an impor-
tant part. However, it is difficult to decide which is most
important - breed or temperature variation. In view of the relatively small variation in Holstein butterfat, it would seem that breed response to temperature variation differs greatly.

The Effects of Heredity on Milk Composition

The literature of hereditary influence on milk composition is very limited indeed and not in the least conclusive. Small numbers of animals on which to base evidence have prevented any assessment of heretability in Lush's sense.

Since 1946, Swedish workers have been publishing papers dealing with variations in the milk composition of identical and fraternal twin cattle. Similar experiments have now begun in New Zealand and Great Britain but little data has yet been published.

Prior to this, no serious attempt has been made to measure hereditary influences on substances other than fat. Yapp, as early as 1925, attempted a simple Mendelian explanation of Bowker Herd results. Bonnier, Hansson and Jarl, together with co-workers, have published results from several studies with twin cattle. Findings on the effects of season and stage of lactation are interesting and largely in line with previous findings. Unfortunately, in an attempt to overcome the limitations of small numbers of animals, Bonnier et al. have used methods of analysis of variance which are unsatisfactory.

In a series of papers (1946, 1949, 1949a, 1950, 1950a)
a hierarchical method of analysis of variance has been adopted to minimise the effects of co-variation of fat, protein and lactose. Milk analyses from each cow are classified according to fat content. A grouping unit of 0.1% is used. This reduces the variation of fat content within each group to such small proportions that it may be ignored. Within these fat classes variation in percentage protein and lactose is computed "within and between cows". The effect of stage of lactation and season of the year on each component is ignored. The method causes each cow to be represented as many as 300 times in the total variance. Thus in a twin study (1949), 29 pairs of twins yield 2,244 degrees of freedom when data is treated in this way. In a further study among cows in the herd of Hamra, 29 full sister pairs, comprising 46 cows in all, give 364 degrees of freedom within, and 578 between cows.

This hierarchic method of grouping reduces the efficacy of the significance test and gives it a false meaning. After all, one cow is still one cow, although she appears 300 times, at different stages of lactation. The description of the components of variance is misleading. The between and within cow variance implies that the variance of the between cow component is entirely genetic. As each cow is represented, not by a simple weighted mean value for each constituent, but by a series of single tests, there is likely to be some random variation in all components in this analysis of variance. Further, there may be a season-stage interaction causing
fluctuation in protein and lactose.

Sommer and Hansson finally present a table of mean square values between cows, showing that as the coefficient of relationship decreases, the mean square increases. On this evidence they postulate genetic determination of the inter-dependency of protein and lactose at fixed percentages of fat. That this is so is more than probable, but it is difficult to see what fresh light a contribution of this kind throws on the problem.

Robertson (1950) in a critique of these experiments points out that the method of analysis used has unequal numbers in the sub-classes. The subdivisions are not, in fact, hierarchical, as they are assumed to be and a subsequent confusing of interactions with main effects results.

A paper by Lonka (1947) is of considerable interest. In studying butterfat and protein figures from Finnish herds, he asks:

"Can the relationship between fat and protein in milk be changed by selective breeding?"

He is interested in this because high protein feeds are very scarce in Finland, whereas starchy feed is plentiful. By selective breeding, he reasons that the protein requirement of a cow for milk production could be reduced for a given fat percentage. Lonka found that among Ayrshire cattle, different cows having the same average fat percentage for a lactation differed by as much as 0.5% in average protein percentage.
compared with a range of variation of 0.64% protein in the whole material. Holsteins with the same average fat percentage differed in average protein percentage by 0.65%. His findings with West-Finnish native cows are similar. He estimates that protein percentage can vary between cows by as much as 1%, independent of fat percentage. Thus, he argues, it is possible to increase total yield and fat percentage without a corresponding increase in protein percentage, provided cows, which are poor protein producers, are selected as dams.

The confirmation of Lonka's suggestion must await the collection of data on which genetic correlations can be calculated. If they are low, then, although a strong physiological relation may apply in the case of the individual cow, it is by no means certain a similar correlation exists between cows.

It can be concluded that little work has so far been done on factors affecting milk composition, which is of any help in animal breeding. We are unaware to what extent variation in milk constituents is due to hereditary or environmental factors. To date, there have been no measures of seasonal effects on milk production which are independent of lactation and vice versa. We do not know to what extent correlations between milk constituents are genetic. We are unaware of the degree of random variation possible among significantly correlated variables. The following study attempts to distinguish between some of these relationships. An attempt to assess their joint behaviour under selection is made.
Section A.

MATERIAL AND METHODS
MATERIAL AND METHODS

The data for this investigation was collected from the herd maintained by the Institute of Animal Genetics, Edinburgh, at Shothead, and from the privately owned herd on the neighbouring farm of Cockburn. The former consists of the remains of a crossbreeding experiment now being graded up to pedigree Ayrshire. The latter is a pedigree Ayrshire herd. Both herds are under common management and the system of husbandry is intensive. All animals are milked three times a day, at eight hourly intervals, and feeding is designed to secure maximum yields. The herds have been closed for several years and all stock bulls have been bred in the herds. Inbreeding has so far been practised on a relatively limited scale and not enough inbred animals are milking to have had any effect on these results. No selection is practised until after the first lactation and only a slight amount before completion of the second. After that, selection is severe and continuous.

The climate is such that cows are housed inside from mid-October to late April. The system of grazing is intensive and high quality grass silage is fed from the end of December until animals are put out to graze. The herd averages are in the region of 1,100 gallons per lactation. The animals, whose milk was tested were mostly by six bulls, all line bred to each other, and two young bulls were sired by two older ones. Selection, both of males and females, is based on total yield of milk. Other causes of disposal may be regarded as fortuitous.
In all, 77 animals were tested throughout one complete lactation, whilst part lactation data were secured from 43 others. 15 animals completing one lactation during the time in which analyses were carried out were tested during 20 weeks (10 tests) of a subsequent lactation. 29 cows were tested over a period of 12 weeks (6 tests).

Samples were taken at three successive milkings, once every 14 days, from each animal. A bulk sample was then made of aliquot portions of each milking.

The following values were determined:

(1) Butterfat percentage - by the Gerber test.

(2) Density - hydrometrically.

(3) Crude Protein - by the Kjeldahl method (as total nitrogen x 6.25).

(4) Lactose - polarimetrically.

(5) Chloride - by the direct method of Sanders (1939).

Further details of analytical methods may be found in Appendix A.

The reasons for carrying out these particular determinations have already been amply justified in the review of the literature. The relationship between the three principal constituents of milk for each cow is well known. We know very little about the relationship between cows. Continuous selection for higher butterfat percentage may or may not have an effect on protein and lactose, depending on the degree of the genetic relationship between them. Chloride is a useful single value for indicating abnormalities in milk secretion and it is thought to play an important role, together with
lactose, in maintaining the osmotic pressure of milk equal to that of blood. Espe (1948) quotes sources to show that they are responsible for 75 per cent of milk's osmotic pressure.

Lactations were standardised at 365 days, those animals going dry earlier being included without correction of lactation averages. Johansson and Hansson (1940) have shown that for total butterfat yield, amputation of lactation records tends to normalise the frequency distribution of percentage of all lactations on butterfat yield. The long 'tails' of the lactation curves are due to extremely long calving intervals and increase non-genetic variation.

Data was entered on sheets recording the sire and dam of the cow, date of calving, date of birth and the number of the lactation. Other information included service dates, remarks on any abnormalities or disease, length of preceding and current calving interval and service period. The total yield at test and the actual total yield for the lactation, based on weighing at each milking were also noted.
Section B.

FACTORS AFFECTING LACTATION AVERAGES.
FACTORS AFFECTING LACTATION AVERAGES.

When studying differences existing between cows, as distinct from differences within cows, it is usual to work with lactation total yield and lactation averages, weighted according to yield. Various factors will affect lactation averages and they are usually split into two groups - genetic and environmental. The present data are not extensive enough to permit a study of genetic differences to be made, but measures of environmental variation can be carried out which will give, at least, an indication of trends. Environmental causes of variation may be subdivided into those which are permanent, e.g., age, and those which are temporary and which vary in extent from season to season. Such factors as length of calving interval, service period and season of calving are examples of seasonal causes of variation. One has also to contend with the possibility that the environmental factors may act cumulatively or are antagonistic to each other.

Johansson and Hansson (1940) have carried out an extensive study of causes of variation in butterfat yield and butterfat percentage from records of 13 herds of Swedish cattle. Sanders (1926) contributed a classic paper on the variation existing in the lactation yields of English milk recorded herds in East Anglia. Many other studies have been made of separate aspects of the problem but none are so complete as these. Little work has been done on the variation
of averages of milk constituents other than fat. Apart from
a failure to isolate the effects of each factor, such
studies have been made on small amounts of data. This
last criticism may be levelled at the present study. A
basic group of 75 cows is used in this section but in
measuring certain relationships it has been found impossible
to avoid discarding some of these. Every effort has been
made to preserve the maximum number of degrees of freedom.
At certain points it becomes painfully evident that a great
deal is being asked of the data.

(1) The Effect of Age and Season of Calving.

A glance at Table II shows that the spread of calving
dates over the months of the year varies with age. Apart
entirely from the small number of cows in the higher age
groups, the concentration of heifer calvings in the autumn
and early winter months may give them an advantage in yield
over older animals. That this will be in turn bound up
with variation in composition is more than likely in view
of the fact that the percentage of fat, protein and lactose
are negatively correlated with yield.

As age and season of calving are inter-related, whilst
not necessarily interacting, their effects have been measured
simultaneously by using constants calculated from the data.
Age is measured in lactations as it provides a more realistic measure than age measured in months. Succeeding pregnancies will have a cumulative effect on mammary development, at least for several years.

**TABLE II.**

Seasonal distribution of Calvings of Cows in different age groups.

<table>
<thead>
<tr>
<th>Age (lactations)</th>
<th>Season of Calving (No of Cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

An analysis of variance, involving the fitting of constants to the data, has been used. Kendall (1948) deals with such a situation as the present one in detail and bases his treatment on methods evolved by Yates (1934). The data is first arranged according to age and season of calving. In the present case this yields 36 sub-classes for each of the variables studied. A preliminary analysis was carried out, considering the sub-classes as a one-way classification, to test for significant differences between sub-class means. The results appear in Table III. Significant differences exist
TABLE III.
Analysis of Variance between Sub-classes for Age and Season of Calving.

<table>
<thead>
<tr>
<th></th>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 305 day Milk Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>258023</td>
<td>35</td>
<td>73720</td>
<td>1.47</td>
</tr>
<tr>
<td>Residual</td>
<td>1960192</td>
<td>39</td>
<td>50261</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4540426</td>
<td>74</td>
<td>74.4</td>
<td></td>
</tr>
<tr>
<td>(b) Butterfat %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>4.23</td>
<td>35</td>
<td>0.1209</td>
<td>***2.63</td>
</tr>
<tr>
<td>Residual</td>
<td>1.79</td>
<td>39</td>
<td>0.0459</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.02</td>
<td>74</td>
<td>74.3</td>
<td></td>
</tr>
<tr>
<td>(c) Crude Protein %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>1.07</td>
<td>35</td>
<td>0.0306</td>
<td>**2.03</td>
</tr>
<tr>
<td>Residual</td>
<td>0.59</td>
<td>39</td>
<td>0.0151</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.66</td>
<td>74</td>
<td>74.1</td>
<td></td>
</tr>
<tr>
<td>(d) Lactose %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>1.65</td>
<td>35</td>
<td>0.0471</td>
<td>**2.30</td>
</tr>
<tr>
<td>Residual</td>
<td>0.80</td>
<td>39</td>
<td>0.0205</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.45</td>
<td>74</td>
<td>74.0</td>
<td></td>
</tr>
<tr>
<td>(e) Chloride (mlgrms/100 gr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>5613</td>
<td>35</td>
<td>160.4</td>
<td>**2.27</td>
</tr>
<tr>
<td>Residual</td>
<td>2752</td>
<td>39</td>
<td>70.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8365</td>
<td>74</td>
<td>74.4</td>
<td></td>
</tr>
<tr>
<td>(f) Density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between classes</td>
<td>3112</td>
<td>35</td>
<td>88.9</td>
<td>**1.86</td>
</tr>
<tr>
<td>Residual</td>
<td>1863</td>
<td>39</td>
<td>47.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4975</td>
<td>74</td>
<td>74.4</td>
<td></td>
</tr>
</tbody>
</table>

* denotes significance at 5% level
** denotes significance at 1% level
between class means of all variables except yield. This is surprising in view of the fact that selection is based on yield. As we shall see later, a more detailed examination reveals possible reasons for this, apart from the small number of animals in the higher age groups.

Age and season effects are estimated from constants and the derivation of these values for butterfat percentage is given as an example. The number of cows in each sub-class was entered in a matrix, shown as Table IV. Calvings occurred in every month except December and they are represented in columns $M_1$ to $M_{11}$. Similarly, age groupings are entered in columns $a_1$ to $a_6$. The marginal values on the right of Table IV represent the sum of all fat percentages in each of the equations in the matrix which may be read off from left to right. For example, equation one in the first row of the table reads:

$$6M_1 + a_1 + 2a_3 + a_4 + 2a_5 = 22.80$$

The object is to solve each equation, thus obtaining successive estimates of each value of $M$ and $a$. Single values of $M$ appear in the first eleven equations and single values of $a$ in the remaining six. The other symbols show the distribution of age in each month of calving and the months of calving in each age group.

The totals are divided by the number of cows in each age group, to give mean values for age. The uncorrected mean age values are used to solve each equation in the top section of
TABLE IV
Matrix of Equations for fitting constants to allow for the effects of Age and Season of Calving.

<table>
<thead>
<tr>
<th>M_1</th>
<th>M_2</th>
<th>M_3</th>
<th>M_4</th>
<th>M_5</th>
<th>M_6</th>
<th>M_7</th>
<th>M_8</th>
<th>M_9</th>
<th>M_{10}</th>
<th>M_{11}</th>
<th>a_1</th>
<th>a_2</th>
<th>a_3</th>
<th>a_4</th>
<th>a_5</th>
<th>a_6</th>
<th>Season of Calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>12</td>
<td>1_4</td>
<td>9</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>22.80</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>26</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>19.31</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>25.06</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3.48</td>
</tr>
</tbody>
</table>

\[ M_1 - M_{11} = \text{January - November} \]
\[ a_1 - a_6 = \text{Lactations 1 to 6} \]
### TABLE V - Constants for Age and Season of Calving

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>AGE</th>
<th>SEASON OF CALVING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a1</td>
<td>a2</td>
</tr>
<tr>
<td>Total Yield (lbs/lactn.)</td>
<td>8570</td>
<td>9140</td>
</tr>
<tr>
<td>Butterfat %</td>
<td>3.81</td>
<td>3.74</td>
</tr>
<tr>
<td>Crude Protein %</td>
<td>3.30</td>
<td>3.35</td>
</tr>
<tr>
<td>Lactose %</td>
<td>4.88</td>
<td>4.86</td>
</tr>
<tr>
<td>Chloride mg/100gr</td>
<td>74</td>
<td>78</td>
</tr>
<tr>
<td>Density</td>
<td>299</td>
<td>297</td>
</tr>
</tbody>
</table>
the matrix for m, to m_{10}. By hypothesis, z_{(m)} = 0.

The calculated values for m, to m_{10} are added and any
discrepancy from 0 is entered as the value of m_{11}.

The calculated m values are then taken for solving the
six age equations. The new age values are entered
underneath the original ones and are used in turn, to re-solve
the equations for season of calving. This reciprocal
process is continued until successive estimates of 'a' and
'm' no longer differ appreciably. They are shown for each
variable in Table V.

To obtain an estimate of the reduction in sum of
squares due to constants the values in Table V, which occupy
the position at the foot of the matrix in Table IV, are
multiplied by the totals in the column on the right of the
Table. Taking butterfat percentage as a further
illustration:

\[(3.61 \times 100.01) + (3.74 \times 89.69) \quad \rightarrow \quad (3.62 \times 21.72) + (0.256 \times 22.60) \quad \rightarrow \quad (0.158 \times 34.91) - (250.00)^2 \times 1.95\]

The last term is the 'correction factor', removing the
effect of including the mean among the 'a' values and
converts the answer into sum of squares of deviations from
the mean, estimated from constants.

This estimate is subtracted from the 'Between sub-class'
sum of squares in Table III. The remainder is a measure
of the 'Interaction' between age and season of calving. Interaction mean square is tested for significance against the 'Residual' mean square in Table III. If interaction proves significant it is used in the analysis of variance, to test age and season separately. Where interaction is not significant, then the original 'Residual' term is used throughout.

The next step in the analysis of variance is to test for significant differences between each age and season of calving separately. The sum of squares for age and season of calving estimated from constants, has subtracted from it the 'Between Season' sum of squares, calculated from the marginal totals on the right of Table IV. The remainder is the sum of squares due to age alone, holding the effect of season of calving constant. The mean square is tested for significance against the 'Residual' mean square. The 'Season of Calving' term is obtained by subtracting the 'Between age groups' sum of squares, calculated from Table IV totals, from 'Age and Season' sum of squares and is tested in the same way.

The results of the analyses of variance are presented in Tables VI A to VI F. Whilst there is a statistically significant difference in milk yield between seasons of calving, this is not so for age. A glance at Table V shows
TABLE VI

Further Analyses of Variance of Age and Season of Calving on Milk Yield and Composition.

A. Milk Yield (lb in 305 days)

<table>
<thead>
<tr>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between classes</td>
<td>2580234</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1779975</td>
<td>15</td>
<td>84042</td>
</tr>
<tr>
<td>Interaction</td>
<td>319609</td>
<td>20</td>
<td>15980</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1779975</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Between seasons (estimated from constants)</td>
<td>1260626</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>519349</td>
<td>5</td>
<td>103869</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1779975</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>299957</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>1480018</td>
<td>10</td>
<td>148002</td>
</tr>
</tbody>
</table>

Residual mean square from Table III = 50261
(d.f.) = 39

*P < 0.05
TABLE VI (continued)

B. Butterfat $3$

<table>
<thead>
<tr>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between classes</td>
<td>4.23</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1.95</td>
<td>15</td>
<td>0.1300</td>
</tr>
<tr>
<td>Interaction</td>
<td>2.28</td>
<td>20</td>
<td>0.1140*2.48</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1.95</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Between seasons (estimated from constants)</td>
<td>0.88</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.07</td>
<td>5</td>
<td>0.2014 1.77</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>1.95</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>0.89</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>1.06</td>
<td>10</td>
<td>0.1060 0.93</td>
</tr>
</tbody>
</table>

Residual mean square from Table III = 0.0459 (d.f. = 39)

* P < 0.05
### TABLE VI (continued)

#### C. Crude Protein %

<table>
<thead>
<tr>
<th></th>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between classes</td>
<td>1.07</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>0.97</td>
<td>15</td>
<td>0.0647</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>0.10</td>
<td>20</td>
<td>0.0050</td>
<td>0.33</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>0.97</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between seasons (estimated from constants)</td>
<td>0.43</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.54</td>
<td>5</td>
<td>0.1080</td>
<td><strong>7.15</strong></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>0.97</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>0.06</td>
<td>5</td>
<td></td>
<td><strong>6.03</strong></td>
</tr>
<tr>
<td>Season of Calving</td>
<td>0.91</td>
<td>10</td>
<td>0.0910</td>
<td><strong>6.03</strong></td>
</tr>
</tbody>
</table>

Residual mean square from Table III = 0.0151 (d.f. = 39)

** P < 0.01
### Table VI (continued)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual mean square from Table III</td>
<td>0.005</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season of Calving</td>
<td>1.65</td>
<td>15</td>
<td>0.11</td>
<td>0.1480</td>
</tr>
<tr>
<td>Age and season (estimated from 1.03 constants)</td>
<td>0.19</td>
<td>10</td>
<td>0.0190</td>
<td>1.44</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.62</td>
<td>20</td>
<td>0.0310</td>
<td>1.44</td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>0.0580</td>
<td>2</td>
<td>0.0290</td>
<td>0.01</td>
</tr>
<tr>
<td>Between seasons (estimated from constants)</td>
<td>0.0480</td>
<td>7</td>
<td>0.0068</td>
<td>0.01</td>
</tr>
<tr>
<td>Between classes (estimated from 1.03 constants)</td>
<td>0.0800</td>
<td>15</td>
<td>0.0053</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*p < 0.05

* * *
TABLE VI (continued)

E. Chloride content

<table>
<thead>
<tr>
<th></th>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between classes</td>
<td>5613</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>3665</td>
<td>15</td>
<td>244.5</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>1948</td>
<td>20</td>
<td>97.4</td>
<td>1.38</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>3665</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between seasons (estimated from 1157 constants)</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2508</td>
<td>5</td>
<td>501.6</td>
<td>7.10</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>3665</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>3310</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Season of Calving</td>
<td>355</td>
<td>10</td>
<td>35.5</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Residual mean square from Table III = 70.6  (d.f. = 39)

* p < 0.01
### TABLE VI (continued)

#### F. Density

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sums of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between classes</td>
<td>3112</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>2061</td>
<td>15</td>
<td>137.4</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>1051</td>
<td>20</td>
<td>52.5</td>
<td>1.09</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>2061</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between seasons (estimated from constants)</td>
<td>362</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1699</td>
<td>5</td>
<td>339.8</td>
<td>166.93</td>
</tr>
<tr>
<td>Age and season (estimated from constants)</td>
<td>2061</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between age groups (estimated from constants)</td>
<td>1384</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season of Calving</td>
<td>677</td>
<td>10</td>
<td>67.7</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Residual mean square from Table III = 47.8 (d.f. = 39)

**p < 0.01**
2. The inter-relation of Calving Interval and Service Period

Johansson and Hansson deal with three possible sources of variation in 300 days lactation yield. They are the Current Calving Interval, during which the lactation is made; the Preceding Calving Interval and the association of calving interval with the Dry Period between lactations. They find Dry Period to be significantly repeatable and suggest that it is to a considerable extent genetically determined. Consequently, no correction can be made for it without eliminating genetic as well as environmental variation.

Fig. 1 gives a graphical illustration of the time factors operating on lactation yield and possibly on milk composition, assuming a negative relationship between yield and the percentage of each milk constituent.

Fig. 1

Factors Influencing the Length of Calving Interval and Lactation.

The interval between calvings may be divided into the Service Period, which is the time elapsing between calving and conception and Gestation, whose length is fixed within fairly narrow limits. As the Dry Period length is to some extent fixed it will have an effect on the length of the calving interval. The successful service of a cow will determine the
length of lactation insofar as it fixes the time of the next calving. Sanders (1927) also suggests that intensity of yield is lowered after conception, but this latter assertion is not well substantiated.

In the first Report of the Production Division of the Milk Marketing Board (1950) it is stated that calving interval length is also affected by age and season of calving. Heifers have, in general, a calving interval a fortnight longer than cows in their second and third lactations. Only a slight difference is noted between autumn and spring calvers but summer calvers have consistently longer calving intervals than the other two groups.

Table VII shows the statistical relationships existing between Preceding and Current Calving Intervals and Service Period. As the number of degrees of freedom is so small there is a distinct possibility that the first two relationships - between Preceding and Current Calving Interval and Preceding Calving Interval and the ensuing Service Period - would become significant with the collection of more data.

We can only turn to Sanders (1927) and Johansson and Hansson (1940) who have studied a large number of cows. They find that the correlation between successive Calving Intervals is small and suggest it is largely environmental. If we accept this evidence, then a tendency of successive calving intervals to be of similar length must be due to management.
Johannson and Hansson note that amputation at 300 days causes "a decrease of total variance by 26.6 per cent calculated on the original variance of the lactation; the correction for age, calving interval and season of calving causes only 13.3 per cent. reduction, or exactly half that caused by amputation." The effect on average butterfat percentage among milk recorded herds in England and Wales was to reduce it by 0.048 per cent. and to quote the first Report of the Production Division of the Milk Marketing Board, "it appears unlikely that, when all lactations are taken into consideration, the results based on the 305 days standard are materially affected." The correlation between PCI and COI in the present data was + 0.17 for 44 degrees of freedom. Whether additional data would have made this significant or not, it appears, from the extensive evidence offered by the Swedish workers and much earlier by Sanders, that such a correlation would be largely environmental and due to management.

Having discussed the relationship between each of these time factors we now turn to a study of their effects on milk yield and milk composition.

3. The Effect of Preceding Calving Interval on Milk Yield and Composition.

Johannson and Hansson have suggested that events occurring during the calving interval preceding the one during which a lactation is made, may cause changes in lactation
yield and lactation averages. They measure the correlation between length of FCI and the 300 days yield of fat and fat percentage and find no correlation. There is a suggestion here that an exceptionally long FCI may affect the yield in the next lactation and through that, the lactation averages of the various constituents. The mean values associated with various lengths of FCI in Table VIII suggest trends in lactose, chloride and density but the statistical relationships in Table IX are not in any instance significant. That density and lactose percentage might show a significant relationship with FCI if more data were available cannot be ruled out. In calculating regression coefficients the degree of linear association is measured. On the evidence available it has been decided to ignore any effect of FCI.

**TABLE VIII**

The Mean Values for 305 days yield and composition of milk associated with varying Preceding Calving Interval.

<table>
<thead>
<tr>
<th>FCI (days)</th>
<th>No. of Cows</th>
<th>Milk Yield (lb/305 days)</th>
<th>Butter-fat %</th>
<th>Crude Protein %</th>
<th>Lactose %</th>
<th>Chloride mlgr/100 gr.</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>315-354</td>
<td>3</td>
<td>8609</td>
<td>3.60</td>
<td>3.43</td>
<td>5.08</td>
<td>66</td>
<td>315</td>
</tr>
<tr>
<td>355-394</td>
<td>19</td>
<td>9956</td>
<td>3.64</td>
<td>3.30</td>
<td>4.79</td>
<td>85</td>
<td>295</td>
</tr>
<tr>
<td>395-434</td>
<td>16</td>
<td>11238</td>
<td>3.73</td>
<td>3.29</td>
<td>4.86</td>
<td>84</td>
<td>292</td>
</tr>
<tr>
<td>435-</td>
<td>11</td>
<td>9170</td>
<td>3.33</td>
<td>3.33</td>
<td>4.73</td>
<td>86</td>
<td>272</td>
</tr>
<tr>
<td>Averages and Total</td>
<td>49</td>
<td>9693</td>
<td>3.52</td>
<td>3.34</td>
<td>4.87</td>
<td>79</td>
<td>294</td>
</tr>
</tbody>
</table>
Regression Coefficients - Yield and Composition of Milk on Preceding Calving Interval (days) (degrees freedom = 48)

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Yield (lbs/305 days)</td>
<td>-2.980 ± 5.771</td>
<td></td>
</tr>
<tr>
<td>Butterfat (%)</td>
<td>+ 0.0001 ± 0.0003</td>
<td></td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>+ 0.0002 ± 0.0005</td>
<td></td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>- 0.0007 ± 0.0006</td>
<td></td>
</tr>
<tr>
<td>Chloride (mlgr/100gr)</td>
<td>- 0.0033 ± 0.0240</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>- 0.0438 ± 0.0516</td>
<td></td>
</tr>
</tbody>
</table>

4. The Relationship between Current Calving Interval, Milk Yield and Milk Composition

The effect of amputation of lactation milk yield at 305 days did not have the same effect on the present data as it did in the studies of Johansson and Hansson (1940) and that by Tyler and Hyatt. The positive correlation between 305 day yield and length of CCI remained highly significant and successive amputations reduced it but little.

Johansson and Hansson not only found a marked reduction in the correlation and in total variance but an effective normalisation of the frequency distribution of lactation length on yield. Before amputation this had a significant negative skewness.

The mean values associated with varying length of CCI are given in Table X and the statistical relationships in Table XI. It is clear that Crude protein and lactose percentage show little or no association with CCI but the
same is not so certain of butterfat and chloride content and density. Fat and density show a negative correlation which, while not significant, are very nearly so. Again, with small numbers we must make the distinction between statistical and biological significance, however unsatisfactory this may be. These relationships are tied up with yield, for yield and length of CCI show a high positive correlation.

Evidently high yielding cows are being served to calve at longer intervals or are proving more difficult to get in calf.

**TABLE X**

The relation between Current Calving Interval, Milk Yield and Composition

<table>
<thead>
<tr>
<th>CCI (days)</th>
<th>No. of Cows</th>
<th>Milk Yield (lbs/305 days)</th>
<th>Butterfat %</th>
<th>Crude Protein %</th>
<th>Lactose %</th>
<th>Chloride (mlgr/100gr)</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>315-354</td>
<td>7</td>
<td>764.7</td>
<td>3.86</td>
<td>3.34</td>
<td>4.88</td>
<td>75</td>
<td>302</td>
</tr>
<tr>
<td>355-394</td>
<td>17</td>
<td>10270</td>
<td>5.67</td>
<td>3.23</td>
<td>4.86</td>
<td>78</td>
<td>296</td>
</tr>
<tr>
<td>395-434</td>
<td>14</td>
<td>11192</td>
<td>3.80</td>
<td>3.31</td>
<td>4.89</td>
<td>73</td>
<td>300</td>
</tr>
<tr>
<td>435-</td>
<td>7</td>
<td>12087</td>
<td>3.69</td>
<td>3.20</td>
<td>4.86</td>
<td>77</td>
<td>297</td>
</tr>
<tr>
<td>Averages &amp; Totals</td>
<td>45</td>
<td>10299</td>
<td>3.76</td>
<td>3.27</td>
<td>4.87</td>
<td>75</td>
<td>299</td>
</tr>
</tbody>
</table>
Variation in CCI has already been shown to be a direct function of Service Period length. Delay in service not only increases calving interval but tends to prolong lactation. Those cows giving greatest 305 day yields have longest calving intervals because their service is delayed longer than those cows giving lowest yields. That this is not due to any difficulty in getting higher yielding cows in calf is confirmed by the correlation coefficient between the length of service period and the number of services required for conception. This was + 0.165 (degrees freedom = 65). Longer service periods for higher yielding cows is therefore either deliberately or unconsciously due to management. In herds in general such a feature is evidently not important. Otherwise a significant correlation would exist between 305 day yield and CCI.

5. The Relation between Calving Interval, Season of Calving and Age.

In view of the findings on the relation between yield and CCI it is necessary to comment upon the possible relationships between calving interval, season of calving and age. This proves of particular interest where 305 day
milk yield is concerned.

The values for constants, calculated for age and season of calving are abstracted from Table V and are as follow:

\[
\begin{array}{ccccccc}
  a_1 & a_2 & a_3 & a_4 & a_5 & a_6 \\
  8570 & 9140 & 10660 & 11650 & 10060 & 9250 \\
\end{array}
\]

\[
\begin{array}{cccccccccccc}
  m_1 & m_2 & m_3 & m_4 & m_5 & m_6 & m_7 & m_8 & m_9 & m_{10} & m_{11} \\
\end{array}
\]

As the difference between the first and fourth lactation means is of the order 30 per cent., which is as great as that found by other workers working on more extensive material. The lack of statistical significance was therefore probably due to the small number of animals in higher age groups. As higher yielding animals have longer calving intervals an additional reason for a lack of significant difference may be due to highest yielding animals moving into an unfavourable season of calving more quickly than those animals giving lower yields. Heifers are served to calve in autumn. The values for constants at this period are highly positive. Second lactation cows will in
general calve for the second time in 14 months, whereas
those heifers with lowest yields will, in general, calve in
12. By the third calving this will have moved the
best cows into March or April where seasonal constants
show the largest negative values. Similarly, as cows
get older the best ones are saved and having longest calving
intervals, will complete the annual cycle of calvings
by the fifth or sixth lactation. Although by this time,
there will be an age decrease in lactation yield, it
will be to some extent offset by an autumn calving.
The overall effect will be to flatten the age curve
of lactation yield.

The values for fat, crude protein, lactose and
chloride content and density are not significantly
associated with calving interval length, although fat
and density values might easily show a significant negative
correlation if more data were available. That this
would be a function of yield, rather than calving
interval, has already been suggested, i.e., higher
cows give milk with lower fat content. This suggests
a possible reason for the age-season of calving interaction shown to exist in Table VI B. Before proceeding to discuss this it is necessary to mention that the effect of variation in calving interval length will be included in the constants estimated for age and season of calving. Thus the negative yield values for April calvings \( (m_b) \) whilst free of age effect will include part of the effects of calving interval length. This effect is to move highest yielding cows in their second or third lactation into March or April. If this is so, then the constants for yield will show a smaller negative value. The net result of longer calving intervals for higher yielding cows will be to reduce the value of constants estimated for season of calving. Part of the effect of calving interval will, however, be included in the constants for age and will have a similar effect.

The interaction between age and season of calving for butterfat percentage is difficult to explain. It might well be a function of change in total yield. As fat percentage is negatively correlated with yield factors reducing yield will tend to increase fat percentage. When the differences between the actual
values for age and season of calving were compared with the expected values, calculated from constants, the pattern of positive and negative differences for yield were matched in almost every case by a negative or positive difference for fat percentage. Heifers and lower yielding older cows tend to calve in more favourable seasons than high yielding cows. The result of such a state of affairs would be to increase the yield of heifers and poor yielding cows, causing a corresponding fall in fat percentage. The reverse would be the case for high yielding cows. Their yield would tend to be lower and average fat percentage higher. This might lead to an age-season of calving interaction for butterfat. Naturally, such an explanation is tentative in the extreme.

-47-
Section C.

THE EFFECT OF STAGE OF LACTATION AND SEASON OF THE YEAR ON MILK YIELDS AND MILK COMPOSITION.
THE EFFECT OF STAGE OF LACTATION AND SEASON OF THE YEAR ON MILK YIELDS AND MILK COMPOSITION.

The relative effects of stage of lactation and season of the year are studied by means of an analysis of variance of monthly differences. The analysis was carried out by means of a two-way table in which cells were arranged by month of lactation and season of the year. The average values for stage and season are virtually free of the effects of each other. A detailed account of the method may be found in Appendix B.

The values are presented in a series of graphs showing monthly changes in composition, total yield and the yield of each of the principal constituents. Further graphs provide an estimate of the effect of season of calving on the shape of the lactation curve for each constituent.

(1) The Effect of Stage of Lactation on Milk Composition.

Fig. 2 presents lactation curves for percentage butterfat, crude protein and lactose, chloride in milligrams percent and density. At the foot of the first two graphs on pps. 49 and 51 the number of animals represented at each graph point is shown. The numbers on the stage of lactation graph, fig. 2, apply to subsequent graphs on the effect of season of calving.

Butterfat and crude protein percentage show a steep and almost synonymous decline for the first month of lactation. Then follows a steady rise from the third month of lactation, more rapid in crude protein, until the sixth month. After that, the crude protein and butterfat percentages rise in a closely parallel manner until the tenth month. Lactose percentage remains steady for the first four months and then steadily falls until the ninth month when a sharper decline occurs. Chloride shows an inverse variation to lactose, but
VARIATION IN MILK COMPOSITION WITH STAGE OF LACTATION

- BUTTERFAT PERCENTAGE
- CRUDE PROTEIN PERCENTAGE
- LACTOSE PERCENTAGE
- CHLORIDE MG PERCENTAGE
- DENSITY

MONTHS OF LACTATION

DENSITY

MG PERCENT

DEVIATIONS PERCENT

1 2 3 4 5 6 7 8 9 10

115 120 120 115 105 95 77 59 44 31

NO. OF ANIMALS

Fig. 2.
falls slightly until the fourth month, whilst lactose remains steady. It then gradually rises to the ninth month and falls away slightly to the tenth month.

Density falls considerably in the first month, recovers to a considerable degree, flattening out in the fourth to fifth month and then rises steadily for the rest of the lactation. After the fourth month variation in density and chloride appear to be closely associated with crude protein, but this may be more apparent than real. Taking the first month as zero, protein and butterfat do not reach the same percentage until the eighth to ninth month, when total yield is very much lower.

(2) The Effect of Season of the Year on Milk Composition.

The effect of season is revealing. The most striking fact is that chloride shows much greater variability due to seasonal effects than it does due to stage of lactation. The range of variation in density is as great as that due to lactation effects. The principal constituents do not vary as much because of season. Crude protein shows a greater variability than either butterfat or lactose. (Fig. 3).

Butterfat, taking January as zero, shows little variation until August, apart from a depression of 0.06\% in February and a rise of 0.04\% in June. There is a pronounced 'hump' during the Autumn, rising to 0.11 in November and falling to zero again in January.

Crude protein percentage shows a pronounced 'hump' in Spring, rising from April to July then showing a slight, but steady, fall to the end of December. There would appear to be
SEASONAL VARIATION IN MILK COMPOSITION

Fig. 3.
## TABLE XIV.
The Magnitude of Random Differences with Stage of Lactation and Season of the Year.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>Monthly Difference</th>
<th>Random Error</th>
<th>Season of the Year</th>
<th>Monthly Difference</th>
<th>Random Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Butterfat %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>±0.023</td>
<td>January</td>
<td>0</td>
<td>±0.025</td>
</tr>
<tr>
<td>2</td>
<td>-0.35</td>
<td>±0.022</td>
<td>February</td>
<td>-0.06</td>
<td>±0.025</td>
</tr>
<tr>
<td>3</td>
<td>-0.35</td>
<td>±0.022</td>
<td>March</td>
<td>-0.01</td>
<td>±0.026</td>
</tr>
<tr>
<td>4</td>
<td>-0.30</td>
<td>±0.023</td>
<td>April</td>
<td>0</td>
<td>±0.033</td>
</tr>
<tr>
<td>5</td>
<td>-0.31</td>
<td>±0.024</td>
<td>May</td>
<td>+0.01</td>
<td>±0.031</td>
</tr>
<tr>
<td>6</td>
<td>-0.19</td>
<td>±0.025</td>
<td>June</td>
<td>+0.04</td>
<td>±0.032</td>
</tr>
<tr>
<td>7</td>
<td>-0.13</td>
<td>±0.028</td>
<td>July</td>
<td>-0.02</td>
<td>±0.035</td>
</tr>
<tr>
<td>8</td>
<td>-0.09</td>
<td>±0.032</td>
<td>August</td>
<td>-0.02</td>
<td>±0.037</td>
</tr>
<tr>
<td>9</td>
<td>-0.01</td>
<td>±0.037</td>
<td>September</td>
<td>+0.13</td>
<td>±0.039</td>
</tr>
<tr>
<td>10</td>
<td>+0.09</td>
<td>±0.044</td>
<td>October</td>
<td>+0.10</td>
<td>±0.035</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>November</td>
<td>+0.15</td>
<td>±0.028</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>December</td>
<td>+0.12</td>
<td>±0.027</td>
</tr>
</tbody>
</table>

(b) Crude Protein %

| 1                  | 0                  | ±0.019       | January            | 0                  | ±0.020       |
| 2                  | -0.35              | ±0.018       | February           | -0.02              | ±0.020       |
| 3                  | -0.35              | ±0.018       | March              | -0.09              | ±0.021       |
| 4                  | -0.21              | ±0.019       | April              | -0.02              | ±0.027       |
| 5                  | -0.16              | ±0.021       | May                | +0.16              | ±0.026       |
| 6                  | -0.09              | ±0.023       | June               | +0.22              | ±0.026       |
| 7                  | -0.09              | ±0.042       | July               | +0.23              | ±0.028       |
| 8                  | -0.05              | ±0.036       | August             | +0.11              | ±0.030       |
| 9                  | -0.04              | ±0.050       | September          | +0.12              | ±0.030       |
| 10                 | +0.14              | ±0.036       | October            | +0.02              | ±0.029       |
|                    |                    |              | November           | +0.02              | ±0.023       |
|                    |                    |              | December           | +0.03              | ±0.022       |

/continued
TABLE XIV continued.

<table>
<thead>
<tr>
<th>Stage of Lactation</th>
<th>Monthly Difference</th>
<th>Random Error</th>
<th>Season the Year</th>
<th>Monthly Difference</th>
<th>Random Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c)Lactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>±0.022</td>
<td>January</td>
<td>0</td>
<td>±0.025</td>
</tr>
<tr>
<td>2</td>
<td>+0.01</td>
<td>±0.022</td>
<td>February</td>
<td>-0.04</td>
<td>±0.025</td>
</tr>
<tr>
<td>3</td>
<td>+0.01</td>
<td>±0.022</td>
<td>March</td>
<td>-0.05</td>
<td>±0.026</td>
</tr>
<tr>
<td>4</td>
<td>-0.01</td>
<td>±0.023</td>
<td>April</td>
<td>-0.01</td>
<td>±0.032</td>
</tr>
<tr>
<td>5</td>
<td>-0.05</td>
<td>±0.023</td>
<td>May</td>
<td>-0.04</td>
<td>±0.030</td>
</tr>
<tr>
<td>6</td>
<td>-0.07</td>
<td>±0.025</td>
<td>June</td>
<td>-0.09</td>
<td>±0.031</td>
</tr>
<tr>
<td>7</td>
<td>-0.08</td>
<td>±0.027</td>
<td>July</td>
<td>-0.12</td>
<td>±0.034</td>
</tr>
<tr>
<td>8</td>
<td>-0.12</td>
<td>±0.031</td>
<td>August</td>
<td>-0.11</td>
<td>±0.037</td>
</tr>
<tr>
<td>9</td>
<td>-0.18</td>
<td>±0.036</td>
<td>September</td>
<td>+0.02</td>
<td>±0.036</td>
</tr>
<tr>
<td>10</td>
<td>-0.35</td>
<td>±0.043</td>
<td>October</td>
<td>-0.10</td>
<td>±0.035</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>November</td>
<td>-0.16</td>
<td>±0.028</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>December</td>
<td>-0.15</td>
<td>±0.026</td>
</tr>
<tr>
<td>(d)Chloride mg %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>±0.531</td>
<td>January</td>
<td>0</td>
<td>±0.584</td>
</tr>
<tr>
<td>2</td>
<td>-2.8</td>
<td>±0.520</td>
<td>February</td>
<td>-2.8</td>
<td>±0.584</td>
</tr>
<tr>
<td>3</td>
<td>-3.3</td>
<td>±0.520</td>
<td>March</td>
<td>-3.7</td>
<td>±0.611</td>
</tr>
<tr>
<td>4</td>
<td>-4.0</td>
<td>±0.536</td>
<td>April</td>
<td>-7.6</td>
<td>±0.768</td>
</tr>
<tr>
<td>5</td>
<td>-2.6</td>
<td>±0.555</td>
<td>May</td>
<td>-13.3</td>
<td>±0.729</td>
</tr>
<tr>
<td>6</td>
<td>-2.2</td>
<td>±0.591</td>
<td>June</td>
<td>-13.6</td>
<td>±0.711</td>
</tr>
<tr>
<td>7</td>
<td>-1.2</td>
<td>±0.648</td>
<td>July</td>
<td>-15.2</td>
<td>±0.814</td>
</tr>
<tr>
<td>8</td>
<td>+0.7</td>
<td>±0.711</td>
<td>August</td>
<td>-11.1</td>
<td>±0.868</td>
</tr>
<tr>
<td>9</td>
<td>+1.1</td>
<td>±0.858</td>
<td>September</td>
<td>-7.2</td>
<td>±0.858</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>±0.825</td>
<td>October</td>
<td>-5.9</td>
<td>±0.822</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>November</td>
<td>-3.0</td>
<td>±0.653</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>December</td>
<td>-3.3</td>
<td>±0.625</td>
</tr>
</tbody>
</table>

NB The derivation of the random error term is dealt with in Section 5, p. 745.
a marked effect of Spring grass on protein percentage. There is an overall rise of 0.29% from March to July, which, for protein, is a considerable environmental variation.

Lactose percentage shows no definite trend but in common with butterfat, shows a sudden rise in September. Whereas fat continues to rise in September lactose falls away again. There is a depression of 0.12 in Lactose in July and 0.16 in November. The highest value occurs in January.

The low density value for October is associated with a month in which 50% of the total number of cows are newly calved. In no other month do the number of newly calved cows form such a large percentage of the total that they are likely to cause a pronounced bias in the average value.

(3) The Effect of Stage of Lactation on Total Yield of Milk, Butterfat, Protein and Lactose. (Fig. 27)

The effect of stage of lactation on the yield of the different factors studied is very similar. Total yield and lactose yield are closely associated throughout lactation, a very slight divergence occurring after the fifth month. A variation of 10 lb. in total yield is accompanied by a similar variation of 1 lb. in lactose yield in the same period, i.e., until the fifth month, 10 lb of milk is associated with 1 lb of lactose. This has been noted by Tocher (1925) and Gaines (1931), but Gaines finds an association of 1 lb. of lactose with 18 lb. milk water. In later lactation, the figure shows an association of 15 lb. total yield and 1 lb. lactose. Clearly, advance in lactation has a specific effect on lactose.
EFFECT OF STAGE OF LACTATION ON TOTAL YIELD OF MILK AND PRINCIPAL MILK CONSTITUENTS.

Fig. 34
yield which causes it to fall away a little quicker than total yield. The divergence in the lactose and total yield curves towards the end of lactation is due to the slower decline in butterfat and crude protein yields which make up the bulk of the total solids in milk, apart from lactose.

Butterfat and crude protein show a shallower decline in amount with advance in lactation and Edwards (1936) and Gaines, in many papers, have used these facts to strike an Energy Value for each cow. Edwards points out that the fall in milk energy values is much less than the fall in total yield and this is doubtless due to the slower decline in the yield of protein and fat. It is obvious that lactose is having an effect on water movement from blood to milk and the lower rate of secretion towards the end of a lactation causes a lowering of total yield. Protein and fat, which are not in solution, and therefore, as far as is known, exert no osmotic effect, vary in rate of secretion quite independently, declining at a slower rate and therefore increasing their percentage in the milk with advance in lactation.

(4) The Effect of Season of the Year on Total Yield of Milk, Fat, Protein and Lactose.

Seasonal effects appear to operate equally on total yield of milk, fat, protein and lactose. Fig. 6 shows how closely associated they are, in contrast to the different variation in percentage of fat, protein and lactose. Both figs, 6 & 7 were derived from the graphs showing percentage variation.

The only unusual feature of fig. 6 is the sudden rise in
VARIATION IN TOTAL YIELD OF MILK AND ITS CONSTITUENTS WITH SEASON

Fig. 5.
the amount of fat, protein and lactose from mid-June to mid-August. This would appear to be directly associated with either temperature or with nutrition. Becker and Arnold (1935) found a pronounced effect of temperature on fat percentage in Florida, but no such effect could be ascribed here. Maximum temperatures recorded on the farm during the summer months showed no effect on yield. The cause of the rise in the yield of fat, protein and lactose would seem to be entirely due to the quality of grazing at that period.

(5) The Effect of Season of Calving on Milk Composition

On pp 36-35 the results of an analysis of variance which was carried out on lactation averages are shown. A significant difference between monthly calving groups was found.

By superimposing the graph of seasonal variation on that of stage of lactation variation, an idea can be gained of the likely effect on lactation of a particular season of calving. This ignores the effect of a possible stage-season interaction. We do not know and it is impossible to test this data for the effect of season on each stage of lactation. Different stages of lactation may not react to the same extent in conditions associated with a particular month of the year. Bearing this in mind, it is still felt that a useful idea may be obtained of the relative effects of different months of calving by adopting the above method. This has been done for butterfat, crude protein and lactose percentage separately.
(a) The Effect of Season of Calving on Butterfat Percentage

Fig 75 gives five curves, one of which is the lactation curve with effect of season eliminated. The other four consist of lactation curves with the seasonal effect curve superimposed from a given month of calving. This method has been adopted for each of the graphs measuring the effect of season of calving.

The four months were chosen to represent Autumn, Winter, Spring and Summer calvings. The January calving deviates least from the base line and shows little seasonal effect. October calvings deviate considerably from the base line, and minimum fat percentage is not reached until the fifth month of lactation. Thereafter, October calvers show little effect of season.

Cows calving in seasons other than Autumn show a steep drop in butterfat percentage during the first month of lactation. Cows calving in July have highest butterfat percentage from the second to seventh month of lactation, whilst April calvers show a steady rise in fat percentage from the fifth to the final month.

(b) The Effect of Season of Calving on Crude Protein Percentage (Fig. 76)

All cows, irrespective of season of calving, show a steep decline in crude protein percentage during the first two months of lactation. Thereafter, July calvers, which show a high value at calving, depart little from the base line. Both January and April calvers show a fairly
EFFECT OF SEASON OF CALVING ON BUTTERFAT PERCENTAGE

---

**Fig. 6.**

---

**LACTATION CURVE (SEASONAL EFFECT ELIMINATED)**
- **JANUARY CALVING**
- **APRIL CALVING**
- **JULY CALVING**
- **OCTOBER CALVING**

---

MONTHS OF LACTATION

---

BUTTERFAT PERCENTAGE
EFFECT OF SEASON OF CALVING ON CRUDE PROTEIN PERCENTAGE

Fig. 46.
rapid rise in crude protein percentage until the sixth and fifth months respectively. The curves then flatten out and finally coincide with the base line for the last two months of lactation. October calvers show no recovery in crude protein percentage until the seventh month when the rise is rapid and continuous.

(c) The Effect of Season of Calving on Lactose Percentage

In fig. it will be noted that the effect of season is always to depress lactose percentage and this is evident in fig. where the effect of season of calving is illustrated. While the base line shows a smooth decline with advance in lactation the effect of season is to cause a successive rise and fall in lactose percentage. This applies to all seasons of calving.

(d) Seasonal Variation in Chloride

The seasonal variation in chloride is so great by comparison to that due to stage of lactation, that no attempt has been made to draw up a similar graph for it. The large variation in chloride with time of year would explain the failure to establish significance in the analysis of variance carried out in Table. Variability within months is as great as that from month to month.

(6) The Effect of Season of Calving on Total Yield of Milk

Fig. shows the lactation curve for total yield with the effect of season removed. Together with it are the
EFFECT OF SEASON OF CALVING ON LACTATION CURVE (SEASONAL EFFECT ELIMINATED)

- JANUARY CALVING
- APRIL CALVING
- JULY CALVING
- OCTOBER CALVING

MONTHS OF LACTATION

PERCENTAGE
EFFECT OF SEASON OF CALVING ON TOTAL YIELD LACTATION CURVE

- JANUARY CALVING
- APRIL CALVING
- JULY CALVING
- OCTOBER CALVING

Fig. 9
lactation curves for cows calving at different times of year. On this evidence, April calvings give the lactations with the biggest initial yields, but the lowest persistency. July calvers show a similar response with a small and rather unexpected jump in the last three months which coincides with January, February and March, when the seasonal yield curve (fig.5) is beginning to rise. October calvings benefit little from seasonal effects in early lactation, the yield curve following the base line closely until the turn of the year and the fourth month of lactation. Thereafter, the yield steadily diverges from the base line and at the tenth month shows a level of persistency much higher than any of the other calving groups.

Overall, the January calved cows provided the best lactation curve. Although third in persistency at ten months, the area between this curve and the base line as a whole is greatest. On this evidence January calvings apparently give highest lactation milk yields.

(7) The Effect of Season of Calving on Butterfat, Lactose and Protein Yields.

Butterfat yield and total yield of milk show a similar response to season. However, the initial increase in butterfat yield is absent in the first month of lactation. The results for butterfat may be found in fig 19.

The lactose yield curves (fig 12) show a striking similarity to those for total yield of milk and suggest a very close physiological relationship with it. This subject
EFFECT OF SEASON OF CALVING ON BUTTERFAT YIELD

[- Graph showing the effect of season of calving on butterfat yield.]

Fig. 10.
EFFECT OF SEASON OF CALVING ON CRUDE PROTEIN YIELD LACTATION CURVE

- LACTATION CURVE (SEASONAL EFFECT ELIMINATED)
- JANUARY CALVING
- APRIL CALVING
- JULY CALVING
- OCTOBER CALVING

Fig. 10
EFFECT OF SEASON OF CALVING ON LACTOSE YIELD LACTATION CURVE

- LACTATION CURVE (SEASONAL EFFECT ELIMINATED)
- JANUARY CALVING
- APRIL CALVING
- JULY CALVING
- OCTOBER CALVING

MONTHS OF LACTATION

LB AT TEST

Fig. 123.
is dealt with in considerable detail subsequently.

Protein yield curves follow the general trend but changes from month to month are more exaggerated than any of the other substances. Thus, if fig. A is compared with fig. B for total yield, the two characteristic peaks at four months for April and seven months for January calvers are much more pronounced. Similarly the October calvers' yield curve shows a much greater persistency. So much so, that after flattening out during the seventh to ninth month, it shows a small rise during the ninth to tenth month. Those animals calving in July do not show this exaggerated tendency and the curve of crude protein values differs little in shape from that of total yield.
Section D

THE VARIATION IN MILK COMPOSITION DUE TO RANDOM CAUSES
The variation in milk composition due to random causes.

The analysis of variation into effects due to stage of lactation and effects due to season of the year, takes no account of random variation and a certain amount is included in each. We shall define random causes as unknown or unknowable and random variation as unrelated to any known cause.

In carrying out this estimate, the opportunity is also taken to calculate the correlation between butterfat, crude protein and lactose percentage and between lactose and chloride. This is carried out "within" and "between" cows. The degree of random variation is then treated as an error term for each deviation shown in the graphs for stage of lactation and seasonal variation in milk composition (figs. 2 and 3).

An estimate of the random fluctuation which can occur between such closely correlated variables as fat, protein and lactose is then obtained.

Finally, the random variance between tests is used, together with the variance between lactation averages to provide:

(a) An estimate of the correlation between the observed value of the lactation mean, based on fortnightly tests, and the true value.

(b) Fixing a minimum correlation of 0.90 for the relation between the observed and true value, the number of tests needed during the lactation for each substance is calculated, together with the standard errors to be expected,
THE RELATIONSHIP BETWEEN THE PRINCIPAL CONSTITUENTS OF MILK.

The statistical relationships between the principal constituents of milk are well established. Nevertheless, little is known about the biological implications of these relationships. Determinations have either been carried out on bulked milks or single samples from large numbers of animals. Where regular tests have been carried out on successive samples from the same cows during a complete lactation, no clear distinction is made in most investigations between intra- and inter-cow relationships.

Each value in the graphs (figs. 2, 3) for seasonal and stage of lactation is a within cow value for a considerable number of cows. The stage of lactation effect is largely a physiological variation, whereas the seasonal values are purely environmental.

The weighted lactation averages for each cow represent an overall estimate of both these causes and the relationship of the lactation averages of different cows is partly genetic and partly environmental. By calculating the regression of one constituent on another and vice versa, an estimate of genetic relationship can be made. Ideally, a genetic regression is carried out between dam-daughter pairs, e.g. where the regression of daughter's butterfat percentage on dam's crude protein percentage and vice versa can be used to measure these constituents' genetic interdependence.

(1) An Estimation of Random Differences.

As a preliminary, an estimate of random differences between
successive tests was made for the constituents determined.

Seventy-seven animals with complete lactations were grouped according to the number of their lactation in order to eliminate lactation age effects. The correlation between the lactation averages for butterfat percentage, crude protein percentage and lactose percentage were calculated, and also the correlation between lactose and chloride percentages. A similar correlation of within cow values was carried out for stage of lactation and season of the year.

Two tests were then selected during the third month of lactation for each of the 77 cows. At this stage, the disturbing effects of early lactation are over and the animals have not yet been served. As the two tests are only fourteen days apart, any differences between them are unlikely to show much effect of season or stage of lactation.

The values for each single test were then correlated and also the differences between them. The values for each of the relationships are given in Table XV.

From this table the differences in the degree of relationship within and between cows is at once evident. Butterfat and crude protein percentages show a highly significant positive correlation with stage of lactation, but seasonal variation appears to be quite independent. The between cow correlations for lactation averages also show a highly significant positive correlation as does one of the single test values. However, the degree of relationship from test to test is slight. The other test shows little relationship and the
### TABLE XV.

Correlation Coefficients for Different Relationships of Butterfat, Crude Protein, Lactose and Chloride Percentages.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Degrees Freedom</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) Butterfat % and Crude Protein %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Stages of Lactation</td>
<td>9</td>
<td>+0.951 xx</td>
</tr>
<tr>
<td>Between Seasons of Year</td>
<td>11</td>
<td>-0.012</td>
</tr>
<tr>
<td>Between Random Differences (A-B)</td>
<td>76</td>
<td>+0.018</td>
</tr>
<tr>
<td>Between Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Lactation Averages</td>
<td>76</td>
<td>+0.318 xx</td>
</tr>
<tr>
<td>Between single tests A</td>
<td>76</td>
<td>+0.139</td>
</tr>
<tr>
<td>Between single tests B</td>
<td>76</td>
<td>+0.371 xx</td>
</tr>
<tr>
<td><strong>(b) Butterfat % and Lactose %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Stages of Lactation</td>
<td>9</td>
<td>-0.752 xx</td>
</tr>
<tr>
<td>Between Seasons of Year</td>
<td>11</td>
<td>-0.317</td>
</tr>
<tr>
<td>Between Random Differences (A-B)</td>
<td>76</td>
<td>-0.135</td>
</tr>
<tr>
<td>Between Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Lactation Averages</td>
<td>76</td>
<td>+0.418 xx</td>
</tr>
<tr>
<td>Between single tests A</td>
<td>76</td>
<td>-0.002</td>
</tr>
<tr>
<td>Between single tests B</td>
<td>76</td>
<td>+0.484 xx</td>
</tr>
<tr>
<td><strong>(c) Crude Protein % and Lactose %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Stages of Lactation</td>
<td>9</td>
<td>-0.796 xx</td>
</tr>
<tr>
<td>Between Seasons of Year</td>
<td>11</td>
<td>-0.076</td>
</tr>
<tr>
<td>Between Random Differences (A-B)</td>
<td>76</td>
<td>-0.212</td>
</tr>
<tr>
<td>Between Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Lactation Averages</td>
<td>76</td>
<td>+0.162</td>
</tr>
<tr>
<td>Between single Tests A</td>
<td>76</td>
<td>-0.028</td>
</tr>
<tr>
<td>Between single tests B</td>
<td>76</td>
<td>+0.075</td>
</tr>
<tr>
<td><strong>(d) Lactose % and Chloride %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Stages of Lactation</td>
<td>9</td>
<td>-0.753 xx</td>
</tr>
<tr>
<td>Between Seasons of Year</td>
<td>11</td>
<td>-0.035</td>
</tr>
<tr>
<td>Between Random Differences (A-B)</td>
<td>76</td>
<td>+0.051</td>
</tr>
<tr>
<td>Between Cows</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Lactation Averages</td>
<td>76</td>
<td>-0.607 xx</td>
</tr>
<tr>
<td>Between single Tests A</td>
<td>76</td>
<td>-0.366 xx</td>
</tr>
<tr>
<td>Between single tests B</td>
<td>76</td>
<td>-0.080</td>
</tr>
</tbody>
</table>

* xx Significant at 5% level

xx Significant at 5% and 1% level.
correlation of random differences from test to test, which is a within cow variable, is also very small. Evidently, the relationship for butterfat and crude protein percentage is very high during lactation. Nevertheless, on the evidence of the lack of correlation of random differences, the ability of each to vary independently of the other from test to test, would appear to be complete.

The relationship of butterfat and crude protein to lactose is the most interesting feature of this table. The relationship within cows is negative and highly significant for stage of lactation. Seasonal variations are small and the correlation of random differences is not significant, although it is very much larger than for butterfat and crude protein. This is particularly so of crude protein and lactose percentage, where the random correlation coefficient is -0.212, virtually significant at 5%. It would seem that there is a degree of physiological relationship between butterfat and crude protein secretion on the one hand and lactose on the other, but not for butterfat and protein with each other.

In both cases, purely environmental effects, as represented by seasonal variation appear to affect each of the constituents in different ways.

Turning to between cow correlations, the most surprising fact is the positive correlation between all three substances. In the case of butterfat and lactose this is highly significant, but this is not so for crude protein and lactose. The genetic
implications are at once evident. There is a highly significant negative relationship between butterfat and crude protein percentage on the one hand and lactose percentage on the other, for each individual cow. It has been assumed that the same relationship holds good between cows. In this case it is not so. Those cows producing milk with the highest butterfat content also produce milk with higher protein and lactose content. However, this statement may be only partly true, because, as Lonka (1947) has shown, the ratio of each of these constituents varies from cow to cow — animals producing milk with identical butterfat content giving different crude protein percentages.

The extent to which selection for one value affects the others, is of great importance in livestock improvement. This is particularly so in view of the economic importance of butterfat and the value placed on milk with a high butterfat percentage. If the genetic relationships for each of these constituents is high then selection for one will affect the others. If non-existent or slight, then selection for one will have no effect on the others. These relationships are discussed later.

The inverse relationship between lactose and chloride content of milk has long been known. It is assumed to be of considerable physiological importance, and to play an important role in milk secretion. The values presented in Table XV show a marked negative correlation, both within and between cows. In view of the observations in the last paragraph this
would seem obvious. However in figs. 2 and 3 the variation in chloride content with season of the year is shown to be much greater than with stage of lactation. The correlation between random differences of lactose and chloride in Table XV is very small. Thus, even between two such physiologically important and compensatory factors, there is almost complete independence of variation in certain circumstances. The relative contributions of lactose and chloride to total osmotic pressure bears little relation to their weight. As osmotic pressure depends on the number of molecules or ions in solution rather than their weight, a given weight of chloride has a much higher osmotic pressure than the same weight of lactose. An opportunity is taken to examine this subject further in section E. Although the percentages of lactose and chloride in milk are in the ratio 50:1, chloride as NaCl exerts roughly twelve times the osmotic pressure of lactose, weight for weight. Thus the ratio of osmotic pressure contributed by each will be 3:1 and the ratio may be reversed in certain circumstances.

(2) The Random Variation of Stage of Lactation and Seasonal Differences.

The season and stage differences in figs. 2 and 3 include variation due to random causes. A separate estimate has been made of the degree to which random fluctuations from test to test affect each estimate. A random error term is then provided for each monthly value in the two graphs. The random error is determined as the standard deviation of
the random differences, divided by the square root of the number of observations at each graph point.

The mean square difference is based on the difference between two successive tests of each animal. Consequently, it has a value equal to the sum of the variances of each of the tests, i.e., is $2\sigma^2$

As an example, the calculation of the random error for butterfat percentage is considered.

\[
\text{Mean square difference} = 2\sigma_p^2
\]

\[
\text{Sums of squares of differences} = 8.93
\]

\[
\text{Random error} = \frac{\sigma_p}{\sqrt{n}}
\]

\[n = \text{the number of cows represented in any one month, e.g. first month of lactation is 115.}\]

\[
2\sigma_p^2 = \frac{8.93}{77} = 0.116
\]

\[
\sigma_p = \sqrt{\frac{0.116}{2}} = 0.24
\]

\[
\text{Random error} = \frac{0.24}{\sqrt{115}} = \pm 0.023
\]

The standard errors due to random differences from test to test are given for each month of lactation and each month of the year. They may be found in Table XIV on page 51ab immediately following figs. A and B. Each monthly deviation is the same as that on the graphs. They represent a deviation from the first month of lactation and the first month of the year and are based on the mean of two fortnightly tests.

Each random error value is seen to be small and only in months showing a very small change does it approach or exceed
the size of the seasonal or stage of lactation differences. This is partly due to the relatively slight differences caused by stage and season at certain points in the graph and partly due to the smaller number of animals milking at these times, causing an increase in the error term. It would seem that the variation during the summer months and at the end of lactation for butterfat and crude protein is not a true seasonal and stage of lactation difference, but a random one. At other times of year and stage of lactation, this is not so and random variation makes up only a minor part of the total effect. A similar effect for lactose is found during the first four months of lactation and the first five months of the year. For chloride it occurs during the last four months of lactation but at no season does the random variation make up more than a fraction of the total effect.

(3) The Correlation between the Observed and True Value for Lactation Averages of Butterfat, Crude Protein and Lactose Percentage.

An estimate of the accuracy of the lactation mean, based on a given number of tests may be made, using the observed lactation mean square, $\sigma_1^2$, and the mean square of random differences, $\sigma_r^2$.

The formula is as follows:
\[ 
\text{r}_{nT} = \frac{\sigma^2_l}{\sqrt{\sigma^2_l (\sigma^2_l + \sigma^2_r/n)}} 
\]

\[ 
\text{r}_{nT} = \text{correlation between the observed value, based on } n \text{ tests, and the true value.} 
\]

\[ 
\sigma^2_l = \text{mean square of the lactation means of } 77 \text{ cows with complete lactations.} 
\]

\[ 
2\sigma^2_r = \text{mean square difference between two successive tests of each cow in the third month of lactation.} 
\]

\[ 
n = \text{actual number of tests made during each lactation.} 
\]

Taking butterfat percentage as an example, the calculation is as follows:

Sums of squares of lactation means = 5.395 (from the data)

Mean square of lactation means = \( \sigma^2_l \) = \( \frac{5.395}{76} = 0.0710 \)

Sums of squares of differences between two successive tests = 8.925 (from p. 75)

As Mean Square difference = \( 2\sigma^2_r \) between two successive tests

Then \( \sigma^2_r = \frac{8.925}{2 \times 76} = 0.0587 \)

\[ 
\text{r}_{nT} = \frac{0.0710}{\sqrt{0.0710 (0.0710 + 0.0587)}} = \frac{0.0711}{0.072} = 0.986 
\]
TABLE XVI.

Correlation between the Observed and True Value ($r_{HT}$) of the Lactation Mean.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Correlation between true &amp; observed lactation mean</th>
<th>Standard Deviation of lactation mean</th>
<th>Standard Error of lactation mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfat $%$</td>
<td>$+0.936$</td>
<td>$\sim 0.30$</td>
<td>$+0.07$</td>
</tr>
<tr>
<td>Crude Protein $%$</td>
<td>$+0.966$</td>
<td>$\sim 0.16$</td>
<td>$+0.04$</td>
</tr>
<tr>
<td>Lactose $%$</td>
<td>$+0.958$</td>
<td>$\sim 0.17$</td>
<td>$+0.04$</td>
</tr>
<tr>
<td>Chloride mg $%$</td>
<td>$+0.992$</td>
<td>$\sim 0.94$</td>
<td>$+0.05$</td>
</tr>
</tbody>
</table>

(4) The Number of Tests during a lactation needed to obtain a Lactation Mean with a given Standard Error.

It is of value to know the number of tests, needed during a lactation, to obtain a mean value with a standard error not greater than a given amount. From Table XVI a relationship between a given standard error and the correlation between the observed and true value may be obtained. By postulating a minimum correlation coefficient, the corresponding standard error can be calculated. Similarly the minimum number of tests needed to achieve this value of $r_{HT}$ can also be determined. Using the values for $r_{HT}$ and the standard errors in Table XVI a minimum value for $r_{HT}$ has been adopted, equal to $0.90$. For butterfat percentage, this will give a standard error for the lactation of:

$$\frac{0.07 \times 0.936}{0.90} = \pm 0.076$$
Similarly, \( r_{MT} = 0.9 \)

\[
\frac{0.076D}{\sqrt{0.076D (0.076D + 0.058D) / n}}
\]

\[
\sqrt{\frac{0.005D + 0.0012}{n}} = \frac{0.076D}{0.90} = 0.078
\]

\[
0.005D + 0.0012 = 0.0061
\]

\[
n = \frac{0.0042}{0.0061 - 0.005D} = 3.24
\]

Repeating the calculations for each constituent the values for \( r_{MT} = 0.9 \) in Table XVII were derived.

**Table XVII.**

The Standard Errors and Number of Tests Needed for an Observed Lactation Mean showing a Correlation of 0.90 with the True Value.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Standard Error</th>
<th>Number of Tests required during a lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfat %</td>
<td>±0.076</td>
<td>4</td>
</tr>
<tr>
<td>Crude Protein %</td>
<td>±0.043</td>
<td>5</td>
</tr>
<tr>
<td>Lactose %</td>
<td>±0.043</td>
<td>8</td>
</tr>
<tr>
<td>Chloride mg %</td>
<td>±3.36</td>
<td>8</td>
</tr>
</tbody>
</table>
Section E.

THE OSMOTIC PRESSURE OF MILK.
THE OSMOTIC PRESSURE OF MILK.

It is generally recognised that milk is isotonic with blood and that both have a common osmotic pressure of approximately 6.6 atmospheres. The two chief osmotic agents in milk are lactose and chloride. Other mineral constituents are in many cases associated in colloidal complexes and are not completely in solution. However, they no doubt vary in the extent to which they are in solution. Several studies (Black and Veris (1934); Porcher (1929); Davies (1939)) report a marked positive correlation between the sodium and chloride content of milk and it is supposed by many authorities that chloride in milk is chiefly sodium chloride, largely ionised. Espe (1948) suggests that potassium is the only other element in true solution and quotes Davies (1939) to the effect that only 25 per cent. of calcium and phosphorus is in solution.

If it is accepted that lactose and sodium chloride together are the chief osmotic agents, then a measure can be made of their relative contributions. The graphs presented as figs. 2 and 3 show the variation occurring in lactose and chloride as deviations from zero in the first month of lactation and the first month of the year. Using
the values in Table XIV on page 51b, the potential osmotic pressure of each of the substances can be calculated from their molecular weights using the principle that:

"...the molecular weight in grams of a non-electrolyte dissolved in 22.4 litres of solvent exerts an osmotic pressure of 760 mm. at 0°C."

This principle applies to lactose but sodium chloride is an electrolyte and some allowance must be made for dissociation in solution. Van't Hoff inserted a factor i, into the osmotic equation

\[ PV = RT, \]

\[ PV = iRT, \]

where \( P \) is the osmotic pressure.

The implication is that each ion exerts the same osmotic pressure as one molecule of an undissociated substance. Dissociation is only complete in very dilute solutions. As sodium chloride is present in small amounts and is a binary electrolyte, \( i \) has been taken as 2. The osmotic pressure of each molecule of sodium chloride has therefore been assumed to exert twice that of one molecule of lactose.

A 1 per cent solution of lactose will therefore exert
the following osmotic pressure:

\[ \text{Molecular weight} = 342 \]

\[ \therefore 342 \text{ gr. in 22.4 litres} = 760 \text{ mm.} \]

\[ 1 \text{ gr. in 22.4 litres} = \frac{760}{342} \text{ mm.} \]

\[ 1 \text{ gr. in 100 ml.} = \frac{760 \times 22,400}{342 \times 100} = 498 \text{ mm.} \]

A solution containing 1 percent. of Sodium chloride would exert the following osmotic pressure:

\[ \text{Molecular weight} = 58.46 \quad 1 = 2. \]

\[ 58.46 \text{ grams dissolved in 22.4 litres} = 760 \text{ mm.} \times 2 \]

\[ 1 \text{ gr. dissolved in 22.4 litres} = \frac{760 \times 2}{58.46} \text{ mm.} \]

\[ 1 \text{ gr. dissolved in 100 ml} = \frac{760 \times 2 \times 22,400}{58.46 \times 100} = 5824 \text{ mm.} \]
Thus weight by weight chloride as NaCl exerts 12 times the osmotic pressure of lactose.

In these calculations the temperature has been assumed equal to 0°C. The values for chloride in Table XIV were first converted into per cent. Sodium chloride. It was then a simple task to calculate the potential osmotic pressure exerted in each month of the year and each stage of lactation. The results for stage of lactation appear in Table XVIII.

**TABLE XVIII**

Variation in Osmotic Pressure (mm) due to changes in Chloride and Lactose content with stage of lactation

<table>
<thead>
<tr>
<th>Month of Lactation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>0</td>
<td>+5</td>
<td>+5</td>
<td>-3</td>
<td>-25</td>
<td>-35</td>
<td>-40</td>
<td>-60</td>
<td>-90</td>
<td>-175</td>
</tr>
<tr>
<td>Chloride (NaCl)</td>
<td>0</td>
<td>-27</td>
<td>-32</td>
<td>-39</td>
<td>-25</td>
<td>-21</td>
<td>-12</td>
<td>-8</td>
<td>+12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>-22</td>
<td>-27</td>
<td>-44</td>
<td>-50</td>
<td>-56</td>
<td>-52</td>
<td>-68</td>
<td>-78</td>
<td>-175</td>
</tr>
</tbody>
</table>
The results of this calculation suggest a slight decline in osmotic pressure with advance in lactation. Assuming the average osmotic pressure to be of the order 6.6 atmospheres, it is equivalent to 3.5 per cent. from the first to last month. However the fall in lactose content from the ninth to tenth month is considerable and between the first and ninth month the fall is only equivalent to approximately 1.6 per cent.

The values are free of seasonal changes and in figure 3 variation in milk composition with season of cytokines shows that variation in chloride content is considerable. It declined 15 milligrams per 100 grams milk between January and July which is equivalent to 20 per cent. of the average chloride content of 75 milligrams.

The picture presented by Table XIX is very different from stage of lactation changes.
**TABLE XIX**

Variation in Osmotic Pressure (mm) due to changes in Chloride and Lactose content with Season.

<table>
<thead>
<tr>
<th>Months of the Year</th>
<th>Lactose</th>
<th>Chloride (NaCl)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J</td>
<td>F</td>
<td>A</td>
</tr>
<tr>
<td>Lactose</td>
<td>0</td>
<td>-20</td>
<td>-25</td>
</tr>
</tbody>
</table>

The range of total osmotic pressure values is greater and a steady decline occurs up to July and a regular rise to the following January. If the average osmotic pressure of milk is taken as 6.6 atmospheres, then the maximum range of variation is 4 per cent.

No doubt stage and season affects to some extent cancel each other out and are rarely completely cumulative. Although these variations in osmotic pressure due to lactose and chloride are small by comparison with the total, osmotic pressure may not be such a constant character as is supposed. In order to balance the joint decline in lactose and chloride other inorganic substances may come into solution. The only direct measurement
of changes in osmotic pressure due to stage and season has been made by Aschaffenburg and Temple (1941). They present data from two cows. The average freezing point values varied little with advance in lactation. As only two cows were used in the experiment no measure of the relative effects of season and stage of lactation could be made. They note a maximum deviation of 3.5 per cent. from the mean, which is in fair agreement with the values obtained here by calculation. Namely, 3.5 per cent. for stage of lactation and 4 per cent. for season of the year, as maximum deviations. Therefore the chloride and lactose data from the 120 lactations and part lactations used in Section C, may be regarded as confirmatory to Aschaffenburg and Temple's direct findings.
Section F.

The Genetic Contribution of Sires, &
The Genetic Contributions to Variability.
THE GENETIC CONTRIBUTIONS TO VARIABILITY.

It has so far been found impossible to distinguish between the parts played by heredity and environment on milk composition in the individual cow. The technique of Lush and his co-workers have provided a powerful tool for doing this for large groups of cows. However, their value of $h^2$ (heritability) characterises populations. How valid it is as an indication of genetic variance in one herd at one period is another matter. Nevertheless, as the predominant number of cows on which data has been collected is by six sires, line-bred to each other, a value of $h^2$ has been calculated. This measures the genetic contributions of these bulls to total variance.

Where inheritance is governed by many genes, correlations between half-sisters will be 0.25, as they have one-quarter of their genotype in common. Dominance, interaction and modifications due to environment will tend to reduce this value. Where the environment for all cows is similar, i.e. when they are contemporaries, as is the case here, $r_{EE}$ will tend to be high and will raise the correlation of half-sisters. However, the effect of environment alone on the various constituents has been determined (fig. 73). In each case it has proved small; with the exception of chloride. If variation due to stage of lactation is largely physiological and that of season completely environmental, the values in Table XVIII give an idea of the effects of $r_{EE}$ on half-sister variance. The variance due to season is expressed as a percentage of that due to stage of lactation.
As chloride percentage is so intimately linked with environmental effect, it has been omitted from the calculations.

TABLE XVIII.

The Effect of Season (Environment) on Milk Composition

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Environmental variation as a percentage of physiological variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfat</td>
<td>19.3</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>39.4</td>
</tr>
<tr>
<td>Lactose</td>
<td>26.1</td>
</tr>
<tr>
<td>Chloride</td>
<td>984.7</td>
</tr>
</tbody>
</table>

(1) The contribution of Sires to the Total Variance of Butterfat, crude protein and lactose percentage.

Lush (1949) denotes total observed variance by $\sigma_0^2$. That due to genetic differences is $\sigma_H^2$ and that caused by environment $\sigma_E^2$. If non additive interactions between heredity and environment are absent, then

$$\sigma_0^2 = \sigma_H^2 + \sigma_E^2$$

and

$$h^2 = \frac{\sigma_H^2}{\sigma_H^2 + \sigma_E^2}$$

where $h^2$ is the fraction of total variance due to heredity.

As all data is contemporary, $r_{EE}$ will be high and the interactions will tend to be the same for all cows.

The cows which were half sisters were grouped "within bulls" and an analysis of variance was carried out within and
between bulls. The results, given in table XIX were used in an estimate of $h^2$. This was arrived at as follows:

\[ \sigma_0^2 = \sigma_W^2 + \sigma_B^2 k_0 \]

\[ \sigma_B^2 = \sigma_W^2 + \sigma_S^2 k_0 \]

\[ \sigma_S^2 = \left( \frac{\sigma_B^2 + \sigma_W^2}{k_0} \right) \]

\[ h^2 = \frac{\sigma_S^2 \times 4}{\sigma_S^2 + \sigma_W^2} \]

\[ \sigma_0^2 = \text{observed total variance} \]

\[ \sigma_W^2 = \text{variance "within bulls"} \]

\[ \sigma_B^2 = \text{variance "between bulls"} \]

\[ \sigma_S^2 = \text{genetic variance due to the sires as a whole,} \]

\[ k_0 = \text{correction for unequal numbers of daughters "within bulls".} \]

$\sigma_S^2$ is multiplied by four as half-sisters only have one quarter of their genotypes in common. The purely genetic variance, $\sigma_S^2$, is included in $\sigma_B^2$, together with environmental effects and represents the difference between $\sigma_B^2$ and $\sigma_W^2$, after allowing for the effect of unequal values of $k$ for each bull.

Lush (1935) shows that the number of daughters by each bull should never be less than 5. Below this, the standard error of estimate becomes too large.

Snedecor (1946), p. 234, gives a suitable method of allowing for unequal class numbers. This is given by calculating an average number $k_0$ for the varying values of $k$ in each
"within bull" class.

\[ k_0 = \frac{1}{n-1} (S_k + \frac{S_k^2}{3k}) \]

For this data

\[ k_0 = \frac{1}{78 - 1} (78 + \frac{134.8}{78}) \]

\[ = 0.12 \]

On allowing for this, the value \( h^2 \) for e.g. butterfat percentage, may be calculated as follows:

\[ \sigma_w^2 = \sigma_w^2 + \sigma_B^2 k_0 \]

\[ \sigma_B^2 = \sigma_w^2 + \sigma_S^2 k_0 \]

\[ 0.25 = 0.12 + 12\sigma_s^2 \]

\[ \sigma_s^2 = \frac{0.25 - 0.12}{12} = 0.01 \]

\[ h^2 = \frac{0.01 \times 4}{0.01 + 0.12} = 0.31 \]

The value for \( h^2 \), where \( h^2 \) represents the contribution of the six bulls to total observed variance is:

- Butterfat percentage .. .. 0.31
- Crude protein percentage .. 0.04
- Lactose percentage .. .. 0.60

As bulls are line bred to varying degrees, the relationship of half-sisters will be slightly greater than 0.25. The multiplication of \( \sigma_s^2 \) by 4 will therefore enhance \( h^2 \) to some extent but not to such a degree that corrections would make much difference.
TABLE XIX.

Analysis of Variance within and between bulls.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>Degrees Freedom</th>
<th>Sums of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Butterfat percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ($\sigma_0^2$)</td>
<td>77</td>
<td>9.81</td>
<td>0.12</td>
</tr>
<tr>
<td>Within bulls ($\sigma_w^2$)</td>
<td>72</td>
<td>8.54</td>
<td>0.12</td>
</tr>
<tr>
<td>Between bulls ($\sigma_B^2$)</td>
<td>5</td>
<td>1.27</td>
<td>0.25</td>
</tr>
<tr>
<td>(b) Crude protein percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ($\sigma_0^2$)</td>
<td>77</td>
<td>2.18</td>
<td>0.028</td>
</tr>
<tr>
<td>Within bulls ($\sigma_w^2$)</td>
<td>72</td>
<td>2.02</td>
<td>0.028</td>
</tr>
<tr>
<td>Between bulls ($\sigma_B^2$)</td>
<td>5</td>
<td>0.16</td>
<td>0.032</td>
</tr>
<tr>
<td>(c) Lactose percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ($\sigma_0^2$)</td>
<td>77</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>Within bulls ($\sigma_w^2$)</td>
<td>72</td>
<td>2.80</td>
<td>0.04</td>
</tr>
<tr>
<td>Between bulls ($\sigma_B^2$)</td>
<td>5</td>
<td>0.62</td>
<td>0.12</td>
</tr>
</tbody>
</table>
(2) **The Within Cow Repeatability of Butterfat, Crude Protein and Lactose Percentage.**

By calculating the correlation between the values obtained in two successive lactations of the same cow, some idea may be gained of the genetic determination of a character. If there is a high correlation from lactation to lactation, then the character is, to a considerable extent, genetically determined. If there is little correlation, environment must play the dominant role in its expression.

Of the 77 animals with complete lactations, 29 were tested six times during a subsequent lactation, covering a 12 week period and 15 were tested 10 times, covering 20 weeks. The values obtained were compared with the first six and first ten tests of the previous lactations of the same cows. The correlations obtained were as follows:

<table>
<thead>
<tr>
<th>TABLE XX.</th>
<th>Repeatability of Butterfat, Crude Protein, Lactose and Chloride Percentages.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) Six tests - 29 cows</td>
</tr>
<tr>
<td></td>
<td><em>Butterfat percentage</em>  ( * ), <em>Crude protein percentage</em>  ( * ), <em>Lactose percentage</em>  ( * ), <em>Chloride percentage</em>  ( * )</td>
</tr>
<tr>
<td></td>
<td>( r )  ( *0.314 )  ( *0.123 )  ( *0.367 )  ( *0.666 )</td>
</tr>
<tr>
<td></td>
<td>(b) Ten tests - 15 cows</td>
</tr>
<tr>
<td></td>
<td><em>Butterfat percentage</em>  ( * ), <em>Crude protein percentage</em>  ( * ), <em>Lactose percentage</em>  ( * ), <em>Chloride percentage</em>  ( * )</td>
</tr>
<tr>
<td></td>
<td>( r )  ( *0.482 )  ( *0.323 )  ( *0.644 )  ( *0.772 )</td>
</tr>
</tbody>
</table>

There is a considerable increase in \( r \) between 12 and 20 weeks. If there is a corresponding increase in the correlation
coefficient in the succeeding weeks of lactation, then the 
repeatability will be significant for all the above constituents. 
As it is, the value of $r$ at 5% for 29 cows is 0.3494, 
and for 15 is 0.4321. Those correlation coefficients 
marked x are significant.

(3) The genetic determination of milk composition.

Until adequate numbers of daughters of a large number of 
bulls, or at least one hundred dam-daughter pairs have been 
tested in the present manner no clear measure of genetic inter-
dependence can be made. Meanwhile, the between cow regression 
of the lactation means has been used from each of the 77 cows 
with complete lactations. These regressions appear in Table 
XXI together with their standard errors. The standard error 
from regression was derived as the mean residual square after 
elimination of the effect of the independent variable, divided 
by the number of degrees of freedom.

This is given by:

\[
SE = \frac{1}{n-1} (\frac{\sum y^2 - (\frac{\sum x'y'}{\sum x'^2})^2}{\sum x'^2})
\]

$\sum y^2$ = sums of squares dependent variable 
$\sum x'^2$ = sums of squares independent variable, 
$\sum x'y'$ = sum of products.

The distribution of $t$ (Fisher and Yates (1948)) shows that 
for 76 degrees of freedom, $t = 2.00$ at the 5% level of proba-
bility. Consequently, in Table XXI where the regression 
coefficient is more than twice the size of its standard error,
TABLE XXI.

The Regression of Lactation Means on Each Other, between cows

<table>
<thead>
<tr>
<th>Dependent Variable (per cent)</th>
<th>Independent Variable (per cent)</th>
<th>Regression Coefficient (per cent)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>Butterfat</td>
<td>+0.1833</td>
<td>±0.0212</td>
</tr>
<tr>
<td>Butterfat</td>
<td>Crude protein</td>
<td>+0.5523</td>
<td>±0.0638</td>
</tr>
<tr>
<td>Lactose</td>
<td>Butterfat</td>
<td>+0.2724</td>
<td>±0.028</td>
</tr>
<tr>
<td>Butterfat</td>
<td>Lactose</td>
<td>+0.6426</td>
<td>±0.0586</td>
</tr>
<tr>
<td>Lactose</td>
<td>Crude protein</td>
<td>+0.1832</td>
<td>±0.0294</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Lactose</td>
<td>+0.1435</td>
<td>±0.0217</td>
</tr>
<tr>
<td>Chloride</td>
<td>Lactose</td>
<td>+0.0457</td>
<td>±0.0124</td>
</tr>
<tr>
<td>Lactose</td>
<td>Chloride</td>
<td>+0.0075</td>
<td>±0.0010</td>
</tr>
</tbody>
</table>

it is regarded as evidence of a significant relationship between constituents.

It is important to emphasise that this is not a purely genetic regression as no relationships are involved. To effect a genetic regression, the animals should, ideally, fall into dam-daughter pairs, so that animals have half $\alpha_h^2$ in common. The regressions are carried out between dam's butterfat and daughter's crude protein; daughter's butterfat and dam's crude protein; and so on. The result is then multiplied by two to give an estimate of $h^2$.

The results obtained here are all significant and to that extent point towards a genetic relationship between these constituents.
(4) The Factors Causing Variation with Advance in Lactation.

Under the analysis of variance distinguishing between seasonal variation and that due to stage of lactation, the temporary environmental effects of the former were separated from the more permanent effects of the latter.

Lecky (1951) defines phenotypic (observed) variance as:

$$\sigma_p^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{Ge}^2 + \sigma_{Eg}^2$$

$\sigma_p^2$ is the phenotypic variance, $\sigma_G$ the genetic variance and $\sigma_E$ the environmental. $\sigma_{Ge}^2$ and $\sigma_{Eg}^2$ is that part of the variance due to interactions between the environment and the genotype.

As the present analysis was carried out within cows, the effects of $\sigma_G^2$ were automatically eliminated leaving variation due to $\sigma_E^2$ (seasonal effects) and $\sigma_{Ge}^2$ and $\sigma_{Eg}^2$ (variance with advance in lactation). Thus, table XV (p. 71) gives an idea of the relative importance of each component. Alim (1950) represents the total variation in milk yield as an oblong figure:

\[
\begin{array}{ccc}
G & R & T \\
\end{array}
\]

G represents the genetic, R the permanent environmental fraction ($\sigma_{Ge}^2 \sigma_{Eg}^2$) and T the temporary environmental fraction.

Table XVIII shows that temporary environmental variation forms much less than half the total variance in the present data on milk composition. However, assuming G and R are constant from lactation to lactation, then T will be successively reduced in the same way, to $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{4}$ of its original value as means are calculated on two, three or four lactations of the same cow.
The repeatability between lactations, calculated in Section F (2), p. 9, Alim shows to represent the correlation between values of $\sigma_{ge}^2$ and $\sigma_{Eg}^2$ for successive lactations of the same cow, i.e., it is a measure of the within cow repeatability of permanent environmental variation and its interactions with the genotype.
GENERAL DISCUSSION AND CONCLUSIONS.
General Discussion and Conclusions

The measurement of total variation and its distribution on different causes has been the answer of the biometrical geneticist to the complexities of quantitative inheritance. Most quantitative characters, of which milk yield is one, are governed by many pairs of genes. It is impossible to assign a given effect to one gene. Therefore the combined effect of all hereditary factors is measured, together with those of environment. It is due to Lush and his co-workers that the methods of Wright, Fisher and Haldane have been so successfully applied to livestock problems. The findings have still to receive practical application on any scale. Now that Artificial Insemination Centres are becoming more widespread the proving of sires should be a relatively rapid and simple matter. The estimates of heritability obtained by Lush and his co-workers in America and Robertson and Rendel here, provide a basis on which probable genetic gain may be calculated and enable a planned breeding programme to be constructed.

Lush (1945) defines the components of total observed variation symbolically, in terms of components of variance.
The equation
\[ \sigma_0^2 = \sigma_H^2 + \sigma_E^2, \]
assuming dominance and epistasis negligible and genetic variance largely additive, reduces to
\[ h^2 = \frac{\sigma_H^2}{\sigma_H^2 + \sigma_E^2}. \]

The development of the equation to allow for dominance and epistasis has been dealt with in a previous section (p. 95). This brief reference to the biometric approach will at once impress upon the reader how far it has outstripped the slower methods of direct experimentation. The latter approach involves not only the measurement of phenotypic expression but an attempt to relate it to the chain of physiological events behind it. The various estimates of heritability of milk yield have provided a figure of roughly 25 per cent. If a measure at an earlier stage in the physiological process were possible, by relatively simple means, and this event proved to have a much higher heritability, then genetic improvement in milk yield could be much more rapid.

The existing information available on milk composition is of little value for a biological study as data has not been
collected in a way that lends itself to modern methods of analysis of variance. The present study, whilst limited in scope, has provided an opportunity to investigate the various factors collectively called 'environmental'.

The lack of knowledge of factors affecting the principal non-fatty solids of milk makes the legal minimum of 8.5 per cent. an arbitrary one, with perhaps little basis in reality. If breeders, for instance, are encouraged to breed cattle giving milk richer in fat, then it is important to know what effect such selection will have on the different components of solids-not-fat. The findings in two herds provide a restricted basis for generalisation but shed interesting light on the various causes of environmental variation in lactation yield and lactation averages and also on monthly variations in yield and composition.

It has been shown that changes in 305 day yield and milk composition with increasing age can be affected by season of calving. A further complication is introduced by the fact that highest yielding cows had longest calving intervals, irrespective of age. This had the effect of moving highest yielding cows into the early months of the year in their peak lactations, reducing the differences
existing between each age group, as spring calving reduces 305 day yield.

The effect of Preceding Calving Interval on subsequent lactation was measured but no association existed between its length and 305 day yield.

Espe (1948) states that within reasonable limits, length of calving interval has little effect on the subsequent lactation, provided a cow has been fed according to production. Where calving intervals are too short the added stimulus to increased production which results from frequent calving is partially offset by the depressing effect of carrying a calf during a larger percentage of the lactation period. It has been shown in the present study that service period is the controlling factor in determining calving interval length and that cows giving highest yields were served at a later date after calving than loweryielders. Service has effects which are not measurable. Sanders (1927) has suggested that it causes a definite drop in daily yield, although the writer has been unable to find much supporting evidence in the literature. Ragsdale et al. (1924) estimate that a cow carrying a calf produces only three per cent. less milk than a cow which is barren, during the first 200 days of any lactation. Nevertheless, this
takes no account of the energy used in building up the foetus, only a portion of which may be represented by the foetus itself.

The age changes in lactose and chloride content confirm the findings of Tocher (1925) and Overman (1929). The former finds trends in butterfat percentage with age and so does Bailey (1951) in a recent study, but both work on restricted data. Johansson and Hansson (1940) find no changes in fat percentage with age among Swedish cattle and quote other sources to the same effect. The fall in density can only be attributed to the decline in lactose percentage and consequently this is likely to be the chief cause of low solids-not-fat in the milk of older cows.

In measuring the amount of variation in each constituent according to advance in lactation and season of year, variation in the actual production of each constituent at each test was also calculated. Tocher (1925), Davies (1936) and many other workers have studied milk composition in detail in Great Britain.

Tocher has estimated changes due to stage in lactation and Davies, changes due to season. Neither, however, has separated these effects. Overman (1929, 39, 44) in the U.S.A. has made several contributions, analysing milk
of the Bowker herd in Illinois. In studying seasonal effects, he used animals calving at intervals over a twelve month period so that stage of lactation effects in any one month of the year will operate at random. Von Patow (1930) and Buchanan Smith (1939 and in earlier papers) stress the need for study of the relationship between the amounts of each substance secreted, rather than their percentages. Gaines (1928) on the other hand, emphasises that percentages give a reliable index of the way in which energy is used in milk secretion. This investigation has carried the investigation along both lines in an attempt to illustrate the difference in response, between total yield of milk and each of its constituents, to the joint stimuli of advance in lactation and season of year.

Becker and Arnold (1935) in a study of seasonal and stage of lactation differences in fat percentage, use a similar approach to the present one. They note a big seasonal response and ascribe it to temperature change. A spread of 0.71 per cent. was found over the range 57 - 81°F. No relation between temperature and composition was established in the present data and the autumn rise in fat percentage would appear to be chiefly nutritional.
Overman (1944) reports a drop of 0.51 per cent in fat percentage from January to July, so it would seem that temperature can be an important source of environmental variation in climates where the temperature range is more extreme than in Scotland. Tocher reports fat percentage at a minimum, 14 - 16 weeks after calving. This does not fit in with other results where fat falls to a minimum during the first 4 to 6 weeks of lactation. However, in fig. 6, where seasonal effects are superimposed on those for stage of lactation, the curve constructed for October calvings is in line with Tocher's findings. It is suggested that the cows taken for Tocher's study calved in Autumn and that this causes the delayed fall in fat percentage. Bonnier (1946) presents lactation curves for fat, crude protein and lactose percentage which are in good agreement with those in fig. 2.

The striking relationship between total yield of milk and lactose yield has been noted by Gaines (1936) in a study of Illinois data. He classified results according to fat percentage, as he advances the theory that fat, having the highest calorific value of any milk constituent, will exert a dominating influence on milk composition. Within these fat percentage groups Gaines found an association of 1 lb. of lactose with 18 lb. milk water. Purely on the evidence of fig. 4, a relationship between roughly
1 lb. of lactose and 10 lb. total yield is noted throughout the greater part of lactation. The yield curves for butterfat and crude protein are of quite a different shape and suggest that there is not the same relationship between colloidal solids and yield as exists between lactose and yield. Lactose is in solution and is thus capable of exerting osmotic pressure. The potential osmotic effect of lactose and chloride have been dealt with in Section 5. It was found that the maximum change in osmotic pressure which could be caused by variation in lactose and chloride (as NaCl) was 3.5% for stage of lactation and 4.5% for seasonal effects. The only direct evidence on variation in osmotic pressure due to advance in lactation and season of year is from the milk of two cows, on which freezing point determinations were carried out by Aschaffenburg and Temple (1940). They noted a maximum variation of 3.5 percent from the mean. The implications of change in secretion of lactose and chloride could be considerable, if their osmotic properties are taken into account. Variation of 1 lb. in lactose would cause a difference of 10 lb. in total yield. As sodium chloride exerts twelve times the osmotic pressure of lactose, weight for weight, a variation of less than 1/2 ounces would have the same effect. Neither fat nor crude protein are in solution to any extent. Consequently the osmotic pressure they exert is negligible,
and the variation in their secretion will have no effect on water movement.

Gaines' work suggests that each animal is capable of sacrificing so much energy in the formation of milk but within this energy limit a wide variation in composition is possible. This metabolic limit is determined partly by heredity and partly by environment. As a high yielder may only be a good lactose producer then total yield may be a misleading measure of a cow's performance. Gaines therefore devised a formula to standardise milk yield according to the amount of energy represented in each cow's milk. As the calorific value of butterfat, crude protein and lactose is of the order 9:4:1, Gaines suggests that the rate of fat secretion will determine the percentage of the other constituents. This is based on the correlations existing between fat, crude protein and lactose percentages. Gaines values represent within cow relationships. As fat increases, so does crude protein, whilst lactose decreases. However, by carrying out a correlation of the differences between two successive tests in the third month of lactation (see Table XV), the writer has shown that the variation of each of these three constituents can be quite independent for fat and crude protein percentage. The same is not so certain of fat and lactose and crude protein and lactose percentages. For 76 degrees of freedom the correlation coefficients were -0.135 for the former and -0.212 for the latter. Provided more
data were available these relationships might well be significant statistically.

In postulating a physiological limit to milk production Gaines bases his assertions on intra-cow correlations. In Table XV the results of correlations carried out both 'between' and 'within' cows are shown. 'Within' cows the usual positive correlation was found between fat and crude protein percentage and a negative correlation between fat and lactose and crude protein and lactose content. 'Between' cows all correlation coefficients were positive. Thus the cow which gives milk richest in fat, gives milk which is richest in crude protein and lactose as well. It is widely supposed that lactose percentage decreases as fat and crude protein percentages increase. That this occurs in the milk of each individual cow is not open to reasonable doubt but in this case at least, the situation is different between cows. Gaines and Overman (1938) roundly attack Goodale (1937) for suggesting that 'between' cows of the Jersey breed there is a positive relation between fat, protein and lactose. In view of the present findings it seems that Goodale was, in all probability, quite correct in his findings. Goodale goes on to suggest that as this is the case, a simultaneous increase in all three values can be brought about by selection. It is not
yet certain if this is possible. Genetic correlations have not been carried out on such data as not enough exists to give reliable estimates. The calculation of genetic correlations is a recent development which holds out great possibilities.

That simple correlation coefficients may be misleading as guides to variation in milk composition is raised by results presented by Lonka (1947), whose paper was referred to in an earlier section. He was attempting to find an answer to the problem of low protein feeding stuffs in Finland. Unfortunately, Lonka's method of calculating regression coefficients between fat and crude protein percentages confuses 'within' and 'between' cow differences. He computes on the basis of all analytical results from each of 57 cows. Consequently, he measures the correlation between tests at different stages of lactation for each cow rather than the differences between cows. Further, he makes no distinction between herds. Thus possible environmental differences will be included which might otherwise have been eliminated.

These considerations aside, Lonka poses several interesting questions which need answering. By grouping his data according to fat percentage he has shown that variation of crude protein percentage within each fat
percentage class is very nearly as great as that existing in the data as a whole, irrespective of fat percentage. We have to decide whether the association of fat and the other principal constituents of milk can indeed be changed to meet different nutritional regimes. If this can be done by selective breeding then genetics may make a considerable contribution to some of the difficulties confronting breeders of dairy cattle in various parts of the world. The deliberate adaption of livestock to unfavourable conditions, whilst maintaining an economic level of production has progressed little although much has been written and said of the need for such schemes. Within our own country temperature does not constitute an important factor but nutrition does. High protein feeding stuffs have to be imported and might well be done without or be reduced in amount if cattle were selected under the condition actually existing on the farms where they are kept.

Korkman (1950) reaches several important conclusions from a study of the effects of advance in lactation on butterfat percentage. They are as follow:-
(1) Fat percentage rises faster during the lactation in young cows than in older ones.

(2) The fat percentage of milk is mainly independent of the length of the calving interval until the seventh month of lactation. After that fat percentage of the milk rises in relation to the time from parturition as well as in relation to the decreasing daily yield, faster in shorter calving intervals.

(3) The differences between fat percentage in successive lactations of the same cow depend chiefly on differences between fat percentage in the first part of the lactation.

(4) Fat percentage rises during lactations more in cows with genotypically high mean fat percentages than in those cows with low genotypic fat percentages.

(5) The variation with advancing lactation in fat percentage is partly genetic. Korkman arrives at a value for heritability of 0.4.

This is perhaps the first real attempt to get at the underlying factors causing variation in butterfat percentage with advance in lactation. The study was made on 241 cows with at least eight complete lactation periods.

In a previous section of this thesis (p. 95) it was argued that the lactation curves for season represented that
part of the total variance due to temporary environmental causes \((\sigma_E^2)\), whilst that due to stage of lactation represented the interactions between heredity and that part of the environment having a permanent effect on the individual cow and remaining with her all through her productive life. This was symbolised by \(\sigma_{Ge}^2 \sigma_{Eg}^2\). The remainder of the phenotypic or observed variance \((\sigma_p^2)\) was regarded as due to genetic differences between cows \((\sigma_G^2)\). The equation is then as follows:

\[
\sigma_p^2 \text{ or } \sigma_0^2 = \sigma_G^2 + \sigma_{Ge}^2 \sigma_{Eg}^2 + \sigma_E^2
\]

Korkman arrives at an estimate of \(\sigma_G^2\) by carrying out an analysis of variance between herds \((\sigma_B^2)\), between sires (within herds) \((\sigma_S^2)\), between cows (within sires) \((\sigma_C^2)\) and within cows (within sires) \((\sigma_W^2)\). He considers that \(\sigma_S^2\) is probably the best basis for calculating heritability. An almost identical method was used for calculating the genetic contribution to variance (p. 87) in the present study. However, here the between cow, or observed variance, forms part instead of the total variance. Korkman concludes that the permanent differences between cows are chiefly genetic. This means that the variation in fat percentage with stage of lactation has a relatively small temporary environmental fraction, a fact confirmed by the present findings (p. 88, Table XVIII).

A steady trend occurs in all substances, with advance in lactation and according to the season of the year. Two of these, lactose and chloride have been generally regarded as accounting for 75 per cent of the osmotic pressure milk is
capable of exerting. The experimental evidence on the osmotic relationships between blood and milk is scanty and often unsatisfactory. Blackwood and Stirling (1932) comment:

"Beyond the fact reported by Van der Laan (1915) that milk is isotonic with blood, no data on the osmotic relationships of the mammary gland appear to have been established."

Simms (1931) in a paper to the International Dairy Congress reported that the osmotic pressure of cow's blood plasma equalled 260 millimols per litre and that of milk, 282 millimols. Making allowance for calcium caseinate, which is not in solution, the osmotic pressure of milk is brought into equivalence at 260 millimols, with blood (equal to 6.6 atmospheres at 37°C.). Simms considers the mammary gland freely permeable to water. His experiments also tend to show that the distribution of inorganic cations between blood and milk is determined by proteins.

Perhaps the two most interesting approaches to the problem of isotonicity are made by Blackwood and Stirling (1932) and Davies (1932). The latter made detailed analyses of representative samples of bulked milks and reported that the principal cause of low solids-not-fat was lactose. Previous findings suggested that high chloride values were associated with low quality. Davies argued that in all cases this was probably due to the substitution of ionised chlorides for lactose where factors caused a deficiency. He therefore postulated an 'isotonic diluent' of inorganic salts in solution, principally
consisting of sodium chloride, which diffused through the basement membranes of the udder when the synthesis of organic lactose broke down, in order to maintain isotonicity. This implies that the membrane is freely permeable to inorganic ions, but under normal conditions the concentration of lactose in milk is sufficient to prevent them diffusing in large quantities. Further, it suggests that as milk is formed, the osmotic pressure is always higher than that of blood.

Blackwood and Stirling take the opposite view. They reject the idea of a freely permeable membrane on the grounds that milk ratios of amino-nitrogen: sugar: sodium chloride are 1: 11: 0.35, whilst in blood they are 1: 6: 65. They also aver that histologically the mammary gland is not equipped for re-absorption of constituents into the blood. Their results show that in 12 hours experimental animals secreted 10 litres of milk containing 450 grams lactose. To provide this \[ \frac{450,000 \times 100}{40 \times 1000} = 1125 \] litres of transudate would have to be filtered from the blood, of which only 10 litres would appear as milk, in order to form this amount of lactose from blood glucose. They therefore postulate preferential absorption of blood glucose. It is suggested that a transudate separates from the blood containing organic milk precursors in the proportions occurring in blood, and inorganic constituents in much smaller amounts. The mechanism of diffusion against the high osmotic pressure of \( \text{NaCl} \) in the blood is not accounted for.
The subsequent conclusion, that osmotic pressure must be reduced as a result of synthesis is the most intriguing. Two molecules of glucose produce one of lactose. Osmotically active amino-acids produce inactive colloidal protein. Colloidal calcium phosphate is formed from calcium and phosphorus ions. The effect of fat synthesis is vague. By gathering freezing point data a calculation was made that the transudate from blood must have three times its osmotic pressure to allow for hydrolysis and reduction of osmotic pressure in milk formation. If the osmotic pressure is higher than that of blood at the end of synthesis water will be absorbed, if lower, it will be returned to the blood. From these interpretations a fresh hypothesis is made that at no point does osmotic pressure rise above that of blood during synthesis. Therefore water will return to the blood during synthesis.

If the variation in lactose and chloride in the present data are converted into the osmotic pressures they are capable of exerting, it can be demonstrated that variation in osmotic pressure due to these two causes is slight. A maximum range of 3.5 per cent, from the first to last month of lactation was noted. If the last month of lactation is omitted, then the
range from the first to ninth month is only 1.6 per cent. This represents stage of lactation effects free of seasonal changes. The range in osmotic pressure values due to season was only 4.0 per cent. The changes due to variation in chloride content were at least as great as those due to variation in lactose content, with advance in lactation. The seasonal changes in chloride content were even greater. The variation in chloride content with season is of particular interest as it bears little relationship to variation in lactose percentage ($r = -0.035$ compared with $-0.753$ between stages of lactation). Thus, whilst chloride makes a big contribution to osmotic pressure its chief source of variation is seasonal and its random correlation with lactose is only $+0.051$. Random differences are due to just those factors - sudden attacks of mastitis, chills, udder damage, - which might be expected to show a strong negative relationship between lactose and chloride. However, the osmotic implications appear to be slight.

The theory advanced by Davies (1932) of an 'isotonic' diluent suggests that the basement membrane of the udder is freely permeable to inorganic ions. The argument of Blackwood and Stirling that osmotic
pressure is maintained whilst synthesis to compounds with higher molecular weights is going on, does strike one as convincing. The inflow of inorganic ions suggested by Davies in a different context would at least allow for this to occur whilst maintaining isotonicity between milk and blood.

The selective permeability concept advanced by Blackwood and Stirling is not so convincing in view of the disparity in molecular weight between amino-acids and glucose, which can diffuse, and inorganic ions which, by hypothesis, cannot. The idea of a transudate possessing a higher osmotic pressure than either milk or blood seems to introduce an added complication. The wide variation in chloride values with season of the year seems to suggest that the secretory cells of the udder are freely permeable to it. Chloride decreases from January to July in the present data but in hotter climates it has been shown that there is a strong tendency for it to increase.

A new approach to the study of factors affecting milk secretion and milk composition is needed. There is need of much closer co-operation between physiologist, bio-chemist and geneticist. A broad approach demands a long-term policy and considerable resources.
Experimentation is difficult and the generation length of dairy cattle is such that only a slow advance can be made. The use of smaller and faster breeding animals and of twin cattle goes some way towards a more rapid solution of the problem. These methods are now being used and offer great possibilities.
1. Analytical data was collected on the variation in milk composition of 120 cows in two herds of Ayrshire and Ayrshire cross cattle.

77 cows were tested at 14 day intervals during one complete lactation. 29 of these were tested during 12 weeks of a subsequent lactation and 15 during 20 weeks.

2. Values were obtained for:— butterfat, crude protein, lactose, chloride and density. Tests were on composite samples of three daily milkings.

3. The joint effects of age and season of calving on 305 days yield and milk composition was measured by the use of constants calculated from the data. The results were as follow:

(a) 305 days yield — a rise of 3080 lb. occurred from the first to fourth lactation followed by a decline of 2400 lb. to the sixth lactation. This represents a variation of approximately 30 per cent. Due to the small number of cows in the higher age groups, the differences between age group means did not prove to be statistically significant but the range of variation is such that an age effect almost certainly exists.

The constants calculated for season of calving showed negative values in the months January to April and June to August. Positive values existed in May and from September to November.

(b) Butterfat $S$ — the measurement of variation in fat
percentage was complicated by the existence of an age-season of calving interaction which is difficult to explain. An attempt to account for this interaction is made after the implications of variation in calving interval length have been summarised.

(c) **Crude Protein** - a significant difference between age groups is not supported by a pronounced trend in the data and it is suggested that this difference is due to chance rather than age.

The constants calculated for seasons of calving are virtually the opposite to those of yield.

(d) **Lactose** - a decrease in lactose content with increasing age occurs. Constants for season of calving are the same as for crude protein. They are probably a function of variation in total yield as the percentage of each constituent is negatively correlated with yield.

(e) **Chloride** - shows a behaviour exactly the reverse of lactose percentage.

(f) **Density** - declines with increasing age and inversely with yield between seasons of calving.

4. Preceding Calving Interval appears to have no effect on subsequent lactation. The lengths of Preceding and Current Calving Intervals, whilst not significantly correlated, would perhaps reach significance if more data were available.
As length of service period, which is the time elapsing between calving and conception, determines calving interval length, the lengths of successive calving intervals are likely to be similar because of management. Delayed service was associated with high yield but not with the number of services needed for conception.

5. Heifers are served to calve in Autumn, which is the most favourable time of year for high lactation yield. The rise in yield due to increase in age will be to some extent offset by the fact that highest yielding cows, having longest calving intervals move into an unfavourable season of calving more quickly than lower yielding cows.

6. The interaction between age and season of calving for butterfat percentage, may be explained on a basis of fat and negative relationship with yield. Lowest yielding cows and all heifers, irrespective of yield, calve at a time of year most beneficial to high lactation yield. Highest yielding cows calve at a time of year least beneficial to high lactation yield. As fat is negatively correlated with total yield it will tend to be increased among high yielding cows by season of calving and decreased among lower yielding animals.

7. The relative effects of advance in lactation and season of the year were shown graphically for the percentages and yields of butterfat, crude protein, lactose and chloride and for total yield of milk and density.
By superimposing the graph of seasonal variation on that for stage of lactation a series of estimates were made of the effect of season of calving on milk composition and yield.

The principal milk constituents showed a greater effect with stage of lactation than with season. Chloride, by comparison, displayed a greater seasonal variation.

High values for protein in Spring and butterfat in Autumn were found.

8. A close association of total yield and lactose yield was noted (1 lb. lactose = 10 lb. total yield). The yield of butterfat, protein and lactose showed a closely similar response to seasonal effects.

9. An estimate was made of the variation in milk composition due to random causes. An error term was provided for each monthly value in the graphs showing variation in composition with advance in lactation and season of the year.

10. A correlation of random differences revealed that such highly associated variables as butterfat, crude protein, lactose and chloride could vary independently.

11. Correlations between stages of lactation were high, but not between seasons of the year.

12. The correlations between the lactation averages of cows are positive for butterfat, crude protein and lactose,
although that between crude protein and lactose is not significant.

13. The relationship between stages of lactation is positive and highly significant for butterfat and protein. For butterfat and protein on the one hand and lactose on the other, the correlation coefficient is negative and highly significant.

14. The correlation between the observed and true value \( (r_{MT}) \) of the lactation mean for each constituent, was calculated and the standard error determined. Postulating a minimum correlation, the number of tests needed to achieve it, were calculated, together with the standard error. It was found that four butterfat and four protein tests during a lactation were adequate and eight lactose and chloride determinations.

15. The osmotic relationships of milk were considered. The variations in lactose and chloride percentages, with season and stage of lactation caused little change in osmotic pressure. Milk has the same osmotic pressure as blood. The average value commonly accepted is 6.6 atmospheres. Change in osmotic pressure due to variation in lactose and chloride only amounted to 1.6% up to the ninth month of lactation. Changes due to season amounted to 4.0%, chiefly due to variation in chloride.

16. The genetic contribution of the six sires represented in the data was calculated. The values obtained show
lactose to be more highly heritable than butterfat. Protein showed a low heritability.

17. For those animals tested during a subsequent lactation the repeatability was significant at 20 weeks for all but protein which was very nearly so. It was concluded that all factors were highly repeatable for a complete lactation.

18. The regression coefficients of butterfat, crude protein and lactose percentage on each other, was calculated between cows. In each case values were positive and significant.

For lactose and chloride percentages values were negative and significant (regression coefficient was more than twice its standard error).

19. The variation in composition with stage of lactation was shown to be due to genetic and permanent environmental effects. Seasonal variation is temporary and purely environmental. Variations in milk composition with advance in lactation appear to be chiefly genetic.
APPENDIX A.

Analytical Methods Used to Determine Each of the Values.

The methods used had to be rapid as the work of analysis was done single-handed. Fortunately, regular butterfat tests and density determinations were carried out by the farm staff for record purposes. The determination of crude protein, lactose and chloride was carried out on the same samples.

1. **Butterfat Percentage**
   Determined by the Gerber test.

2. **Density**
   Using a hydrometer and correcting readings for temperature to 20°C, allowing for butterfat percentage.

3. **Crude Protein**
   Reagents:  
   - N/100 Hydrochloric acid
   - 0.5% Boric Acid
   - 60% Sodium hydroxide solution
   - Alcoholic Brome Cresol Green
   - Methyl red indicator.
   Method: 10 ccs. of well mixed milk were weighed and transferred to a 300 cc Kjeldahl flask. 10 grams anhydrous sodium sulphate and 1 gram copper sulphate crystals were added plus 30 ccs. concentrated sulphuric acid. The contents of the flask were then digested over a naked bunsen flame.

   The milk digest was then transferred to a 250 cc graduated flask and made to volume with distilled water, followed by a vigorous mixing. The ammonium salts present were then determined in a Markham micro-still as follows:

   5 ccs. of the solution of milk digest in the graduated flask were pipetted into the still and the stopper was inserted. Approximately 2 ccs. 60% sodium hydroxide were then pipetted into the cup. This was allowed to flow into the still, at the
same time placing a 50 cc. conical flask under the condenser, with the condenser tip under the liquid in the flask. The distillate, containing the freed ammonia, was collected in the 0.5% boric acid, to which had been added 0.45 cc. alcoholic brome cresol green – methyl red indicator. The distillation time allowed was two minutes.

The distillate was back titrated with N/100 hydrochloric acid. (Replicates should agree to 0.1 cc.).

Crude protein percentage was then calculated from the titre as follows:

\[
\text{1 cc. } \text{N/100 HCl} = 0.00014 \text{ gms Nitrogen}
\]

\[
\text{Crude Protein} = 0.00014 \times \frac{100}{10 \text{ccs}} \times \frac{250}{5} \times 6.38 \times \text{titre.}
\]

(4) **Lactose**

25 ccs. Milk were used and the proteins precipitated with 25 ccs. of dilute mercuric nitrate solution. The filtrate was then read off in a saccharimeter, the sucrose reading on the scale being multiplied by 2 to allow for dilution. This value was then converted to lactose percent by allowing for the lower rotatory power of lactose.

(5) **Chloride.**

The direct method of Sanders (1939) was used. 10 ccs. milk were taken and 15 ccs. special silver nitrate was then added and titrated with potassium thiocyanate solution of equivalent strength.
Reagents:- silver nitrate 0.0291 N

4.9438 grams AR AgNO₃ were dissolved in distilled water, 200° cc. saturated ferric alum solution added. The solution was then made up to 1 litre.

Potassium thiocyanate 0.0291 N was made by dissolving about 3.2 to 3.5 grams in 1 litre distilled water and adjusted to equivalence with the silver nitrate solution.

% Chloride = ccs. thiocyanate used in titration x Cl⁻.

With increasing proficiency, smaller amounts of milk and reagents can be used.
APPENDIX B.

The Analysis of Monthly Differences into those due to Season and those due to Advance in Lactation.

A form of the analysis of variance, involving a two-way classification with unequal class numbers, was used.

Animals were first classified according to the month of the year in which they calved. All those animals having tests in January and February then had the differences between these monthly values calculated in groups according to their month of calving. These values were then entered in a Table similar to A.

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<th>January/February Differences.</th>
<th>Butterfat percentage.</th>
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<tr>
<td>Difference</td>
<td>Month of Lactation</td>
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<td>-0.04</td>
<td>6 - 7</td>
</tr>
<tr>
<td>+0.11</td>
<td>5 - 6</td>
</tr>
<tr>
<td>-0.04</td>
<td>4 - 5</td>
</tr>
<tr>
<td>-0.12</td>
<td>3 - 4</td>
</tr>
<tr>
<td>+0.03</td>
<td>2 - 3</td>
</tr>
<tr>
<td>+0.55</td>
<td>1 - 2</td>
</tr>
<tr>
<td>-0.04</td>
<td>7 - 8</td>
</tr>
<tr>
<td>Total &amp; Average</td>
<td>+0.03</td>
</tr>
</tbody>
</table>

This Table was repeated for each of the twelve monthly differences.

The differences were inserted in a two-way table, divided according to the months of the year and months of lactation. Such a table is shown as B.

The values in Table A form a column of values under Jan-Feb in Table B.

The average values for season and stage differences
### TABLE B.

**Analysis into Season and Stage of Lactation Differences.**

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<td>-</td>
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<td>-.11</td>
<td>+.06</td>
<td>-</td>
<td>-.37</td>
<td>-.32</td>
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<td>-</td>
<td>-.16</td>
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<td>9 - 10</td>
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<td>-.18</td>
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<td>Av</td>
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<td>+.01</td>
<td>-.04</td>
<td>+.03</td>
<td>+.11</td>
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</tr>
</tbody>
</table>
consist of the mean value of cows at all stages of lactation. The average values were then used as correction factors for each other. Thus for Jan-Feb, there are cells for the first to ninth month of lactation; the average values for these months over all seasons were added together and subtracted from +0.03, i.e., +0.03 - (-0.033) = +0.063.

This was done for all averages, until successive corrections made no difference to the estimate. The monthly differences were then compounded, taking the first month of lactation and the first month of the year as zero.

Ideally, the graph of seasonal differences should commence at zero in January and return to zero in the following January. However, time changes in environment will generally cause a discrepancy to appear. This discrepancy must be distributed over the months of the year in such a manner that the graph will return to zero. This is done by dividing it by 12 and allocating one-twelfth of it to the February value, two-twelfths to March, three-twelfths to April and so on. The effect is to swing the whole graph into a position where the two values for January will be zero, without affecting the change in values from month to month. The first part of the graph is repeated in figs. 11 and 23 in order to give a better impression of the changes occurring at the turn of the year.

The actual production of each substance may be determined by finding the overall mean from the data. This will pass through the middle of the range of graph points. This value
is then treated as the new origin and the monthly variations
as deviations from it. By doing this, monthly deviations are
converted into actual percentages. The total yield at test
in any one month (the mean of actual yields at each fortnightly
test) is then used to calculate the actual yield of the sub-
stance in that month. Finally, monthly changes in yield are
again expressed as deviations from zero in the first month of
lactation and the first month of the year.

The method of using the average values at the foot and the
side of Table B to correct each other, is analogous to Yates'
(1933) correction for missing data in a latin square.
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