OBSERVATIONS

ON

RUMINANT FAT METABOLISM

WITH

PARTICULAR RELATION

TO

LACTATION

by

J.W.MAYHEAD

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I N D E X

OBSERVATIONS ON RUMINANT FAT METABOLISM WITH PARTICULAR RELATION TO LACTATION

1949.

I INTRODUCTORY.

In that animal fat is a major economic "building block" the mechanism of its production has attracted particular study. Although observations regarding digestion of fats may be traced back as far as those of Asellius in 1622 it was in 1843 that specific investigations into Body fat production were first instituted by Lawes and Gilbert. From 1900 onwards biochemical research has made its greatest advances but, in spite of the volume of literature published on the subject of fat metabolism the state of knowledge in this field may yet be in its infancy.

> Baldwin (1) in 1948 made the following statement " our knowledge of the mechanisms whereby fats are metabolised is far from being complete and the complexion of the whole subject is changing rapidly. The evidence available regarding fat metabolism has in the past been so contradictory and speculation so rife that almost any statement made might be contradicted by as many facts and arguments as could be produced in its support."

The three main experiments to be described relate principally to the changes effected in the degree of unsaturation of milk fat from dairy cows when highly unsaturated oils are included in the daily ration. The immediate effects of short-term inanition are also investigated. Two minor experiments are described; the first concerning the tracing of ingested stained fat into the milk and depot fats of simple-stomached animals; the second relating to the keeping qualities of milk fats of varying degrees of unsaturation.

Before proceeding to detail experiments undertaken it is first necessary to appreciate at least some of the research work that has been direct towards elucidating the manner in which milk fat is synthesised in the ruminant, as well as understanding the present beliefs regarding the digestion of fats. In view of the "dynamic" aspects of physiology it is with caution that one views one aspect singly; but to attempt to integrate theories of fat metabolism, in itself a large field, with those of metabolism of other substances throughout the development of a review of literature would prove too unwieldy. Accordingly the digestion and absorption of fats from the diet into the bloodstream is briefly discussed as preparation for considering the "General Review of Literature" which is confined to papers pertinent to the study of the source of milk fat in the ruminant. For simplicity of consideration alone this has been sectioned further into four main aspects. It is fully appreciated that subdivision of any aspect of metabolism is purely arbitrary and that in actual fact the sections are interdependent without established boundaries.

The General Review of Literature incorporates work which relates to the experiments later to be described but in addition there follows a brief Particular Review of Literature in which several other papers immediately pertinent to the specific research undertaken together with the objects of the latter are discussed.

Thereafter five experiments conducted at Massey Agricultural College in 1949 are described in turn and evidence presented corroborating the observations of several workers whose research is reviewed. Evidence is also presented of a consistent diurnal variation in degree of unsaturation of milk fat from pasture-fed cows.

II THE TRANSFER OF FAT FROM THE ALIMENTARY CANAL INTO THE BLOODSTREAM.

The earlier work on digestion of fats by simple-stomached animals was based on observations of that fraction of blood fat which is most readily apparent following the ingestion of a meal containing fat.

In 1622 Asellius observed the "milky" appearance of lacteals leading from the intestine evidenced when food was present.

1650 Pecquet traced the lacteals to the jugular vein.

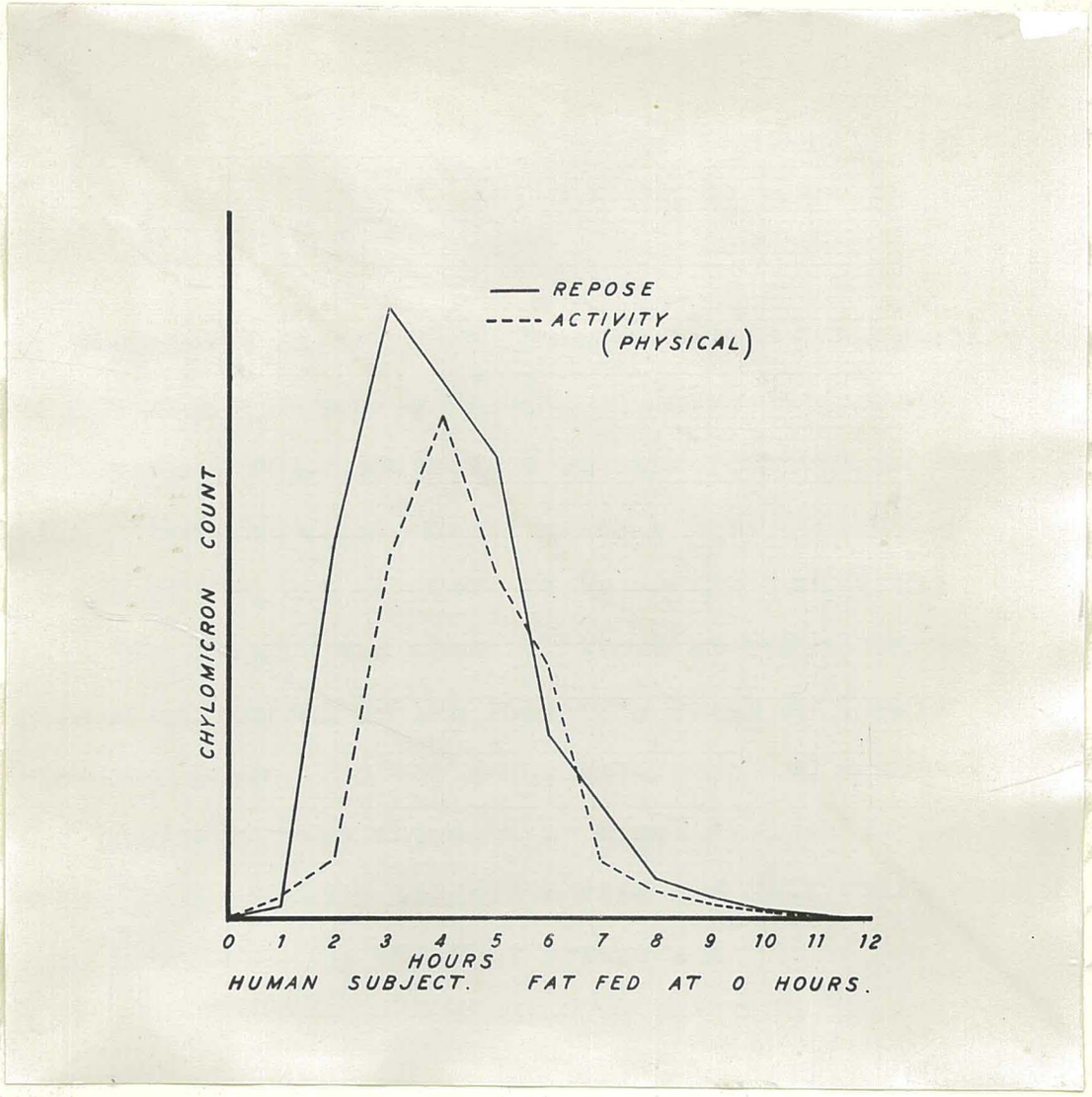
1665 Boyle demonstrated that blood serum assumes a "milky" appearance at the same time as the lacteals, following food ingestion.

1774 Hewson showed that the "milky" fluid of the lacteal chyle contained appreciable amounts of fat.

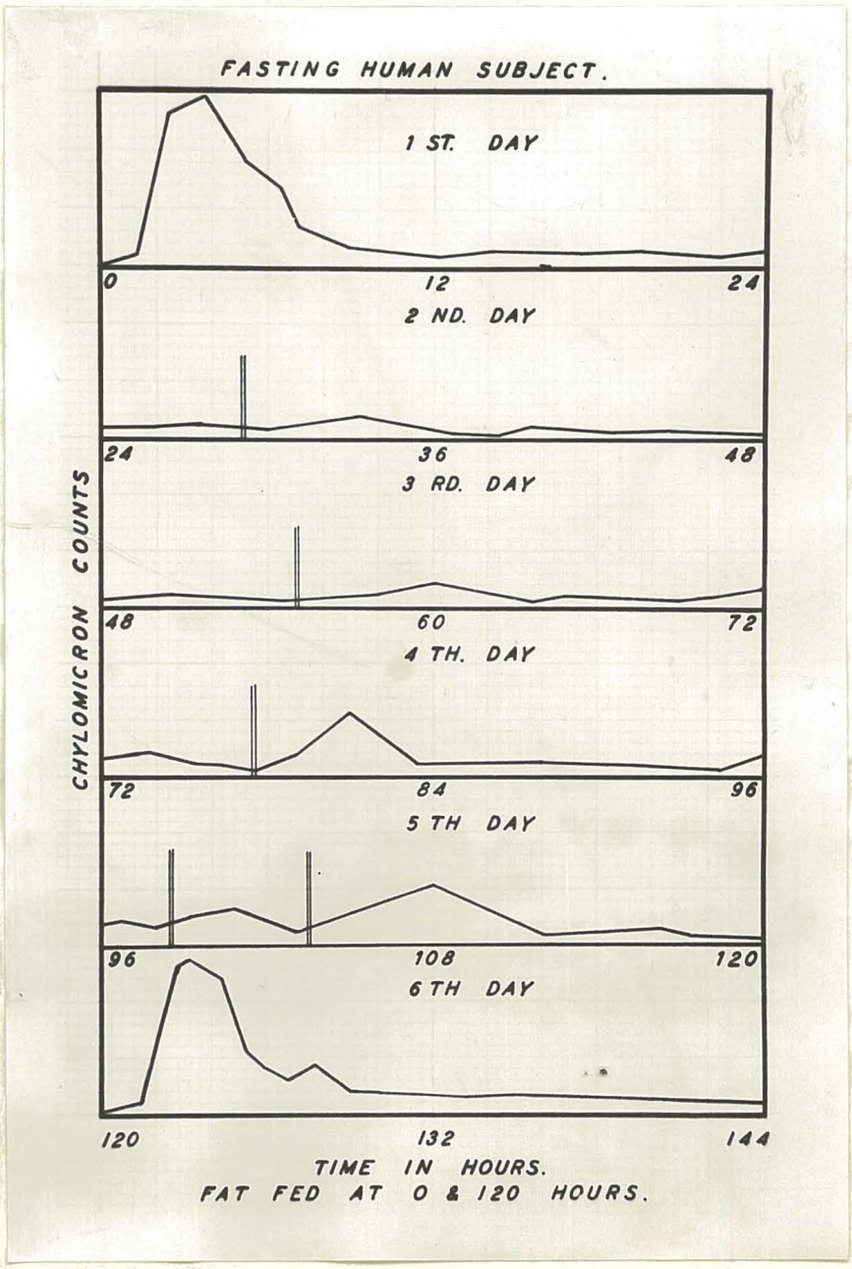
1877 Edwards first noted actual particles in "milky" blood under the microscope.

In the older literature these particles were referred to by various terms such as "blood dust", and "hemakonia" a term coined by Neumann in 1907. Later, in 1924, Gage and Fish (2) (who quoted the above few experimenters) proposed the term "chylomicron" which is now widely used. Gage and Fish (2) devised a system for making comparative chylomicron counts under the microscope. They clearly demonstrated fat to be the only food constituent causing an increase in chylomicron count on ingestion. Pure carbohydrate and protein diets when supplied to humans and various experimental animals caused no alteration in the chylomicron count. Meals containing fat caused marked rises in numbers of particles, reaching a peak up to four hours after the meal and then

declining less rapidly. It was generally assumed that the increased fat in the blood was representative of the fat that had been fed,but the authors were aware that the visible blood fat increment did not account for all the fat fed. Experiments showed the deviations from normal absorption rates caused by physical activity and by mental activity.



An experiment designed to investigate the mobilisation of fat reserves during fasting was also conducted. Employing a human subject, a six-day fast was imposed at the beginning and end of which a meal containing 30 gms of fat was allowed. At intervals during the fast the subject was given physical exercise. These points are marked in the following graph by double vertical lines. The two meals each produced a normal and characteristic increase in blood fat, while the exercise apparently induced a rise and fall which became more pronounced as the fast proceeded.



It appeared in this experiment that fat could be mobilised as required, and that following the cessation of demand the fat supply was not immediately curtailed but tended to carry on with a kind of "physiological inertia". It appeared to these workers that the blood fat picture on drawing on body fat reserves paralleled the digestion lipaemia normally following the ingestion of fatty foods. Baldwin (1) in 1948 states the very antithesis of this in that "when fat is being withdrawn from the depots to be metabolised elsewhere there is no lipaemia" and presumes mobilised fat to be transported as phospholipoids, appreciably soluble in water. He further points out that neutral fat is present in the blood as such only while the condition of post-absorbtive lipaemia persists. However Baldwin does not discuss the situation where a fasting subject is suddenly called upon to perform physical exercise, or where a demand such as that of lactation is superimposed. Gage and Fish found that for simple stomached animals a meal containing fat induced a characteristic single peak curve when chylomicron count is graphed against time as already shown. When cows were given a small quantity of fat in the ration the subsequent blood picture proved very fluctuating, yielding a many-peaked curve. A similar result was noted for goats. The authors concluded that the fluctuations in absorption in the ruminant were associated with intermittent passage of food into the intestine, Although in general it has been assumed that the increased blood fat consequent upon fat ingestion is the same fat that has been fed, later workers have shown that certain modifications occur during absorbtion. One example of this is the work of Wilson and Hanner (3) who compared the degree of saturation of the blood fat increments

resulting from feeding cream, and cod liver oil respectively to children. The iodine values of the blood fat increments were markedly inclined towards those of the fed fats, but it appeared that the butterfat had undergone a certain amount of de-saturation during absorbtion whereas the cod liver oil initially of very high iodine value had been partially saturated.

Although, as shown in the foregoing brief review of earlier work, it at first appeared that food fat could give rise to blood fat in a simple manner, the fact that the food fat could not all be accounted for in the visible blood fat increment, and the fact that there were indications of the occurrence of saturation and de-saturation of food fats during absorbtion led workers to realise that the mechanism of fat absorbtion is far more complex.

Frazer (4), in 1946, integrating the results of other workers with those of his own in vitro, and in vivo experiments using rats, put forward the presently accepted <u>tentative</u> hypothesis of fat absorbtion, of which the following is a precis.

Frazer's (4) Hypothesis of Particulate Fat Absorption.

Diet fat is mainly in the form of mixed triglycerides incorporating various fatty acids having various lengths of even-numbered carbon chains. It has been shown that the properties and behaviour during absorption, of triglycerides differ.

Emulsification:- The first alteration of ingested triglyceride is that of emulsification occurring in the first part of the small intestine. The resulting particle size is 0.5μ and less, which agrees with Gage and Fish's earlier observations (2). Emulsification is known to occur in a medium of pH 6.5 In vitro experiments showed that the only effective emulsifying system is that of a triple combination of fatty acid/ bile salt/ monoglyceride. Frazer assumes the emulsification of triglycerides in the intestinal lumen to be dependent upon partial hydrolysis, resulting in fatty acids and monoglycerides, and that these two products together with bile salts provide the basis of the emulsifying system.

Hydrolysis:- Optimum hydrolysis of triglycerides in vitro occurs at pH8.5 and hydrolysis is restricted at higher acidities. The upper two-thirds of the small intestine has a pH of less than 7.0 while a more alkaline reaction prevails at the lower end of the ileum. Short-chain triglycerides are readily hydrolysed even in the more acidic upper intestine. It appears that the hydrolysis of long-chain triglycerides is restricted in the upper two-thirds of the intestine but more extensive hydrolysis may be expected in the lower end of the ileum whereas short-chain triglycerides as exemplified by tributyrin are likely to be adequately hydrolysed irrespective of pH.

Particulate Absorption:- The final products of emulsification consistof lipid particles of less than 0.5μ diameter.

In order to accept an hypothesis of particulate absorption Frazer considers that five major aspects must be elucidated.

1. The presence of an effective emulsifying system:- Already discussed.
2. The receptiveness of the intestinal cell membrane to particulate absorption:- Baker, quoted by Frazer has shown fine canals to exist in this membrane at right angles to the surface, but is not yet demonstrated in mammals.
3. The ability of the intestinal cell membrane to absorb finely dispersed particles such as unhydrolysable paraffin of particle size less than 0.5μ :- Frazer has been able to show this experimentally.
4. The ability of negatively charged particles to pass through a membrane of this type:- If particulate absorption is allied to known systems of electrolyte absorption then an artificially induced disturbance of electrolyte metabolism may be expected to interfere with particulate fat absorption. Frazer quotes Verzar and Laszt who demonstrated the depression of fat absorption following double adrenalectomy, which is known to induce electrolyte imbalances. Replacement therapy corrected the induced faulty fat absorption.
5. The ability of the intestinal cell membrane to absorb substances in a preferential

manner:- Seeing that canals readily admitting relatively large globules of lipid material may exist, it is difficult to appreciate why carbohydrates and proteins have to be so greatly simplified prior to absorption. This latter is not yet explained.

Some evidence has also been shown that under certain circumstances complete hydrolysis to glycerol and fatty acids in the intestinal lumen may occur, followed by resynthesis to triglycerides within the intestinal cell. The fat observed in the chyle of the lacteals is mainly in the form of triglycerides. If a considerable amount of the diet fat is absorbed in the incompletely hydrolised or un-hydrolysed form as seems very likely from the foregoing considerations then theories of resynthesis diminish in importance.

Phosphorylation:- During fat absorption phospholipids are known to be formed in the intestinal cells but it is only a small portion of the absorbed fat that takes part in the synthesis. The purpose of phosphorylation remains obscure. The indications are that phospholipids formed in the intestinal cell become incorporated in the interfacial structure of the fat globules being absorbed, and are responsible for the stability of the particulate fat in the blood.

Entry routes of lipid material into the Organism:-

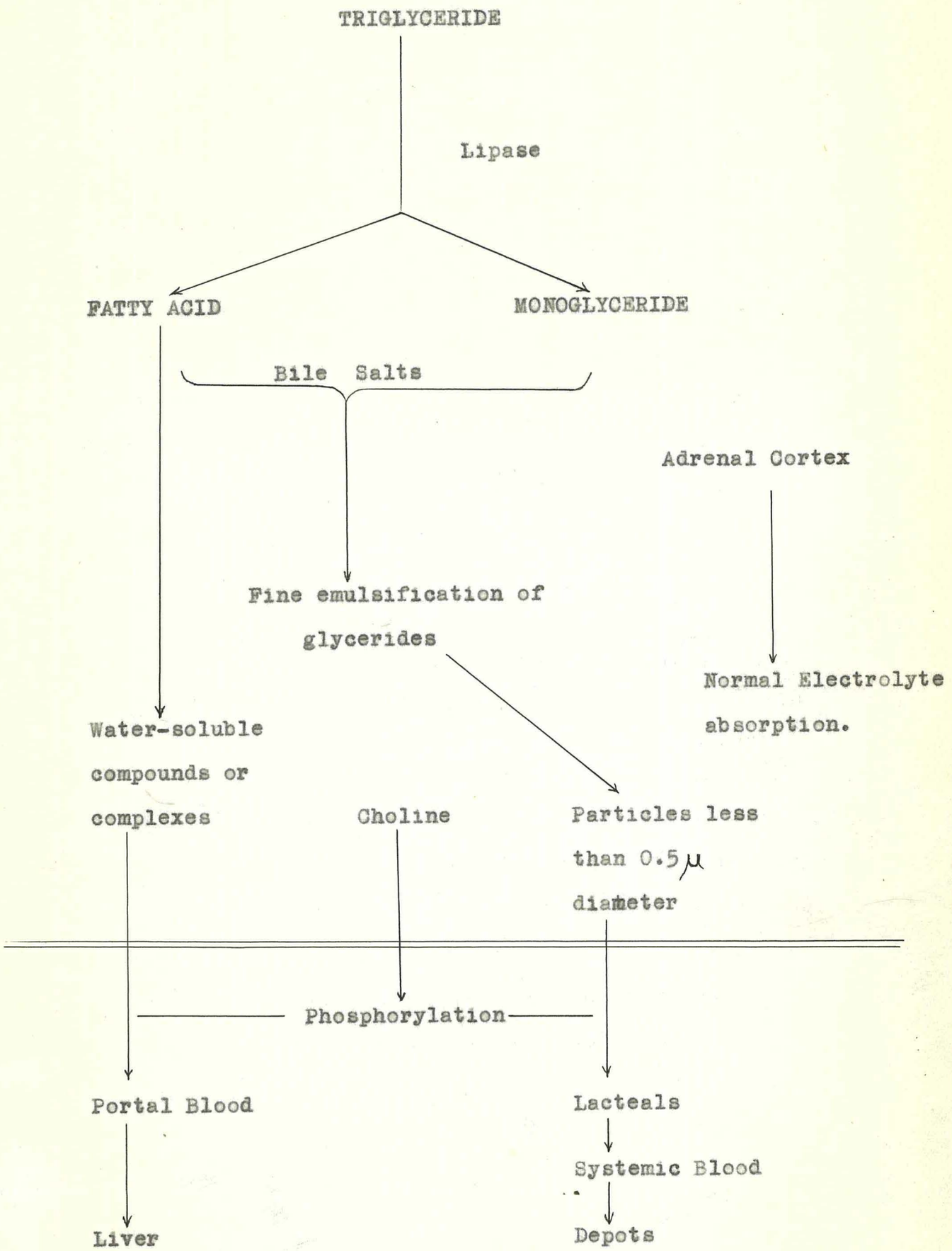
Ingestion of triglycerides in the diet give rise to large lipid globule formation within the intestinal cell, to the "milky" lacteals noted by early workers, to a post- absorptive systemic lipaemia, and to deposition in the fat depots.

Ingestion of fatty acids and glycerol separately produces a "granular" appearance in

the intestinal cell, a portal lipaemia and deposition in the liver, with no change in the systemic blood or fat depots.

It is suggested by Frazer and others that fatty material absorbed unhydrolysed tends to pass by the lacteal lymphatic pathway into the systemic circulation, and may be largely deposited in the fat depots, while on the other hand hydrolysed material travels by the portal route to the liver. Short-chain triglycerides being readily hydrolysed behave more like long-chain fatty acids than long-chain triglycerides. For instance, tributyrin, following ingestion, cannot be recovered from the chyle nor does it appear to be deposited in the fat depots.

Frazer presents the following table in illustration of his hypothesis:-


TRIGLYCERIDE
Lipase
FATTY ACID
MONOGLYCERIDE
Bile Salts
Adrenal Cortex
Fine emulsification of glycerides
Normal Electrolyte absorption.
Water-soluble compounds or complexes
Choline
Particles less than 0.5μ diameter
Phosphorylation
Portal Blood
Lacteals
Systemic Blood
Liver
Depots

III GENERAL REVIEW OF LITERATURE.

The majority of papers concerning fat metabolism in relation to lactation deal perforce with individual aspects, and in addition, most of the periodic reviews of literature tend to furnish the bare results of research work with little integration of the conclusions of various workers into a logical sequence.

In an endeavour to present such a review the literature, as far as practicable, is discussed under four main headings, the latter being based on the primary assumptions that the mammary gland is the site of milk synthesis, and that the "raw materials" for this process are supplied to the mammary gland by way of the blood.

THE SOURCE OF MILK FAT IN THE RUMINANT.

Considering blood as a fluid tissue acting as a transport agent carrying materials to and from the fixed tissues it is feasible to expect, with particular regard to the fixed tissues of the mammary gland, that milk fat could possibly be synthesised in any or combinations of the following ways:-

1. From lipoid materials of the blood derived via the walls of the alimentary tract from lipid fractions of the diet.

2. From lipoid materials synthesised from non-lipid substances such as carbohydrate, either actually in the mammary gland itself, or elsewhere in the body and transported to the mammary gland by the blood.

3. From lipoid materials of the blood derived from mobilisation of fat reserves in fixed tissues other than the mammary gland, or

directly from fat reserves of the mammary gland.

4. From varying combinations of the above possible sources according to the level of nutrition of the animal, to its state of health, or to its reactions to changes in environmental conditions.

Consideration of the literature has been sectioned into the above broad divisions but it is to be borne in mind that no one section may be regarded as entirely complete in itself without relation to the conclusions from each of the other sections.

1. THE RELATION OF FOOD FAT TO MILK FAT.

(a) The Effects of Varying the Level of Fat Intake:

Maynard and co-workers (5,6) in studying the influence of different levels of fat intake upon milk secretion found that on drastically lowering the level of fat in the ration of dairy cows marked lowering of milk yield also occurred although the percentage of fat in the reduced amount of milk tended to remain the same. Further studies revealed that there was a certain threshold level below which the reduction of fat in the ration commenced to show its effect on the milk yield. Reduction from 7% to 4% level of fat in the ration achieved no significant reduction of milk yield, but below this level of intake milk yield reductions did occur. Where fat in the ration was not lowered below the level of that secreted in the milk no consistent lowering of milk yield occurred. In the experiments the low-fat diets had been made up to equivalent energy level with the normal fat diets by the addition of starch. It would appear from this that the level of fat intake is intimately concerned with the process of

of milk secretion in that as the secreted fat was reduced the other constituents declined as well even though the diet was as before with the exception of the fat content alone.

Nat. N. Allen (7) & (8) experimented with increasing the fat intake levels of lactating dairy cows above normal and at first in short-term experiments, six-day periods of feeding of various oils, found that while milk yield was affected but slightly, an increase of butterfat production occurred equivalent to 10% to 20% of the increased fat intake. Later longer-term trials, (fifty-day periods) also showed the same effects but certain oils exerted a depressing effect not noted in the short-term trials previously.

Gibson and Huffman (9) confirmed Maynard's work that addition of fat to a low-fat ration for dairy cows led to an increase in milk production, although over the range of diet-fat percentage from 2.69% to 4.89% Monroe and Krauss (10) in the course of fairly extensive experiments were unable to show any significant differences in milk or butterfat production.

(b) The Effects of Feeding Stained Fats:

The problem of actually tracing fed fat after ingestion was perhaps first attempted seriously by employing fat-soluble dyes. From 1896 onwards various workers used this technique. Mendel and Daniels (11) using Sudan 111 and other dyes were able to show that stained fats when fed to various small experimental animals were laid down readily in the fat reserves of the body and in the bone marrow. Also when fed to lactating rats, guinea pigs, cats and goats, stained milk fat was readily demonstrated, but when the trial was attempted with a lactating cow stained milk fat was not secreted.

In pregnant animals the stained fat did not traverse the placenta in any instance. Later, in 1924, Gage and Fish (2) published results of extensive experiments on stained fat feeding which were essentially similar to those of Mendel and Daniels and others. They repeatedly attempted to demonstrate stained milk fat in the cow by the feeding of stained fats but entirely without result. Following on these attempts Kelly and Petersen (12) in 1939 again attempted stained fat feeding to dairy cows and failed to secure stained milk fat. They further endeavoured to discover the fate of the stain by histological techniques but could find no evidence of the stain in body fat, udder tissue or milk fat. They suggested that in the cow the dye molecule was changed in some manner to a water-soluble form. In spite of all these previous failures Huffman and Duncan (13) in 1941 repeated the experiment using massive doses of various dyes and in most cases secured positive appearance of the dyes in the milk fat within twelve hours. In view of the previous total failures it seems possible that in the latter experiment the doses were of such magnitude that the normal "mechanism"of dairy cows by which dyes may be eliminated had either broken down or had been unable to operate at sufficiently high a level in order to eliminate the greatly increased amounts of dyestuff. The amounts of the dyes appearing in the milk fat were not estimated quantitatively.

(c) <u>The Tracing of Chemically Identifiable Fats from the Food into the Milk</u>.

Suggesting that dyes fed to animals may dissociate from the fed fat within the organism and recombine with fats already present thereby invalidating the conclusion of the fed fat itself having reappeared in the milk, Bowes (14) in 1915

traced an ingested fat by chemical means. Employing a goat he checked prior to the experiment that no arachidic acid was being secreted in the milk then proceeded to feed small doses (25 cc.) of arachis oil containing 5% arachidic acid along with the normal ration. Twelve and one-half hours later arachidic acid was found in abundance in the milk fat. Quantitative estimation was not attempted and no record was made of how long the arachidic acid continued to be secreted. Similarly Maynard, McCay, and Madson (15) administered food fat of varying degrees of unsaturation to dairy cows and followed the iodine values of the milk fat secreted. On feeding ground flaxseed containing oil of a high degree of unsaturation (iodine value 137) a marked rise was noted in the milk fat iodine value within eighteen to twenty four hours and the value rose to a maximum by the third to fourth day the authors concluding from this that there must exist a close relationship between food fat and milk fat, and that the course of fat metabolism in lactation must be a very direct one or that the various processes must take place very rapidly. Hill and Palmer (16) also, noted that when oils or fats are fed to dairy cows the resulting butterfat assumes some of the characteristics of the fat or oil fed and that apart from deliberate oil feeding the chemical characteristics of the milk fat are more or less specific for the type of ration fed. In greater detail Hilditch, and Thompson (17) in 1936 studied the variations of specific acids of butterfat resulting from ingestion of various unsaturated oils by dairy cows. As their work entailed extensive and exacting chemical analysis they were unable to provide a picture of variations in butterfat composition day by day over a period but relied on taking composite sample from several cows following

one week periods of feeding oils of various degrees of unsaturation. Considering the fact that but nine cows were used and one sample from each taken it is doubtful if such exhaustive chemical analyses were really worth while, especially when it is realised that the individuality of the animals can affect comparative results to such a great extent. Nevertheless Hilditch and Thompson did show clearly that certain fatty acids of the diet fat were readily utilised in the production of milk fat indicating that a highly selective mechanism may be operating in milk fat synthesis. Earlier, Hilditch and Sleightholme (18) in 1930 had already shown by chemical analyses that certain added fats in the diet could influence the composition of bovine milk fat positively, but that other factors could influence fatty acid composition more profoundly than short-term changes in the nature of the diet. e.g. such effects as those brought about by general level of nutrition over longer term periods, and possibly those resulting from seasonal temperature variations.

(d) <u>The Identification of fat fractions of the Blood utilised by the mammary gland</u>.

The study of uptake of nutrients from the blood by the mammary gland was first instituted by various workers just prior to 1900.

To compare blood immediately entering and immediately leaving the mammary gland would necessitate access to the external pudic artery on the "input" side, so deep-seated as to be impracticable and to the subcutaneous abdominal veins which <u>are</u> readily accessible

Kaufmann and Magne (19) quoted by Blackwood and Stirling (22) in 1906 chose to compare the composition of the jugular blood with that of the subcutaneous abdominal venous blood in an endeavour to ascertain by difference what constituents were being utilised by the mammary gland during milk secretion.

They assumed all venous blood in non-lactating animals to be similar regardless of the sampling point. Later this method of comparing input with output was utilised and modified by many other workers.

With specific regard to fat metabolism in relation to lactation interest was aroused in determining just what fraction or fractions of blood lipids of dairy cows are utilised in the synthesis of milk fat. Bloor (20) classifies the constituents of blood lipids as follows:-

(a) Neutral Fat. Mixed triglycerides in small amounts.

(b) Phospholipids. Lecithin predominating in the plasma while cephalin and sphingomyelin constitute most of the phospholipid of the cerpuscles.

(c) Cholesterol. Occurring in amounts paralleling the phospholipids, as free cholesterol in the corpuscles and 60 - 70 per cent in ester combination with fatty acids in plasma.

(d) An "Unsaponifiable" fraction of little-known constituents, similar in amount to cholesterol, mostly complex sterols and sterol derivatives.

(e) Fatty acids in other combination than above plus some free fatty acids.

In 1919 Meigs, Blatherwick and Cary (21) followed the Kaufmann and Magne technique and using cows in lactation discovered that the phospholipid fraction was apparently depressed in blood leaving the mammary

gland and also that the inorganic phosphorus (presumably resulting from the breakdown of phospholipid and otherwise not required for milk secretion,) was apparently increased, and that the apparent decrease in phospholipid was sufficient to account for all the fat in the milk. They concluded from this that blood phospholipid was the sole source of milk fat. Other workers were unable to repeat this work. Blackwood and Stirling (22) quoted Schalk and Armadon (23) who had shown that the salivary glands of the ox can secrete more than sixty litres volume of saliva in twenty-four hours. Blackwood and Stirling (22) considered that this could possibly produce significant concentration of jugular blood. To check on this, using cattle, they compared the composition of jugular blood with radial artery blood and verified that the jugular blood actually was more concentrated that the arterial blood. They attributed this fact to the process of saliva secretion from which area of activity the jugular vein returns to the heart. Blackwood (24) them compared blood from the subcutaneous abdominal artery with blood from the radial artery and examined the variations in phosphorus content. The little difference they found between the bloods from the two sources proved to hold for non-lactating cows as well as lactating animals. They concluded from this experiment that phospholipid was <u>not</u> a precursor of milk fat. Sinclair (25) likewise arrived at a similar conclusion from the results of his experiments using rats.

Petersen, Palmer and Eckles (26) showed that in the dairy cow fat is one of the major constituents of the lactating mammary gland, averaging 40% of the dry matter. Further, they found that the gland fat, (as judged by the three constants, iodine number, Reichert-Wollny number, and the saponification number) is almost intermediate in nature between butterfat and body fat. Later, experiments by the same workers (27)

on perfusion of the surviving bovine mammary gland subsequent to excision, proved that perfusion tends to alter the nature of the gland fat as regards the lower fatty acids towards butterfat but does not alter the iodine number or saponification number. The inference from these experiments was that fat may be laid down first as glandular fat prior to being mobilised for milk fat synthesis. The presence of appreciable amounts of the lower fatty acids, more than could be accounted for in the calculated amount of milk retained, was also noted. This stimulated further investigation to be mentioned in due course (see Kelly and Petersen (12) later).

Maynard, Harrison and McCay (28) and also Blackwood (24) observed in cattle a rapid and proportionate rise in all blood lipoids following parturition, succeeded by a gradual fall as lactation advanced.

Schaible (29) in studying plasma lipids in lactating and non-lactating cows, concluded that lactation apparently requires neither a difference in distribution, or difference in nature of blood lipoids, but merely a greater supply of all. This worker compared the degree of saturation of the fatty acids of blood constituents, finding the fatty acid radicle of cholesterol esters to be more highly unsaturated than those of plasma lecithin, while fatty acid radicles of triglycerides of neutral fat were intermediate. This proved true for lactating and non-lactating animals. Schaible suggested that cholesterol esters might play a part in fat transport.

Maynard, McCay, Ellis, Hodson, and Davis (30) at Cornell in 1938 (in further experiments with dairy cows) improved on the older blood sampling techniques, by sampling ingoing blood to the mammary gland from

the internal pudic artery via the vagina. Results from this method proved far more consistent than with the older methods. No indication was found that either phospholipid fatty acids or those linked with cholesterol were removed by the lactating gland but total lipids proved to be consistently lower in the mammary venous samples. Calculated values for fatty acids present as neutral fat showed that the gland apparently does utilise this fraction, intimating that neutral blood fat possibly plus some free fatty acids may be the precursors of milk fat.

Any method of blood sampling is open to question from the aspect of the excitement caused the animal during actual sampling. Nervous and/ or hormonal responses to pain or fear may rapidly redirect metabolism in other than normal directions. In such cases it is feasible to expect that the blood, working on unusual "errands", may have other than normal composition, to a certain extent invalidating the results of blood analysis.

Shaw and Petersen (31) in 1940 confirmed Maynard's work (30) and added that in general the bovine mammary gland uses only neutral fat of the blood and possibly cholesterol fractions of the plasma. They calculated that the quantity of blood fat used by the gland is sufficient to account for all the fat secreted in milk, concluding that but little milk fat could be derived from other sources.

Voris, Ellis and Maynard (32) developed improved methods of estimating neutral fat by means of glycerol determinations. Their paper deals mainly with chemical technique but includes some arterio-venous comparisons of neutral fat in lactating and non-lactating cows by sampling blood from the sub-cutaneous abdominal veins and internal iliac arteries

via the rectal wall. As before the method may be criticised on the count of disturbance of the animal stimulating abnormal blood composition, and furthermore, excitement could feasibly inhibit the rate of milk secretion, which fact in turn could assist arterial and venous mammary blood to become more nearly alike. That excitement can cause similarity of arterial and venous mammary bloods had been shown by Graham, Kay and McIntosh (33) (quoted by Voris (32) in 1936.) Nevertheless Voris and co-workers were able to demonstrate an arterio-venous difference of about 3 mg. of neutral fat per 100 cc. of plasma in the non-lactating cow, and differences of the order of 19, 16, 12 mg. per 100 cc. of plasma in lactating cows indicating greater removal of neutral fat from the blood by the actively secreting mammary gland.

At first sight the foregoing findings might appear to indicate that in a simple manner neutral fat of blood may account, as a precursor, for fat secreted in milk; but milk fat on critical chemical examination is found to contain short-chain fatty acids C^{4-14} to an extent of 30% of the total fatty acids present, and could not have originated in a simple manner from the neutral fat of blood.

(d) The Problem of Accounting for the Short-chain Fatty Acids of Milk Fat.

Hilditch and Sleightholme (18) suggested that these short-chain acids may result from the breakdown of oleic triglycerides from blood neutral fat. They had observed in studying milk from cows fed with cod liver oil that the lower fatty acids were apparently depressed and that 5 - 7% of the highly unsaturated series C^{20-22} acids were present. This might be interpreted as additional evidence

of the operation of a highly selective mechanism in milk fat synthesis referred to previously. The authors proposed that the presence of the C^{20-22} acids, avid acceptors of hydogen and oxygen, may have inhibited the "normal" oxidation and reduction of oleic acid to lower fatty acids, which furnishes a feasible explanation of the presence of greater than normal amounts of oleic acid they noted in the samples. Kelly and Petersen (12) mentioned earlier in connection with stained fat feeding to dairy cows proceeded, after the stained fat experiment, to two further investigations. Extracting the fat from several mammary glands they estimated the fatty acid content and found markedly high values in the actively secreting gland as compared with dry glands and glands from cows at the end of lactation. In the third experiment they endeavoured to locate just where in the tissue of the mammary gland, the fatty acids predominated. Udder tissue sections were stained with various dyes believed specific for fatty acids and for neutral fat. The fatty acids consistently proved to be situated towards the basal portions of the alveolar secretory cells while the neutral fat, apparently in larger coallesced globules , was located distally. They deduced fatty acids to be present either molecularly or colloidally and that as their synthesis to neutral fat occurs there may be alterations in interfacial tension allowing the developing fat particles to coalesce so that on reaching the alveolar lumen surface they are of normal milk fat particle size. Shaw and Knodt (34) observed during arterio-venous blood comparisons in experiments with cattle that the lactating gland removes from circulation an amount of beta-hydroxybutyric acid sufficient to account for all the lower fatty acids of milk up to C^{14}, but seeing that this acid is so readily oxidised by the tissues the authors but tentatively suggested

that it <u>might</u> be a precursor of lower fatty acids.

If this latter suggestion were correct it would be logical to expect that if the supply of beta-hydroxybutyric acid to the mammary gland were increased, then an increase in the lower fatty acids of milk fat would result.

Shaw (35) noted that in cases of ketosis where the mammary gland took up twice as much beta-hydroxybutric acid, instead of the lower fatty acid content of the milk fat being increased, it was markedly lowered. This seemed to preclude the conception of beta-hydroxybutyric acid being the precursor of lower fatty acids, and indicated greater liklihood that it might be used in the gland for energy purposes.

Paralleling this work in principle Malpress (36) injected sodium butyrate into a goat and a cow, also fed large amounts of silage as a source of butyric acid but found that no increase of lower fatty acids of milk fat resulted,, additionally supporting the view that beta-hydroxybutyric acid is <u>not</u> the precursor of the lower fatty acids of milk fat.

Hilditch and Meara (37) investigated human milk fat and found few fatty acids below C^8, in contrast to cow milk fat. This suggests that the ruminant form of digestion for the cow secreting a greater proportion of the lower fatty acids. Barcroft,McAnally and Phillipson (38) noted a peculiarity of ruminant digestion in that following breakdown of carbohydrate material in the rumen by microfloral action, the resulting short-chain fatty acids may be directly absorbed via the rumen wall and that these acids may circulate in the blood.

In view of Malpress' negative results (36) of sodium butyrate and silage feeding experiments it still seemed improbable that these observed acids could be the precursors of the lower fatty acids of milk fat. So the inclination was to return to Hilditch's hypothesis of the breakdown of oleoglycerides providing the precursors of the lower fatty acids.

Further support was lent to the latter theory by the results of Smith and Dastur's work (39) which demonstrated the effects of inanition on the composition of milk fat. When cows were fasted for periods of twelve days, up to an 80% decrease occurred in the lower fatty acids (up to C^{14}) of the secreted milk fat, compensated by a corresponding increase in the oleic acid fraction. This had actually been noted previously by several other workers, notably Eckles and Palmer (40) in 1916.

Concluding this Section 1 "The Relation of Diet Fat to Milk Fat" tentative to other considerations following, the following ideas have been derived:-

A. The bulk of the milk fat may be derived from neutral fat of blood.

B. The lower fatty acid fractions of milk fat may result from the breakdown in the mammary gland of oleic triglycerides. (later shown to be improbable.)

II. THE RELATION OF NON-LIPID "PRECURSORS" TO MILK FAT.

Despite so much evidence favouring the theory of blood neutral fat being the precursor of milk fat as discussed in section 1 there have been conflicting indications of synthesis of a proportion ofthe milk fat from other sources.

(a)The Respiratory Quotient.

In 1938 Graham, Houchin, Petersen, and Turner (41) showed that the respiratory quotient of the lactating ruminant mammary gland was greater than unity which would appear to indicate synthesis of fat from carbohydrate.

(Respiratory Quotient- $\frac{\text{Volume of Carbon dioxide produced}}{\text{Volume of oxygen absorbed.}}$)

Synthesis of the relatively oxygen-poor fats from the oxygen rich carbohydrates involves liberation of carbon dioxide, resulting in respiratory quotients of unity or temporarily greater than unity in some cases.

Utilisation of fats on the other hand involves more oxygen consumed than represented by the carbon dioxide given off and results in respiratory quotients of less than unity.)

Reineke, Stonecipher, and Turner (42) also showed respiratory quotients greater than unity in the mammary glands of anaesthetised goats. On fasting, the R.Q. declined below unity after the third day, suggesting breakdown of fat for milk fat synthesis on fasting, and conversely that carbohydrate material may have been utilised for milk fat synthesis prior to fasting i.e. during normal lactation.

These observations when linked with Smith's and Dastur's (39) finding that in fasting animals the

lower fatty acids of milk fat greatly decrease, lends support to the idea that the lower fatty acids (up to C^{14}) may be products of synthesis from carbohydrate, an idea conflicting with Hilditch's theory of lower fatty acids being degradation products of oleic triglycerides. Smith and Dastur (39) offered yet another theory opposing Hilditch's, that the lower fatty acids of milk fat might be the intermediate stages or by-products of oleic acid synthesis, and that during inanition when the gland is producing less than the normal amount of fat more oleic acid synthesis is allowed to proceed to completion with consequent reduction of the amount of lower fatty acid "by-products".

This latter theory would be borne out by the R.Q. remaining above unity while readily available carbohydrates reserves last, passing below unity as these reserves are used up and body fat reserves become more heavily drawn upon, if the latter actually does occur.

If reserve body fat is to be drawn upon for milk fat synthesis during fasting (i.e.:- conceivably a condition where a greater-than-normal proportion of lipid material is being offered the mammary gland, by virtue of the liklihood of lipid reserves being the most readily available in any appreciable quantity), and one accepts Hilditch's hypothesis of oleic glyceride degredation providing the lower fatty acids of milk fat, then one might reasonably expect these lower fatty acids to increase during fasting. But such is not the case. Both during fasting, and while the animal is on fat rich diets (Smith and Dastur (39) the lower fatty acid fraction of milk fat remains below normal while the oleic acid fraction remains above normal. These facts would not easily be reconciled with Hilditch's theory. Furthermore "diets rich in

in carbohydrates and poor in fat cause a increase in the lower fatty acids of milk fat, while those poor in carbohydrates and rich in fat have the reverse effect, indicating that with diets on which fat synthesis would most probably be necessary the lower fatty acids increase ". (Smith and Dastur (39).

(b) Experiments on Carbohydrate Feeding.

Kaufmann and Shaw (43) had shown in experiments with dairy cows by means of simplified diets that carbohydrate-rich diets increase the lower fatty acids of milk fat and at the same time depress the iodine value. To determine whether synthesis of lower fatty acids from carbohydrate was direct or not insulin was injected into fasting cows to induce a condition of hypoglycaemia for about thirty hours. If the transformation of carbohydrate into lower fatty acids were a direct process then on removal of available carbohydrate from the blood one would expect the proportion of lower fatty acids of the milk fat to drop correspondingly. But no change in rate of decline from that proceeding while on fasting alone (without insulin) was noted. The authors concluded from this that blood glucose was not the precursor of the lower fatty acids of milk fat. Shaw and Knodt (44) had earlier put forward evidence conflicting with Kaufmann's and Shaw's simplified diet story for in feeding pure carbohydrate in the form of dextrose to dairy cows they found that the lower fatty acids of the secreted milk fat declined and the mammary R.Q. also declined below unity thus paralleling the recognised consequence of inanition as shown by Reineke's work (42). They showed also that in ketosis, blood sugar and lactic acid decrease to less than 50% of the normal values while the mammary R.Q. remains greater than unity indicating the improbability of the mammary gland synthesising milk fat from blood carbohydrate in a direct manner. Kaufmann and Shaw (45) again demonstrated that in ruminants carbohydrate-rich diets stimulated extra secretion of the lower fatty acids and proposed that the mechanism was an indirect one since from their previous experiments they

were convinced that blood carbohydrate was not responsible. They suggested that the carbohydrate of the diet might be converted into the necessary precursors by rumen microflora. This supposition was proven possible by Barcroft and co-workers (38) as has been seen in Section 1. Further Kaufmann and Shaw (45) pointed out the liklihood that the precursors of the lower fatty acids of ruminant milk fat in normal cases may be in demand by many tissues other than the mammary gland and that the presence of carbohydrate in the diet may in some way exert a sparing action on the utilisation by other body tissues of substances which act as precursors of the lower fatty acids of milk fat. Shaw (46), Knodt (47), and Shaw and Powell (48) had all done extensive work on glucose therapy in ketosis without providing any very great advances towards a logical theory of lower fatty acid synthesis. They consistently observed the increase in lower fatty acids of milk fat consequent upon carbohydrate administration, as well as demonstrating the uptake by the mammary gland of beta-hydroxybutyric acid but not of aceto-acetic acid. Mann and Shaw (49) in 1946 evolved a method for the continuous intravenous injection of food materials into cows by drip feed via the subcutaneous abdominal vein. They tested out a number of compounds in an endeavour to find a substance which would arrest the decline in the lower fatty acids of milk fat normally resulting from inanition. Glucose, protein hydrolysate, sodium oleate, and sodium acetate each proved ineffective while sodium butyrate yielded varying results. The fact that sodium oleate failed to prevent the fallin lower fatty acids of milk fat would seem to refute Hilditch's theory of oleic acid breakdown being responsible for the lower fatty acids. Similarly sodium acetate being ineffective would seem at first sight to preclude blood acetate being utilised for synthesis. That glucose failed seemed to corroborate the work of Kaufmann and Shaw (45) which had already indicated that glucose was not the direct precursor. That the residues from deamination of amino acids, (as exemplified by protein hydrolysate) might

provide precursors also appeared to be precluded. The glucose uptake of the mammary gland was found sufficient to account for all the lactose of the milk, but not for all the lactose plus the short-chain fatty acids of butterfat.

The foregoing experiments on carbohydrate feeding all tended to indicate that carbohydrate of the blood is not utilised directly by the mammary gland for synthesis of the lower fatty acids of milk fat in spite of the well recognised but still unexplained high R.Q.

The old idea of certain amounts of carbohydrate being required for a certain amount of fat oxidation was discredited by Stadie (50) who found diabetics to oxidise ketones or fats without simultaneous oxidation of carbohydrates. Stadie in his review quotes several other workers who have confirmed this. Stadie postulated that up to a certain level all fat catabolised may be completely oxidised, and no ketonurea results; beyond this level all fat catabolised may not be completely oxidised, and consequently part of the fat catabolised may be excreted "unburned" in the form of ketone bodies. He suggested that the function of carbohydrate may be simply to spare fat oxidation, which would account for the therapeutic value of carbohydrate administration.

(c) The use of 'Labelled' Fats.

The original theory of successive β-oxidation of fats proposed by Knoop and not accepted in explaining the entire catabolism of fatty acids in Stadie's review (50) stimulated interest in the fate of the 2- carbon degredation products of fat catabolism. Buchanan, Hastings and Nesbit (51) using radioactive C^{11} synthesised acetic, propionic, and butyric acids with the C^{11} as the carboxyl carbon atom. The sodium salts of these acids were fed along with glucose to fasted white rats. Results demonstrated exceptionally rapid utilisation of the short-chain acids fed in that 50% of the radioactive fatty acid absorbed was excreted in the respiratory gases as carbon dioxide over a two-hour period.

Using deuterium Stetten and Schoenheimer (52) synthesised palmitic acid and on feeding the ethyl ester of this in very small quantities (.56% of the diet) found that the bulk of the test material was absorbed, 44% of the deuterium fed as palmitic acid apparently having been deposited in the tissue fats. Some of the fed palmitic acid had been deposited directly in the tissues; some degraded to shorter-chain fatty acids; some desaturated to palmitoleic acid; and some converted into stearic acid by an addition of two carbon atoms to the chain. These observations together with those resulting from other work by Schoenheimer and Rittenberg (53) (54) showed strikingly the interchangability of fatty acids in the tissues.

The shortening and elongation of fatty acid chains, and the saturation and desaturation of fatty acids have been ably demonstrated. Further, the studies with the aid of isotopes have established the fact that the various reactions mentioned above occur continuously even when the total amounts and properties of the body fats do not change.

Rittenberg and Bloch (55) were able to synthesise acetic acid incorporating both C^{13} and deuterium; the former in the carboxyl group, the latter in the methyl group. The sodium salt was fed to rats and mice on a low-fat, high-carbohydrate diet. Fatty acids and cholesterol were separately isolated from the carcases. Both lipids contained both isotopes, strongly suggesting that both carbon atoms of the acetic acid had been utilised in fat formation. There was further evidence that the labelled carbon atoms were distributed at alternate positions along the fatty acid chains.

These results furnish a very good indication of the ability of animals to synthesise fatty acids from acetate "units" if these be supplied.

That appreciable quantities of acetate could normally be present in the blood was not compatible with the then-current beta oxidation-condensation theory of ketone bodies being formed in the liver by degredation of whole fatty acid molecules with little accumulation of free 2-carbon groups

The above research with isotopes was carried out by medical workers and the application of their observations to the study of ruminant fat metabolism does not appear to have been immediately recognised in spite of the established fact (Barcroft and co-workers (38) that in the ruminant short-chain fatty acids are absorbed direct from the rumen and do circulate in the blood.

As we have seen earlier in this section all endeavour to demonstrate synthesis of milk fat from blood carbohydrates within the mammary gland had failed and the well recognised high R.Q. of the lactating gland remained unexplained.

In 1946 Folley and Mapress (56) had conducted an experiment similar to that of Mann and Shaw (49). Sodium acetate was intravenously infused into lactating goats during periods of inanition. The observations were the same as Mann and Shaw's, that the administered acetate failed to arrest the decline of short-chain fatty acids of milk fat. They proposed that this result was possibly due to the avid utilisation of the administered acetate by the fasting tissues overwhelming the demand of the mammary gland to use the acetate for milk fat secretion.

(d) Surviving Tissue Studies.

Recent work with isotopes has re-emphasised the facility with which tissues may utilise short-chain acids in the synthesis of longer-chain fatty acids. Employing surviving artery tissue from rats Chernick, Srere, and Chaikoff (57) studied the synthesis of lipid material from acetic acid in which both carbon atoms were represented by C^{14}, as well as following the utilisation of inorganic phosphate labelled with P^{32}. Ready ability to convert acetate into longer chain fatty acids was demonstrated as well as the incorporation of P^{32} into the phospholipid molecule.

The work of Folley and French (58) using lactating mammary gland slice technique throws new light on the mammary gland R.Q. problem. Lactating mammary gland slices

from non- ruminants (rat, mouse, rabbit, guinea pig) in the presence of carbohydrate (glucose) all showed a high R.Q. indicating the possible formation of fat from carbohydrate. But when the same experiment was conducted on ruminant (cow, goat) mammary gland slices, an R.Q. well below unity was demonstrated. The experiment repeated with acetate as substrate in place of glucose showed the R.Q's reversed, i.e. the non-ruminant slices showing a low R.Q. and ruminant slices a high R.Q.

	Glucose R.Q.	Sodium Acetate R.Q.
Rats	1.66	.75
Goats	.85	1.20

In view of the knowledge that acetic, propionic and butyric acids in appreciable quantities are produced in the rumen from microfloral activity upon ingested carbohydrate, and that these acids are readily absorbed direct from the rumen into the bloodstream, Folley and French's work strongly indicated the possibility that the ruminant mammary gland may utilise such acids of the blood in the synthesis of milk fat.

A fresh problem is revealed on comparing Folley and French's results with those of Popjak and Beeckmans (59). The latter in studying the utilisation of acetate by the foetus for fatty acid and cholesterol synthesis employed the isotopes deuterium and ^{14}C. Deuterium ingested as heavy water, and ^{14}C as sodium acetate parenterally administered to pregnant rabbits showed clearly a higher rate of synthesis of fatty acids and cholesterol in the foetal than the maternal tissues with the sole exception of the mammary gland where the ^{14}C content of the neutral fats indicated a much higher rate of acetate utilisation for fatty acid synthesis than in the foetus or anywhere else. It is noteworthy that the ^{14}C was found to be particularly concentrated in the lower (volatile) fatty acid fraction (60). This indication of ready utilisation of acetate for milk fat formation in the rabbit conflicts with Folley

and French's (60) observations on the utilisation of acetate by lactating rabbit mammary gland slices. As before (58) slices from rat, mouse, rabbit, and guinea pig mammary glands proved almost inert towards utilisation of acetate, but with experiments on rabbit mammary gland slices in particular, addition of glucose markedly raised the previously negligibile acetate uptake. Folley and French suggest that the action of glucose may be to provide glycerol for glyceride synthesis.

In Popjak and Beechman's note (59) the main substance of the diet of the experimental animals is not mentioned but it is conceivable that it would be a highly-carbohydrate one (as would be normal) and that this may have induced a condition more or less paralleling Folley and French's gland slice technique using acetate when glucose was supplied in addition.

However the fact that the in vivo isotope experiments demonstrated ready utilisation of acetate by the lactating rabbit mammary gland, and the in vitro gland slice technique shows the reverse would appear to throw some doubt on one or the other of the techniques. The differences in metabolism between surviving tissue slices and tissue in the living animal constitute an unknown factor; while on the other hand the introduction of even minute amounts of radioactive material into the living animal body may feasibly induce other than normal metabolism to a certain degree.

Nevertheless these recent experiments provide strong indications that short-chain fatty acids resulting from breakdown of carbohydrate material in the rumen may be readily utilised by the mammary gland in the synthesis of milk fat. It is perhaps fitting at this point to recall Smith and Dastur's, (39) work mentioned earlier, in 1938, that the lower fatty acids of ruminant milk fat might be the by-products of oleic acid synthesis, which in the light of these latest experiments may be very nearly correct.

The work of Folley and French, Popjak's observation

of rapid synthesis of neutral fat fatty acids in the mammary gland from acetate, and his noting in using labelled acetate that the highest concentration of ^{14}C occurred in the lower fatty acid fraction of the mammary gland fat largely influence the conclusions to be drawn from the literature reviewed in this section "Relation of Non-Lipid Precursors to Milk Fat" and modify the conclusions of Section 1.

A. Neutral fat of the blood is very possibly the precursor of the bulk of the milk fat.

B. Short-chain fatty acids resulting from breakdown of carbohydrate in the rumen and absorbed direct into the bloodstream, in all probability, are the precursors not only of the lower fatty acids of milk fat C_{4-14}, but also of some of the higher members of the fatty acids of milk fat.

III THE RELATION OF DEPOT FAT TO MILK FAT.

That, in the ruminant, body fat reserves may be drawn upon to participate in the synthesis of milk is tacitly assumed, but as yet the accuracy of this assumption has not been verified.

Familiar indications that depot fats may be mobilised for general utilisation by the tissues are afforded by the obvious depletion of body fats in starved animals, and by the loss of condition of high-yielding dairy cows during lactation.

The work of Gage and Fish (2) (mentioned in Section II with graphs illustrating) demonstrated by direct chylomicron count of a fasting human subject that as special demands arise so neutral fat appears to be mobilised into the blood, acting with a kind of "over-run" effect or "physiological inertia" as the authors described it. Patterson (61) using a more refined technique than that of Gage and Fish verified the latters' observations of blood fat increase in a fasting human subject consequent to severe exercise, and added that blood cholesterol content appeared unaffected by the exercises imposed and thus possibly not closely related to other blood-fat constituent variations.

Baldwin (1) believes however that when fat is being withdrawn from the depots no condition of lipaemia is evidenced, but he makes no mention of special situations obtaining when unusually heavy demands for fat are to be met.

Bloor (20) states such blood-fat increments to be irregular of appearance, sometimes being observable, sometimes not, but that a possible explanation is that the amount of fat being transported is often so small in relation to the volume of the blood and the period of time over which fat may be in process of withdrawal from the depots, that the methods used to measure the increments may be too crude. Further he suggests that where an obvious increase is noted, its magnitude may be dependent on the nature of the stored fat, and "easily

movable" fat being more responsive to a stimulus such as that of starvation, so that larger amounts of lipid are delivered into the blood than can be utilised immediately, resulting in observable accumulation.

Adding weight to the ideas favouring mobilisation of neutral fat, Hilditch and Pedelty (62) in their work on the composition of pig depot fats during starvation emphasise that the problem of mobilisation of fat reserves may only be considered adequately in terms of mixed triglycerides, in that where a specific fatty acid is required from the depot fats its withdrawal involves the simultaneous removal of the other two companion fatty acod molecules of the particular triglyceride.

Mendel and Daniels (11) and Gage and Fish (2) demonstrated clearly that in simple-stomached animals, eg. rat, cat, the feeding of fat stained with a fat-soluble dye induced deposition of coloured fat in the tissues, also that if stained fats were fed while the animals were in lactation then the milk fat secreted was also readily stained.

Feeding of stained fats during gestation induced coloured body fats as before. At parturition these rats placed on normal non-stained diet secreted milk of normal appearance, whereas if starvation were imposed milk containing stained fat was promptly secreted; strongly indicating that body fats may by utilised for milk fat secretion. Gage and Fish point out that although stained body and milk fats may be evidenced as as resulting from ingestion of stained fats fed, these results do not imply that food fat is the only source of milk or body fat or that it has been taken without modification from the food fat.

Mendel and Daniels (11) using a goat showed that 1 gm. of a fat-soluble dye (Sudan 111) in fat of the feed could induce coloured milk fat secretion within 9 hours. On cessation of dye feeding coloured milk fat was no longer secreted although the stain persisted in the adipose tissues. This, in simple manner indicates two of the possible destinations of

ingested fats, and additionally suggests that if body fat is utilised at all for milk fat synthesis at a normal level of nutrition the amounts involved may be insufficient for identification by stained fat techniques: at least it would appear unlikely for milk fat, at normal feeding levels ,to be elaborated wholly from depot fats.

As mentioned in Section III 1 (b), all the earlier experiments of feeding fat soluble dyes to dairy cows failed to demonstrate either coloured fat deposition, or secretion in milk. Huffman and Duncan's experiments (13) successfully demonstrating the secretion of stained milk fat in the dairy cow consequent upon ingestion of stained fat were possibly facilitated by the stage of lactation of the experimental cows and by the heavy concentration of dye used. The cows employed in the experiment were apparently near the end of lactation, producing but 0.5lb of butterfat per day. The dyes were fed in 0.5lb of soybean oil. The oil content of the normal dairy ration at the time is not stated in their brief report but it would appear possible, in the absence of more detailed information, that in effect the experimental animals may have been ingesting a normal amount of digestible oil from the feed <u>plus</u> the 0.5lb of stained oil. The situation obtaining may have been extremely favourable towards spectacular results in comparison with those of experiments of earlier workers who used smaller quantities of dye and who may have employed cows at an earlier stage of lactation.

Gage and Fish (2) suggested that where an amimal is "deluged" with fat following starvation, subjected to "forced" fattening, or fed abnormal amounts of unusual fats, it may not be able to individualise the ingested fat under such adverse conditions as completely as normally, prior to depositon in the tissues or to secretion in milk.

The experiments of Gage and Fish (2) amd Mendel and Daniels (11) while not conducted with ruminants do suggest ready mobilisation of depot fats for purposes of general

tissue metabolism and possibly for milk fat secretion. It seems possible that to a certain extent fasting metabolism of depot fats may amount to an exaggeration of the withdrawal aspect of normal metabolism to a degree sufficient to be differentiated.

In order to avoid repetition evidence more directly pertinent to possible ruminant mobilisation of depot fats for milk fat synthesis is withheld for more convenient consideration in the following Section III No.4 "The inter-relationship of milk fat precursors".

IV. THE INTER-RELATIONSHIP OF MILK FAT "PRECURSORS".

(A) Under grassland farming systems it becomes apparent from considering grass composition that the amount of digestible oil ingested daily by a cow is quite insufficient to account for the total amount of milk fat secreted even if it were a directly quantitative conversion. Further, it is not yet possible to estimate to what extent the short-chain acids, proven to be available in the bloodstream and resulting from breakdown of carbohydrate material in the rumen, may compensate for the deficit.

A lactating Jersey cow may ingest little more than 0.7lb of digestible oil daily and yet at the same time secrete up to 2lbs of butterfat.

More than half the butterfat produced appears to be derived from sources other than that of fat in the ration. Moreover, by Frazer's hypothesis of fat absorption (4) the ingested fat fractions may be differentially absorbed so that in actual fact theremay be far less than 0.7lb of neutral fat of the blood originating from food fat ingested, although, less directly, the fatty acid fractions of the ingested fat may become available via the liver. It may also be expected that the mammary gland will be in competition with other fixed tissues for the utilisation of blood fat: so that, in effect, considerably more than half the butterfat produced may be :-

(a) of non- lipid origin.

(b) of lipid origin within the body.

(a) Possible non-lipid sources would be carbohydrate or protein of which the former predominates in ruminant food and which as has already been described, may give rise, on its breakdown within the rumen, to short-chain fatty acids absorbable direct via the rumen wall into the bloodstream.(38) These acids are mainly acetic and propionic with some butyric

(To face P. 41)

Jarl (85) has shown in controlled experiments that the addition of a protein supplement to rations of dairy cows may induce a lowering in degree of unsaturation of milk fat. This effect is similar to that resulting from the feeding of a higher carbohydrate-content ration but apparently is not as marked.

Sjollema (94) has reported that the feeding of grass of high protein content to dairy cows results in the secretion of milk fat of markedly higher degree of unsaturation than when grass of lower protein content is fed. Frens (95), also, mentions that under conditions experienced in Holland the feeding of young grass to dairy cows frequently increases the degree of unsaturation of milk fat to an extent sufficient to cause difficulties in butter manufacture.

Jarl (96) demonstrated that the above problems resulting from the grazing of protein-rich pastures may be countered by the feeding of supplements of high carbohydrate content.

From the above it appears that although protein to a certain extent, and/ or under certain conditions may provide some milk fat precursors, possibly via an intermediate carbohydrate stage as suggested by Bloor (20), the process is by no means as direct as from carbohydrate itself, as evidenced particularly by the effects resulting from the supplementing of protein-rich diets with feeds of high carbohydrate content.

(To face P. 41)

and small amounts of other acids. Acetic and butyric acids are both recognised "fat-formers" while propionic may become transformed into glycogen. Baldwin (1) considers it very probable that the main reserves of fat and carbohydrate in herbivorous animals are built up from short-chain fatty acids.

It appears, according to Bloor (20), that in simple-stomached animals protein may, when fed in excess, be formed into depot fat with carbohydrate as an intermediate stage so that the resulting fat appears to be identical with that formed direct from ingested carbohydrate. (See P. 40a opposite.)

In view of the fact that the ruminant diet normally contains abundant carbohydrate greatly in excess of protein, it seems unlikely that in the ruminant, protein would be a major precursor of depot or milk fat.

(b) The possibility, also, of lipid origin of milk fat within the body is good in view of Stetten and Schoenheimer's work (52) evidencing the continual interchange of fatty acids, saturation, de-saturation, degradation, and synthesis of fats. Hilditch and Pedelty (62) state "The adipose tissues are now clearly recognised as dynamic, rather than static, reservoirs."

It seems quite feasible to expect that depot lipids, being apparently so readily interchangeable, would also be easily available to demanding tissues such as those of the mammary gland.

(b) Lawes and Gilbert (63) in 1859 had been able to show that carbohydrate in animal foods could be responsible for depot fat formation while later, in 1897, Jordon and Jenter (64) demonstrated that on a virtually fat-free ration dairy cows could gain in weight and secrete milk of apparently normal composition. A cow which yielded 62.9lbs of milk fat over a period of 95 days digested during this time only 5.7lbs of oil from the ration (which was otherwise normal) and gained 47lbs in weight, i.e. 57.2lbs of the milk fat secreted obviously had arisen from a source other than that of food

fat. From this experiment and others on protein intake and excretion the authors concluded that milk fat could be produced in part at least from carbohydrate ingested. Further, they concluded, perhaps rather hastily, that as the animal in the first experiment had increased in weight while lactating, milk fat could not have been derived from previously stored body fat.

(c) Hilditch and Longenecker (65) in analysing ox depot fats found the major component acids to be of relatively constant proportions.

Oleic Acid	38,0 - 40.0%	of total fatty acids.
Palmitic Acid	26.0 - 31.0%	" " " "
Stearic Acid	20.0 - 25.0%	" " " "

(d) Although it has been seen that relatively small amounts of lipid material are normally ingested by grass fed animals, it proves surprising to find existing such a dearth of literature on the subject of grass lipid chemistry. The research on vegetable fats, in the past, has been almost entirely devoted to the chemistry of seed fats of industrial rather than agricultural application. Smith and Chibnall (66) point out that leaf glycerides differ from seed glycerides in that the former are integral components of the protoplasm of physiologically active cells, as compared with the latter which are regarded as possibly more static components of reserve material. The fats present in grasses are known to have a high proportion of unsaturated acids and the presence of these in such relatively large amounts greatly complicates chemical analysis.

Smith and Chibnall (66) examined the glyceride fatty acids of cocksfoot and ryegrass (perennial) and of the total fatty acids separated could not avoid an initial loss of 17 % (later proven to be mainly of unsaturated acids) in endeavouring to separate the saturated group of acids from the unsaturated. They employed various methods of analysis, and pointing out possible inaccuracies resulting from

technique gave the approximate major fatty acid composition of glycerides of the two grasses as :-

Saturated Acids	15%
Oleic Acid	0%
Linoleic and Linolenic acids over	60%
plus the initial loss of	17%

Older methods of analysis whose greater inaccuracies were pointed out by Smith and Chibnall had indicated the presence of up to 17% of oleic acid. Repeated analysis by the latter workers failed to show the presence of any oleic acid.

Hilditch and Jasperson (74) using different technique found values for the saturated acids fraction of mixed pasture grass fats which they realised were subnormal but confirmed Smith and Chibnall's view that linolenic acid and closely allied acids are the principal components of grass fat glycerides. Contary however to Smith and Chibnall they believe the linoleic acid series to be in the minority of the unsaturated acids, and oleic acid to be present in relatively appreciable amounts.

Shorland (71) separated the lipids from samples of mixed pasture and, as regards the proportions of total lipids and glyceride fatty acids, results paralleled those of Smith and Chibnall (66) for cocksfoot and ryegrass. Rewald (72) using a triple-solvent method of extraction showed a rather higher figure for total lipids. The above workers' figures together with Woodman's (73) are shown below:-

	Grass Lipids (Percentage Dry Matter) Sample	Total Lipids.	Glyceride Fatty Acids.
Smith and Chibnall	Cocksfoot and ryegrass	5.8	2.2
Shorland	mixed pasture	5.6	2.5
Rewald	mixed pasture	7.0	-
Woodman	mixed pasture	3.0-6.5	-

Smith and Chibnall appear to be among the few chemists who have conducted lipid analysis on pure species and even in their work the starting point is indefinite regarding such factors as stage of growth, height of cutting, season etc. This applies similarly to the other work reported while in addition the term "mixed pasture" even when qualified by percentage composition as in Shorland's work (90) remains somewhat indefinite.

Although the above workers' results agree in certain aspects it is plain that the chemistry of grass lipids remains at present but little known.

The fact that glycerides of depot fats of ruminants have been shown to contain principally oleic, stearic, and palmitic acids; coupled with the knowledge that the already very minor amounts of grass lipid material ingested by cattle contain barely 15% of the saturated acids stearic and palmitic, and contain possibly no oleic acid (controversial) provides additional emphasis on the concept that ruminant depot fats are the products of biosynthesis from non-lipid materials.

(e) As an adjunct to the immediately foregoing considerations it is of interest to note the effects of greatly increased amounts of oil in the ration on the depot fat of ruminants.

Edwards and Holley (67) using cattle, sheep and pigs added corn oil to the rations fed. The oleic and linoleic acids of corn oil are practically the only unsaturated fatty acids present in quantity and are responsible for the corn oil having a high iodine value of 115 to 120. It was presumed that if this oil in the food were to be utilised in a simple manner and deposited as depot fat then its presence should be revealed by an increase in the iodine value of the animal depot fat. Their results are briefly summarised below:-

	Feeding Period (days)	Ration	Iodine Value of Depot Fat.	Body Fat increase in trial
Cattle	56	Normal oil	34.06 35.40	150%
Sheep	70	Normal Oil	39.06 42.74	160%
Swine	70	Normal Oil	56.72 75.67	400%

From the above it may be seen that the cattle and sheep apparently had failed to deposit the ingested fat as such although the linoleic glyceride content of the sheep depot fat did show a slight increase. In the case of the swine however a marked assimilation of ingested oil is indicated. On analysis this increase also was found to be in the linoleic glyceride fraction of the depot fat.

The increases had to be assessed from comparison with body fats of control animals on "normal" rations and it may be expected that "individuality" would influence the values quoted, for the trial was conducted on but few animals. Longer-term trials had been conducted earlier by Thomas, Culbertson and Beard (68) who used various oils added to the ration of fattening steers for periods up to 260 days. Essentially similar results to those of Edwards and Holley were achieved.

Although the apparently minor role of food fat in ruminants may be appreciated from earlier considerations these latter experiments provide still further evidence that body fat of ruminants may be largely the product of synthesis from substances other than food fats.

(f) Hilditch and Pedelty (62) in discussing the analysis of pig depot fats during starvation emphasise the markedly preferential manner in which oleic acid is "mobilised", (not losing sight of the essentially triglyceride nature of depot fat mobilisation.) It appeared to the authors from the results of previous investigations that minor unsaturated

acids of the depot fats, presumably originating in the food, were less readily mobilised than those acids synthesised from non-lipid sources in the food. Oleic acid appeared to be readily mobilised but the structurally closely related linoleic acid over the same period was withdrawn from the depot fats to less extent than any other acid. Hilditch and Pedelty suggested that in the pig depot fats, acids derived by assimilation from dietary fats may be less readily mobilised than those which are synthesised by the animal from carbohydrate. These analyses, although not repeated specifically with cattle, showing ready mobilisation of oleic acid from depot fat in the pig during starvation, indicate possible applicability to ruminant studies. In the cow, as has been shown, oleic acid is the major component acid of the depot fat glycerides. Further it is apparent that it is synthesised by the animal and not an ingested fat. If the behaviour of oleic acid in the depot fats of the cow is similar to its behaviour in the depot fats of the pig then it is probable that oleic acid may be the "first call " fatty acid available on demand by various tissues.

(g) The effect of abnormal amounts of oleic acid in the ration of dairy cows on the milk fat secreted was shown by Hilditch and Jasperson (69). The feeding of groundnut cake with oleic acid representing 55% of the total fatty acids to lactating dairy cows led to an increase in the oleic acid content of the milk fat and to a fall in the lower fatty acids.

(h) A similar effect was noted by Eckles and Palmer (40) in 1916, by Smith and Dastur (39), and verified more recently in 1941 by Riddet, Campbell, McDowall, and Cox (70) on subjecting dairy cows to reduced planes of nutrition. Smith and Dastur (39) imposed 12-day periods of starvation on lactating dairy cows and noted a decrease of about 80% of the original content of the lower fatty acids of milk fat up to and including C^{14}, conpensated almost quantitatively by a simultaneous increase in oleic acid content.

In sequence of the arbitrary subdivisions of this review the foregoing presentations of evidence, in summary, have shown:-

1. That the tissues of the ruminant mammary gland utilise neutral fat of the blood during milk secretion.
2. That, in the ruminant short-chain fatty acids result from the microfloral degredation of ingested carbohydrate material in the rumen; are absorbed direct from the rumen into the systemic blood; and possibly may be utilised in the synthesis of milk fat with particular but not limited regard to the lower fatty acids fraction C^{4-14}.
3. That, in simple stomached animals, mobilisation of depot fat into the bloodstream as neutral fat occurs during starvation; also, by stained fat techniques, that mobilisation of depot fats for milk fat synthesis is a possibility.

4.(A&B) That, in the ruminant, food fat may be of minor importance in milk fat synthesis, and that depot fats and carbohydrate fractions of food are other likely sources of milk fat precursors; and also that body lipids may undergo continual interchange and alteration.

(C) That oleic is the major component acid of ruminant depot glycerides.

(D) That, in grass lipids, (the chemistry of which is but little known) oleic acid is believed to be virtually absent by most workers.

(E) That ruminants fail to deposit recognisable fractions of unaccustomed ingested oils as such, the depot fats remaining of apparently normal composition.

(F) That, in the pig, triglycerides incorporating oleic acid are mobilised more readily than those incorporating other acids.

(G) That an increase in the oleic acid content of the food of the lactating ruminant stimulates an increase in the oleic acid content of the milk fat secreted, and

a decrease in the lower fatty acid content.

(H) That subjection of a lactating ruminant to a subnormal plane of nutrition has essentially similar effects to those evidenced on feeding a ration containing glycerides of high oleic acid content. viz:- a decrease in the lower fatty acids fraction of the secreted milk fat compensated by an approximately corresponding increase in oleic acid content.

It is here suggested, in view of the evidence presented regarding possible sources of lower fatty acids and higher molecular weight glycerides of ruminant milk fat, that in the ruminant at normal levels of nutrition <u>neutral fat and lipoids</u> of the blood (originating from ingested fats, from synthesis from carbohydrate, and from withdrawal of readily mobilised components of depot fat) and <u>short-chain fatty acids</u> (resulting from microfloral breakdown of carbohydrate fractions of ingesta in the rumen)

may be "competitors" for milk fat synthesis: and that a balance is arrived at, resulting in about 30% of the total fatty acids of milk fat being comprised of the lower members C4-14. It is further suggested that this does not imply that the short-chain fatty acids from the rumen are the sole precursors of the lower fatty acids of milk fat, or that they do not participate in the synthesis of some of the higher members; but rather that the short-chain fatty acids from the rumen may be the precursors of the majority of the lower milk fat fatty acids, and also of some of the higher members.

When the apparently readily utilisable oleic acid is supplied to the mammary gland in abnormal amounts it may exert a depressing effect on the efficiency of the lower fatty acid precursors by virtue of being a superior "competitor" as exemplified by Hilditch's high oleic acid feeding experiment (in division G above).

The depression of the lower fatty acid content of milk fat on subjecting a ruminant to a reduced plane of nutrition or to starvation may be explainable from the point of view that during inanition the supply of short-chain acids, resulting from breakdown of carbohydrate in the rumen, would be reduced to an extent probably in some measure proportional to the degree of inanition.

The latter however does not account for the simultaneous increment of the oleic acid fraction of the milk fat fatty acids during inanition as noted by Smith and Dastur (39).

It has been shown that ruminant depot fat contains a high proportion of oleo-glycerides, and that in a non-ruminant, the pig, (similar information on ruminants being lacking) oleic acid is the most readily mobilised of the depot glyceride fatty acids.

If ruminant depot fat is able to be mobilised in the preferential manner demonstrated in pigs, the lipoids that may be available to the mammary gland during inanition conceivably may be of high oleic acid content. If this is so the situation during inanition may approximately parallel that obtaining when a high oleic acid content ration is fed and thus furnish similar results.

From the considerations in this review relating to the source of milk fat in the ruminant, it would appear possible, although the evidence remains at present circumstantial, that in addition to food fat and lipoids resulting from carbohydrate digestion, depot fat may be a ready source of lipoids for milk fat synthesis.

It is suggested that at normal levels of nutrition the balance of these three main supplies may result in the synthesis of "normal" milk fat, and further, that any alteration in level of supply from one source may be compensated by an adjustment of the level of supply from other sources where this is possible. The supply to the mammary gland of milk fat precursors may additionally be affected by the degree of demand of other fixed tissues in competition. Variations from

normal balance of supplies to the mammary gland may result in deviations from normal of the butterfat constants as markedly demonstrated during inanition.

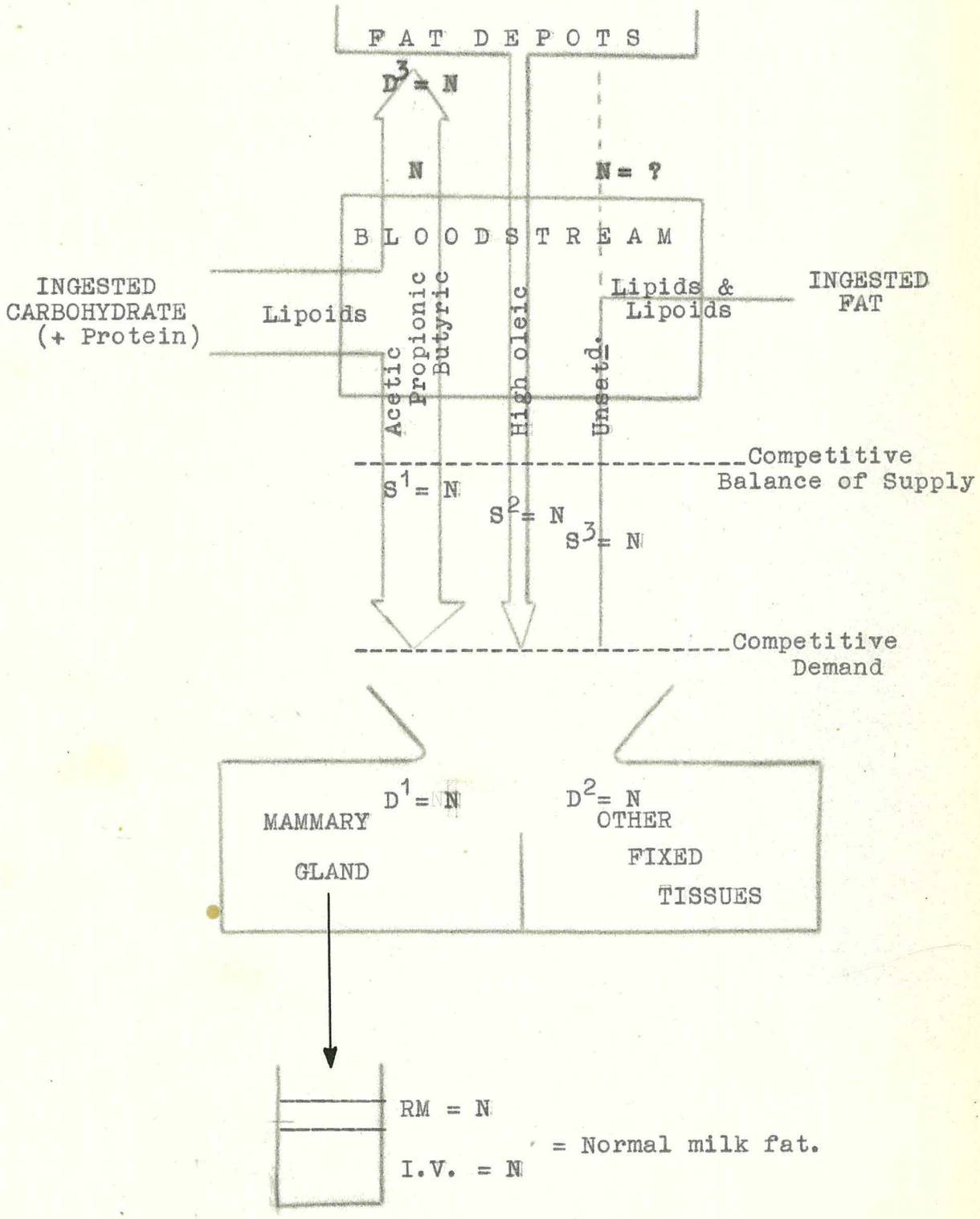
In spite of Stetten and Schoenheimer's work showing the apparently ever-varying interchange and alteration of fats during metabolism it is considered that although alterations may occur to differing degrees according to the composition of the particular fat considered there still appears to be a certain "maintainance of entity" of fatty acids for sufficient periods within the living organism to justify the foregoing endeavour to discuss the source of milk fat in the ruminant.

The foregoing discussion is summarised diagrammatically on pages 50a to 50e, following:-

.....

1. ADEQUATE LEVEL OF NUTRITION

"NORMAL" Rations



S^1 = Supply of lipoids resulting from ingested carbohydrat

S^2 = Supply of lipids and lipoids resulting from ingested fats of the diet.

S^3 = Supply of lipids and lipoids resulting from mobilisation of depot fat.

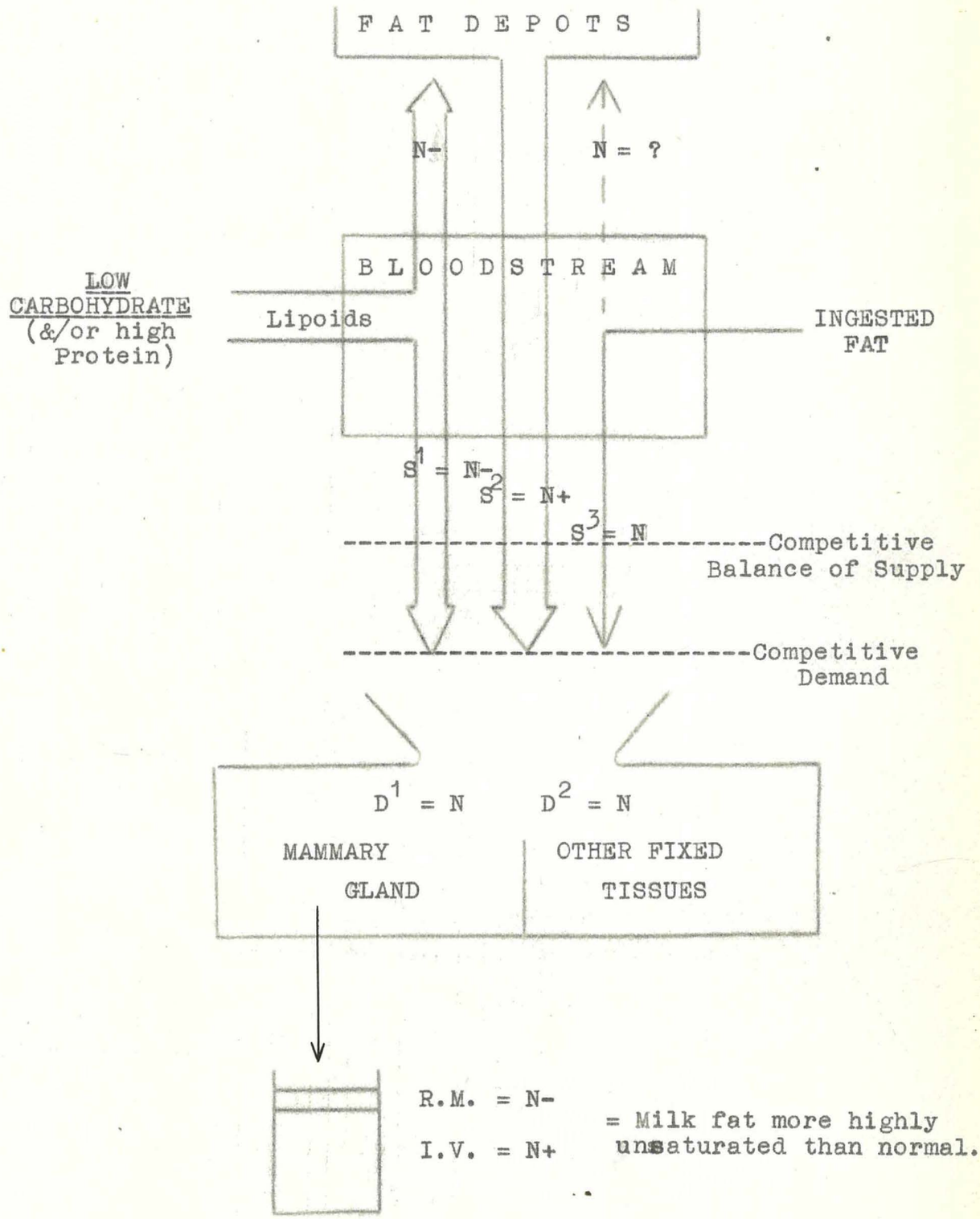
N = Normal

D = Demand

RM = Reichert-Meissl Value

I.V. = Iodine Value

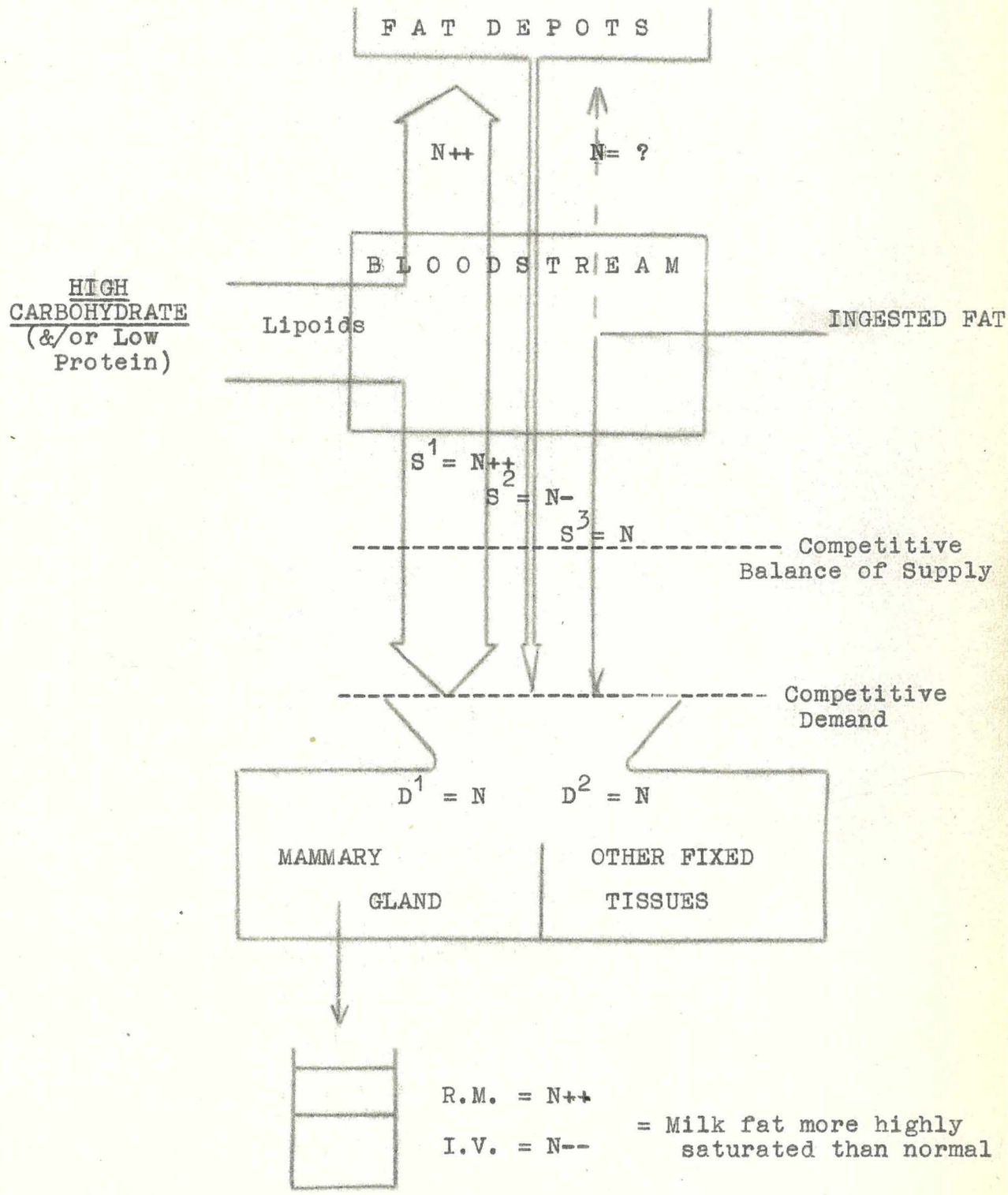
2. THE EFFECT OF RATIONS OF LOW CARBOHYDRATE CONTENT.



N+ = greater than normal

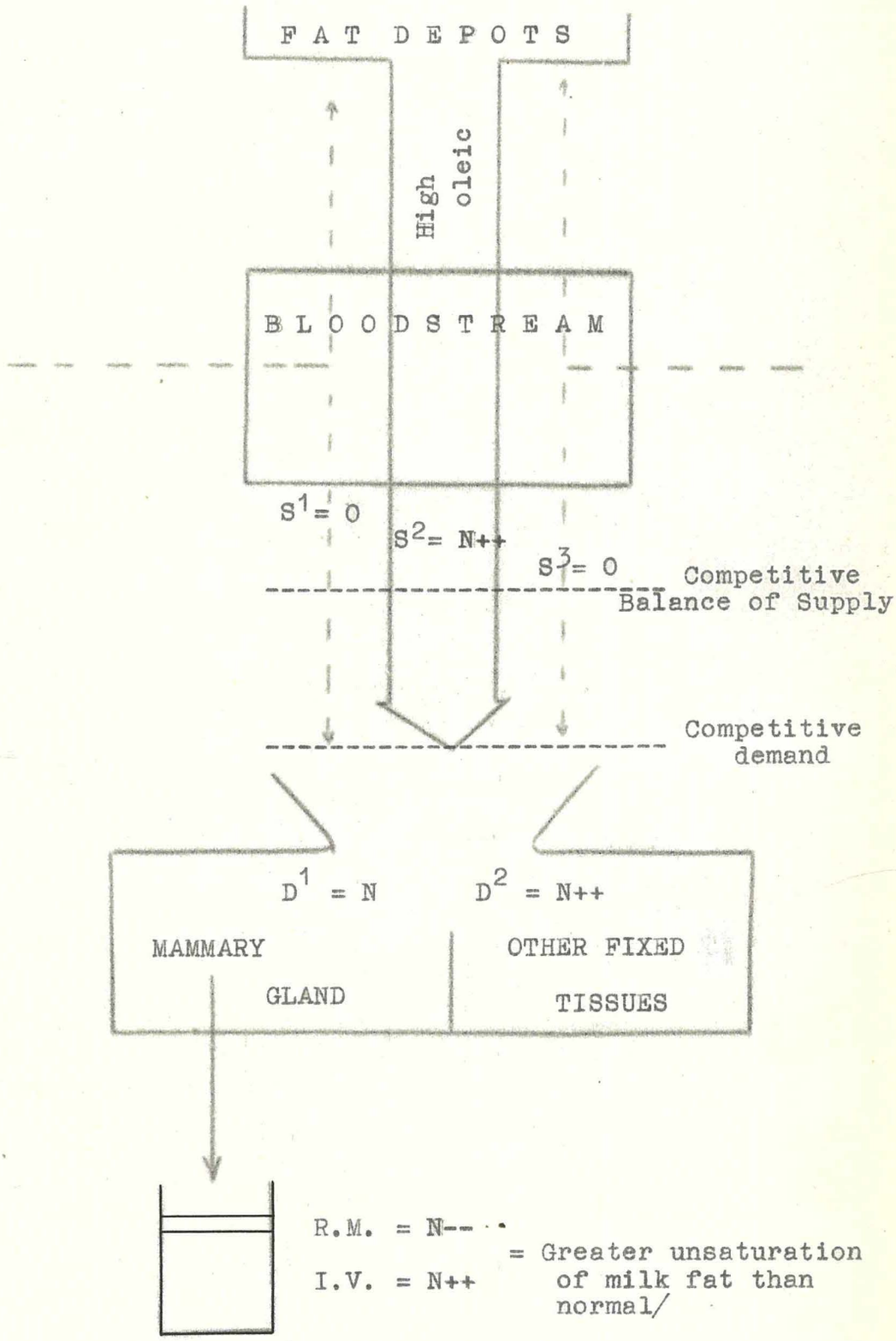
N- = less than normal

3. THE EFFECT OF RATIONS OF HIGH CARBOHYDRATE CONTENT



4. THE EFFECT OF INANITION.

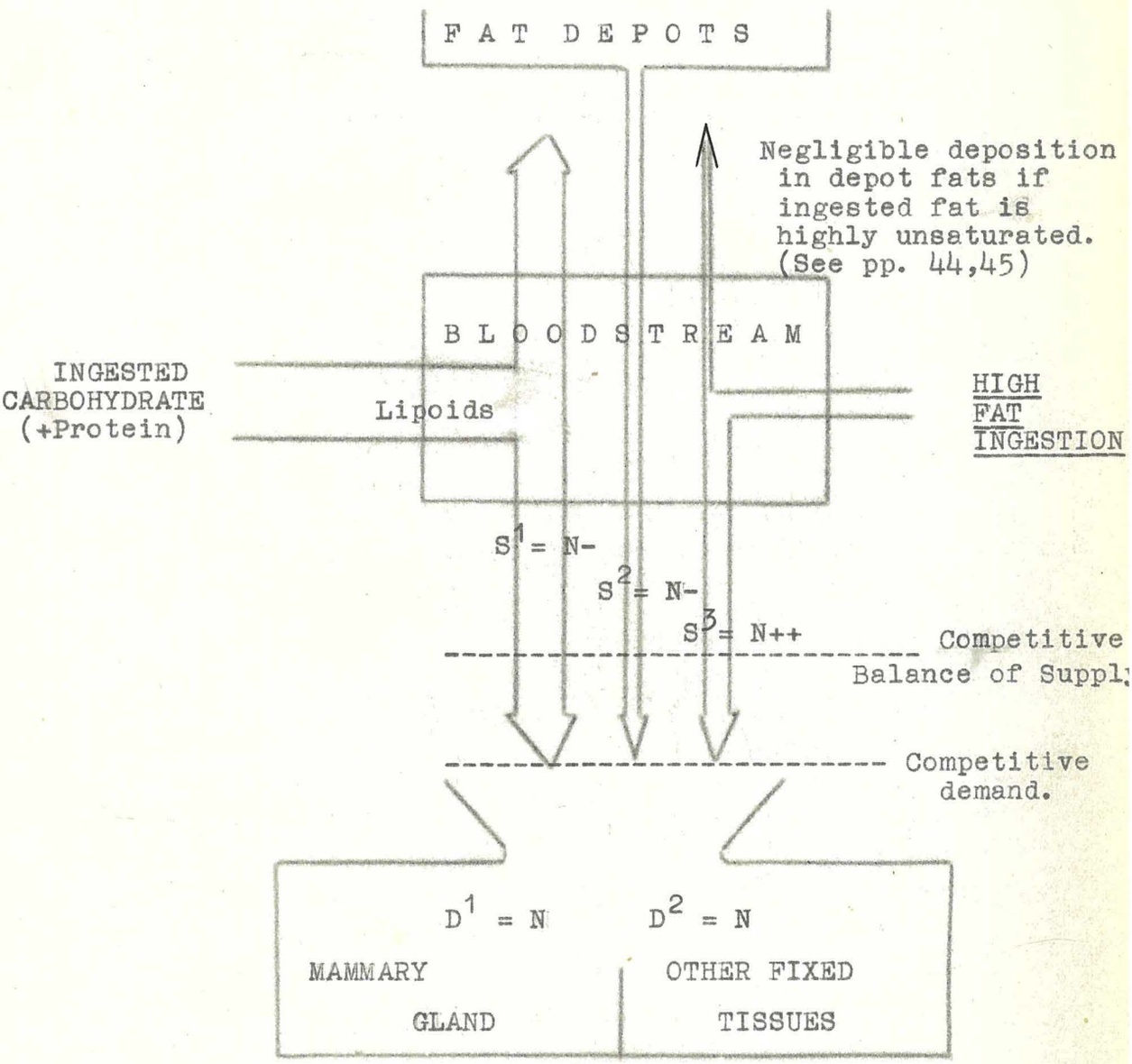
(Extreme case)

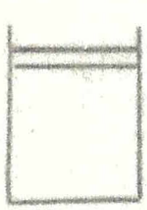


N.B. :-

$S^2 = N++$ implies a greater than normal degree of mobilisation of glycerides from depot fat, but does not imply that $S^2 = (S^1 + S^2 + S^3)$ at a normal level of nutrition as shown in diagram 1. (P.50a)

5. THE EFFECTS OF HIGH FAT INTAKE.

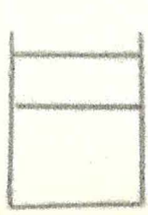




R.M. = N--

I.V. = N++

When ingested fat is highly unsaturated;
= Greater unsaturation of milk fat than normal.



R.M. = N++

I.V. = N--

When ingested fat is highly saturated;
= Milk fat more highly saturated than normal.

IV. PARTICULAR REVIEW OF LITERATURE AND AIMS OF EXPERIMENTS UNDERTAKEN.

EXPERIMENT I.

The Effects of Feeding an Unsaturated Oil to Dairy Cows on the Butterfat Produced.

Early experiments on the feeding of oil to dairy cows were undertaken with a view to discovering an economic supplement which would increase the percentage and yield of butterfat. Differing results with different oils stimulated interest in the phsiology of assimilation and secretion of specific acids of ingested fats.Other work on oil-feeding was directed on the addition of fish oils to dairy rations with a view to arresting the decline of vitamin A content of milk secreted by cows during winter feeding on concentrates, roots, and hay. Certain detrimental effects of cod liver oil feeding being noted attracted further research. A fresh aspect of interest in oil-feeding has been with regard to concentrate dairy feeds in Great Britain and U.S.A. where many constituents of rations are industrial by-products. The extent to which commercial oils are extracted becomes of agricultural interest when the oil content of by-products falls below the optimuum for maximum yield of milk and butterfat, while oil contents above that level in the by-product represents a loss to industry.

In examining experiments relating to oil-feeding of dairy cows the aspect outstanding for comment in practically all cases is the doubtful reliability of the practices of composite or infrequent sampling. While it is appreciated that the amount of work entailed in accurate chemical analysis greatly limits the numbers of samples examined, it is necessary to take into account the well recognised individual variability of animal product samples and to view with considerable reserve figures resulting from extremely critical analyses of small numbers of samples.

The Aim of Experiment I was to trace by frequent sampling (twice daily) the effects on the degree of unsaturation of butterfat resulting from the feeding of peanut oil to dairy cows.

Wing (75) in 1895 fed up to 2lbs of tallow per head to dairy cows without apparent effect on yield or composition of milk, or on health or body weight.

Smith, Wells, and Ewing (76) noted changes in butterfat composition resulting from inclusion of cottonseed oil in dairy rations. Group sampling was employed and experimental group compared with a control group. Only slight changes were noted in that the iodine value of the experimental group increased slightly. The suggestion was made that some of the substances of which the oil was composed had been transferred into the milk in greater amounts than others.

Bowes (14) using a single goat fed 25 cc. of arachis oil and noted the appearance of arachidic acid in the milk 12½ hours later. Continued feeding twice daily brought a maximum arachidic acid content in the milk 24 hours after the first feeding. This level appeared to continue for the remaining three days of the experiment.

Channon, Drummond amd Golding (77) studied the effects of adding coconut, arachis, and cod liver oils to dairy rations on the yield of milk and on the yield and composition of butterfat. Although milk yields and butterfat tests were recorded for every milking, butter samples were taken only at the commencement, middle and end of feeding periods. Three cows were used and no control animals. No effects were noted as resulting from the first two oils but cod liver oil apparently induced high iodine values and lowered saponification and Reichert-Wollny values suggesting that there may have been some transference of the fed oil or fraction of the fed oil into the milk fat.

The effect of feeding Menhaden oil (iodine value 176.6) to dairy cows on the butterfat composition was investigated by Brown and Sutton (78). A fourteen-day feeding period was employed. One Holstein cow was used and butter samples made up from two-day composites. A general steady increase of iodine value from 42 to 57 was noted during the feeding period followed by a period

of about six days during which the iodine value returned to normal. A drying-off tendency was noted. Some of the highly unsaturated acids of the menhaden oil were shown to have passed into the milk fat in small amounts. Butterfat percentage was depressed during the oil-feeding period from 3.0% to 2.1% followed by a return to normal which, from his figures, practically coincided with the return to normal of the butterfat constants. Milk yield decreased markedly during oil-feeding and made poor recovery following the cessation of oil-feeding. Feeding difficulties resulting from unpalatability were experienced. Brown and Sutton (78) noted in this trial that depression of saponification number and Reichert-Meissl number accompanied the rise in iodine value as had been noted by Channon, Drummond, and Golding (77).

A similar effect was demonstrated by Bender and Maynard (79) in supplementing a low-fat diet with linseed oil, Four lactating goats were used. A change back to a low fat ration in all cases had the reverse effect viz:- a lowering of iodine value, and an increase in saponification number. On the low-fat rations marked lowering of milk and fat yields occurred but when supplemented with linseed or coconut oil these yields returned toward normal. In the case of the coconut oil-feeding however the iodine value remained low.

> It may be noted that the above is not, as may seem at first sight, contradictory to the hypothesis of competitive balance of "neutral fat and lipoids already present in the body/ lipoids from carbohydrate/ neutral fat and lipoids from food fat", put forward in the concluding section of the General Review of Literature. In the case of the additional coconut oil-feeding the extra blood lipids and lipoids resulting could still depress the fraction of butterfat resulting from the short-chain acids from carbohydrate degradation and the effects not be noticed: the explanation offered for this being that coconut oil contains over 84% saturated acids from caproic (C_6) upwards. Saturated acids below C_{14} in coconut oil may amount to over 60% (data from Jamieson (80).
>
> From this it may be seen that although the ration may be high in food fat, if the latter be highly saturated then the blood lipids and lipoids resulting could still be "superior competitors" for milk fat synthesis with little noticeable

alteration of butterfat constants.

Allen (7) studying the effects of adding various oils to dairy rations on the fat percentage of milk noted that 12 to 24 hours elapsed before the influence of the added fat became observable in the milk, and also that a time lag of 30 to 42 hours after the cessation of fat feeding was evident before the fat percentage returned to normal.

Maynard, McCay, and Madsen (15) using two cows fed alternate rations containing fats of high and low degrees of unsaturation. As noted in previous experiments above a fat of high unsaturation in the ration induced a higher than normal degree of unsaturation of the milk fat. Samples were 24-hour (2 milkings) composities. On changing rations abruptly from high to low unsaturation marked corresponding alterations were evidenced by the milk fat rising or falling to a relatively constant level from the third to fourth day following the change of ration.

Hill and Palmer (16) in examining the relation of feed consumed by the cow to the composition of milk fat carried out oil-feeding experiments using 7-day feeding periods. Sampling was done at the endof each feeding period. As previously shown highly unsaturated fats when added to the ration caused increase in iodine number, and decrease in Reichert-Meissl and saponification values. Owing to the infrequency of sampling the rate of change of the butterfat constants caused by oil-feeding was not demonstrated.

Using 8 cows and 4-day composite sampling Gibson and Huffman (9) conducted an experiment to show the effects of differing fat intake levels on milk yield and fat test. The addition of soybean oil to a low fat ration caused increases in butterfat percentage which returned to normal from 4 to 12 days following the cessation of feeding. More efficiently planned than any of the foregoing was the experiment of Garner and Sanders(81). Using a Latin square design the effects of eight different oils fed at three different levels, on milk and butterfat yields

were studied. In addition three different lengths of terms of oil-feeding were investigated. Assuming the effects of the oils to be rapid in action periods of only two days without oil were allowed between any two feeding levels which may have been too short for the effects of the previous oil-feeding to be dissipated. They found that experiments with the same oil were not always consistent, that the effects may vary with individual cows, and with the same cow at different times.

Kuhlman and Gallup (82) followed the effect of several feeding levels on the butterfat produced by 8 cows throughout a lactation period. Sampling was infrequent being morning/evening composites of the milks from individual cows taken at 30-day intervals. It was claimed, nevertheless, that the results showed individual variability with respect to the properties of the fat produced by cows on the same feed, and in the same period of lactation.

The depressing effect on milk and butterfat yield resulting from addition of cod liver oil to dairy rations having been noted by various workers and the similar effect resulting from addition of menhaden oil (Brown and Sutton (78), focussed attention on the fractions of these fish oils responsible for the effects. Hilditch and Thompson (17) compared the effects of rape, linseed, and cod liver oils in the dairy ration on the composition of butterfat. A feeding period of one week was used, 9 cows employed. Six specimens of fat were obtained for analysis at the end of the feeding period, three of the samples being three cow-pair composites. The oils were given in 4oz. lots twice daily. Marked changes as indicated by the butterfat constants resulted from the oil feeding, as shown in the following condensed table:-

	Control	Linseed	Rape	Cod Liver Oil.
Reichert-Meissl value	30.0	28.9	28.5	16.0
Saponification value	244.2	249.0	251.2	264.2
Iodine value	34.9	46.0	44.5	51.7

The increase in saponification value as the iodine value increases does not agree with the earlier observations of Channon Drummond and Golding (77).

Hilditch and Jasperson (69) in feeding groundnut oil found similar trends in butterfat constants as had Hilditch and Thompson (17). Hilditch and Jasperson (69) observed that the iodine values of the milk fats (observed at several-day intervals) were variable for the first 2-3 weeks of oil-feeding and then became relatively constant after 34 days when a two-day composite of butter was made up and subjected to detailed analysis.

Brown, Dustman and Weakley (84) examined the relation of the degree of unsaturation of fat in the ration of dairy cows to the iodine number of the butterfat. Eleven cows were used. During a pre-feeding period daily determinations of iodine values of milk fat were carried out to ascertain the normal daily variations. These were not published in their paper. Experimental feeding periods were of six weeks followed by reversals in feeding. Two samples a week from each cow were used for iodine value determinations although for other experiments on development of oxidised flavour milk samples were obtained every morning. The effects of soybean oil and coconut oil added at the rate of 1lb per day were noted as essentially similar to results of previous workers in that on feeding the highly unsaturated soybean oil the degree of unsaturation of the butter fat was increased while on feeding the far more saturated coconut oil, the iodine value of the butterfat was depressed below normal. Brown et al found that on feeding 1lb of oil per day 3-4 weeks were required before the iodine

values reached a maximum.

Moore, Hoffman, and Berry (84) are apparently among the few workers who have employed frequent sampling in tracing the effects of oils added to dairy rations. Cod liver oil feeding by two methods was investigated. One group of cows was fed 5-8oz. of cod liver oil in one dose each day for 3-6 days. the second group received the same amount of oil divided into twelve equal feedings each day. The single feeding each day produced abrupt increase in the iodine values of the milk fat. The "gradual"feeding of the same amount of oil resulted, in general, in a slower and smaller response. Samples examined were daily composites of the morning and evening milks from each cow. Three-day pre-feeding, six-day oil-feeding, and fourteen day post-feeding periods were employed.

From the above brief review it may be seen that although the general effects of feeding unsaturated oils to dairy cows are fairly well known the tracing of these effects by frequent sampling apparently has been done by very few workers, and it may be noted that even Moore, Hoffman and Berry (84) whose paper has just been briefly reviewed used daily composites of morning and evening's milk, i.e. in effect, sampling every 24 hours.
EXPERIMENT I was designed to enable, by frequent sampling, observation of changes in butterfat composition resulting from oil-feeding to dairy cows.

(A short pre-feeding period was employed followed by 7 days of oil-feeding, and 7 days post-feeding. Three cows were used as control animals, and three as experimental. Sampling was carried out twice a day from all cows, except during the first three days of oil-feeding when the experimental group were milked and samples taken.every 6 hours. It was hoped that the more frequent sampling in the early part of the oil-feeding period would provide a clearer picture of the oil-feed-induced changes in butterfat composition.

EXPERIMENT 2.

The Efects of Inanition on the Degree of Unsaturation of Milk Fat Secreted by Dairy Cows.

The fact that world dairy products are subject to variability is manifest upon even the most cursory glance at dairy science literature.

The work already reviewed has demonstrated that variations in quality and quantity of milk fat may result from variations of the constituent proportions and qualities of the diet of dairy cows. In addition it has been shown that under-feeding may also influence the quantity and composition of milk fat.

Comparatively little research has yet been devoted to under-nutrition with particular regard to ruminant fat metabolism. In most under-feeding research projects frequent sampling has been employed recording plainly during inanition the course of changes in composition of milk fat.

Inadequate feeding under New Zealand conditions may occur as a result of insufficient pasture in late winter / early spring, late summer/ early autumn, overstocking, extremes of weather conditions, or instances where cows are fed to capacity on bulky foods of inadequate nutritive value.

The Aim of Experiment 2 was to determine the effects and after effects of short-term inanition on the degree of unsaturation of milk fat secreted by dairy cows.

The "classic" work on the effects of under-feeding dairy cows on the composition of milk and milk fat was that of Eckles and Palmer (40) in 1916. They subjected lactating cows to varying degrees of undernutrition, and for varying terms. Sampling in general was carried out at 2-day intervals and in one or two instances, on consecutive days. In general their work showed that during under-feeding:-

Body weight declines.

Milk yield may remain normal or decline depending on whether underfeeding is imposed early of late in lactation respecitvely.

Fat yield may be variable but in general, subnormal.

Fat percentage may also be variable. In half of the experiments fat percentage was increased.

Iodine value of the milk fat increases.

Reichert-Meissl values decline.

Saponification values decline.

It was also apparent that the effects of underfeeding were subject to modification by :-

The stage of lactation at which underfeeding was imposed.

The previous plane of nutrition and condition of the cow.

The degree and period of the underfeeding.

The nature of the inadequate ration during the period of under-nutrition.

These observations have been verified by subsequent research.

Smith and Dastur (39) studied the butterfat constants and chemical composition of milk fat from two cows during a 12-day fast. Using daily sampling they found similar but more marked trends than did Eckles and Palmer.

Milk fat constants.		Before fast.	First day of fast.	End of fast.
Iodine values	Cow 1.	36.6	46.1	52.5
	Cow 2.	37.1	47.3	54.9
Reichert-Meissl values			Second day of fast	
	Cow 1.	26.0	14.5	9.8
	Cow 2.	33.3	22.9	13.8

It appeared from chemical analysis that during the 12-day starvation there had been a decrease of about 80% in the original content of lower fatty acids of the milk fat up to C_{14}, the decrease apparently being compensated by an increase in oleic acid content.

The effect on milk composition of lowering the plane of nutrition of dairy cows was also investigated by Riddet, Campbell, McDowall and Cox (70). Three consective 30-day feeding periods were employed in the trial, The 6 cows used were housed throughout the 90 days. All received a maintainance ration over the whole period. In addition the control pair of cows received a full production ration. Of the other two pairs, one received half / full / half production rations respectively

in the three consecutive periods, the other receiving full / half / full production rations in the corresponding periods. Milk sampling was carried out twice daily for purposes of studying the milk composition; but with specific regard to butterfat constants 10-day composite morning and evening samples were taken. It would be expected that this method of sampling would be quite satisfactory to reveal definite trends in butterfat constants, but that the short term rate of change of butterfat constants resulting from changeovers in level of ration would be masked.

It was found that in general on reducing the plane of nutrition from a normal level:-

Milk yield decreased.

Fat yield decreased.

Fat percentage was variable.

Iodine value increased.

Saponification value increased. This latter is not in agreement with the majority of Eckles and Palmer's work (40) in which there appeared only a few instances of saponification value increasing at the same time as the iodine value, and were of irregular occurrence.

Eckles and Palmer (40) found that "all types of under-feeding have marked effects on the physical and chemical constants of the butterfat, which are characterised by a decline in the Reichert-Meissl number and saponification value, and an increase in the iodine value."

In the work of Riddet et al (70) the increase in saponification values was positive and coincided with increases in iodine values. As Reichert-Meissl values for the samples were not determined it is difficult to assess the cause of saponification value increase.

At an adequate or high plane of nutrition an exact balance of nutrients may be of no great importance in that all the ration constituents are present in more than necessary amounts, but at a reduced plane of nutrition nutrient balance may be of greater import in that some constituents may be quite insufficient while others are still adequate. In this manner it would be

possible for slightly differing effects to result from underfeeding when the character of the insufficient ration differed.

Jarl (85) has shown statistically that decreases in body weight of dairy cows are correlated with the iodine values of the milk fats, but that increases in body weight are not. Jarl states "the increase in iodine number when cows lose weight is due to catabolism of stored body fat with a high iodine number and its secretion in the milk". It has been pointed out in the General Review of Literature that although the evidence to support such a statement is strong it remains as yet circumstantial. A simple direct proof has not yet been put forward.

The latter work refers to small gains and losses in body weight of dairy cows occurring during apparently well-managed feeding. The previous experiments have illustrated either the effects of lowered planes of nutrition on composition of butterfat over short and long terms, or the effects of the extreme case of starvation over a period improbable in practice. Experiment 2 was designed to follow the changes in butterfat composition during and following short-term starvation such as occasionally may be imposed on lactating cattle in holding yards, at sales, or more occasionally during shipment by rail.

EXPERIMENT 3.

The Effects of Inanition during the Feeding of Unsaturated Oils to Dairy Cows on the Degree of Unsaturation of the Milk Fat Secreted.

The principal literature relating to the two main aspects of this experiment, oil-feeding and inanition, already has been surveyed in the General Review of Literature and in the Particular Reviews of Literature pertaining to Experiments 1 and 2.

The Aim of Experiment 3 was to discover if oils, fed to dairy cows during drastically reduced intake, may be assimilated and utilised for milk fat synthesis to any marked degree.

The previous work reviewed has illustrated that some fractions o ruminant blood lipoids resulting from ingested fats may participate in milk fat synthesis to varying degrees. Some of the oil-feeding work has been conducted more with a view to studying the variations in milk fat percentage induced than the mechanism by which they occurred.

In short-term oil-feeding experiments (6-day periods) with dairy cows Allen (7) showed that certain oils in the ration in excess of normal amounts could induce increased milk fat yields, the latter being of the order of 10-20% of the additional fat that had been fed. Allen and Fitch (8) repeated these experiments using 50-day feeding periods and secured essentially similar results.

Garner and Sanders (81) and Maynard ,Loosli, and McCay, (86) were also able to demonstrate increased milk fat yields resulting from oil-feeding

In many of the experiments so far discussed the feeding of oils was superimposed on normal rations. From these experiments it is apparent that the mammary gland is quite able to utilise greater than normal amounts of blood lipoids resulting from food fat. Under these conditions of normal nutrition or above

this higher-than-normal blood fat fraction resulting from food fat may be expected to be in "competition" with other blood lipoids. If some of the "competition" were to be removed it would seem possible that an even greater proportion of the blood lipoids from fed fat might be utilised by the mammary gland and be reflected in the milk fat composition.

EXPERIMENT 3 was designed to discover if, during inanition in the dairy cow, blood lipoids resulting from absorption of an ingested fat may be utilised for milk fat synthesis more readily and to a greater extent than normally evidenced.

> By feeding peanut oil to dairy cows during a period of drastic reduction in food intake, it was anticipated that if the oil were assimilated and utilised for milk fat synthesis to an abnormal extent this would be reflected in a higher degree of unsaturation of the secreted milk fat than during inanition alone i.e.:- the effect of supplying a "ready-made" fat to an avidly fat-demanding tissue.

Experiment 3 was planned in conjunction with Experiment 2. During the periods of inanition in Experiment 2 the only food allowed was approximately equivalent in food value to the oil in meal which was allowed during otherwise similar periods of inanition in Experiment 3.

EXPERIMENT 4.

The Effects of Feeding Stained Fats to Simple Stomached Animals.

The feeding of stained fats already has been briefly discussed in the General Review of Literature.

The Aim of Experiment 4 was to confirm that the feeding of a stained fat to a lactating simple-stomached animal may result in the secretion of stained milk fat.

Mendel and Daniels (11) credit Daddi with discovering in 1896 that the fat soluble dye Sudan 111 when fed in fat to small experimental animals may be absorbed and laid down in the adipose tissues. Mendel and Daniels conducted stained-fat feeding experiments on rats, cats, guinea pigs, laying hens, a cow and a goat. With guinea pigs they found that 80 mgms. of Sudan 111 fed every second day for 4 weeks gave no evidence of stained tissues but at higher dosage rates slight visible deposition was effected.

The feeding of stained fats to lactating cats and rats resulted in stained milk fat being secreted, seeming to indicate ready utilisation of blood lipoids resulting from ingested fat, by the mammary gland. One feeding of a stained fat to a goat resulted in distinctly faintly pink milk fat being secreted, whereas repeated feedings to a dairy cow failed to result in the secretion of coloured milk fat. By the comparitive times taken to stain depot fats, or milk fats by the feeding of stained fats Mendel and Daniels concluded that "those animals which absorb fat readily give evidence of Sudan-stained fat in far less time than those, like the guinea pig and the cow in which fat forms a smaller factor of the diet".

It would appear from this statement (in 1912) that the herbivora may have limited ability to utilise lipoids resulting from ingested fat; but from the recent work of Allen (7) and (8) and Maynard, Loosli, and McCay (86) it has been shown that the mammary gland of ruminants is able to utilise greater than normal amounts of blood lipoids of food fat origin.

It was found that animals previously fed with stained fat and then subjected to starvation excreted stain by medium of the bile indicating that much of the depot fat mobilised may be utilised or undergo alteration in the liver.

If the stain Sudan 111 is actually soluble in bile it would be reasonable to expect that it would also be soluble in the small intestine where bile is admixed with ingesta. Gage and Fish (2) fed Sudan 111 not in oil to cats and found no evidence of absorption. Stained body fat occurred only when the dye was supplied dissolved in oil. They showed that the dye remains with the fatty acids. On feeding alternate stained and non-stained fats to laying hens concentric rings of alternately stained and non-stained material appeared in the egg-yolks. No diffusion of stain into the parts of the yolk being laid down when no stain was being fed occurred indicating that the stain remained closely associated with the fatty acids with which originally united. Gage and Fish believed that the stain remained with these fatty acids in the living body and could be separated from the fatty acid radicals only when the radicals themselves were broken up in metabolism.

The work of Mendel and Daniels was repeated by Gage and Fish with similar results, notably the repeated failures to secure secretion of stained milk fat by feeding stained fats to lactating cows.

In addition to demonstrating stained milk fat secretion and stained depot fat deposition in small animals during the feeding of stained fats it was shown that lactating rats, known to have a good supply of stained depot fat (from previous feeding) when fed non-stained rations secreted milk of normal colour. On subjecting these rats to starvation stained milk fat promptly resulted.

This would appear to indicate that in the simple-stomached animal depot fats may be readily mobilisable for milk fat secretion when demand occasions. During normal levels of nutrition if depot fats are being drawn on for milk fat synthesis it would seem that it may be at too slow a rate to be

detected by stained fat techniques. As previously mentioned, in spite of the failures of Mendel and Daniels (11), Gage and Fish (2) and Kelly and Petersen (12); Gibson and Huffman (9) were able to demonstrate in the dairy cow positive cases of stained milk fat secretion resulting from the ingestion of stained food fats. These results were secured by administering massive doses of dyestuff. It is believed that, as yet, no further work has been carried out with dairy cows using this method of ingested-fat tracing.

Along with other techniques of tracing ingested material the feeding of stained fat involves the introduction into the organism of a foreign body which may cause metabolic processes to deviate from normal.

EXPERIMENT 4 was purely confirmatory to observe that stained fat when fed to lactating guinea pigs does result in the secretion of stained milk fat.

EXPERIMENT 5.

The Relation of the Degree of Unsaturation of Butterfat to its Keeping Qualities.

Experiment 1 revealed evidence of a consistent difference in degree of unsaturation of milk fat from morning and evening milkings.

The Aim of Experiment 5 was to discover if any marked difference of keeping qualities exists between milk fats from morning and evening milkings.

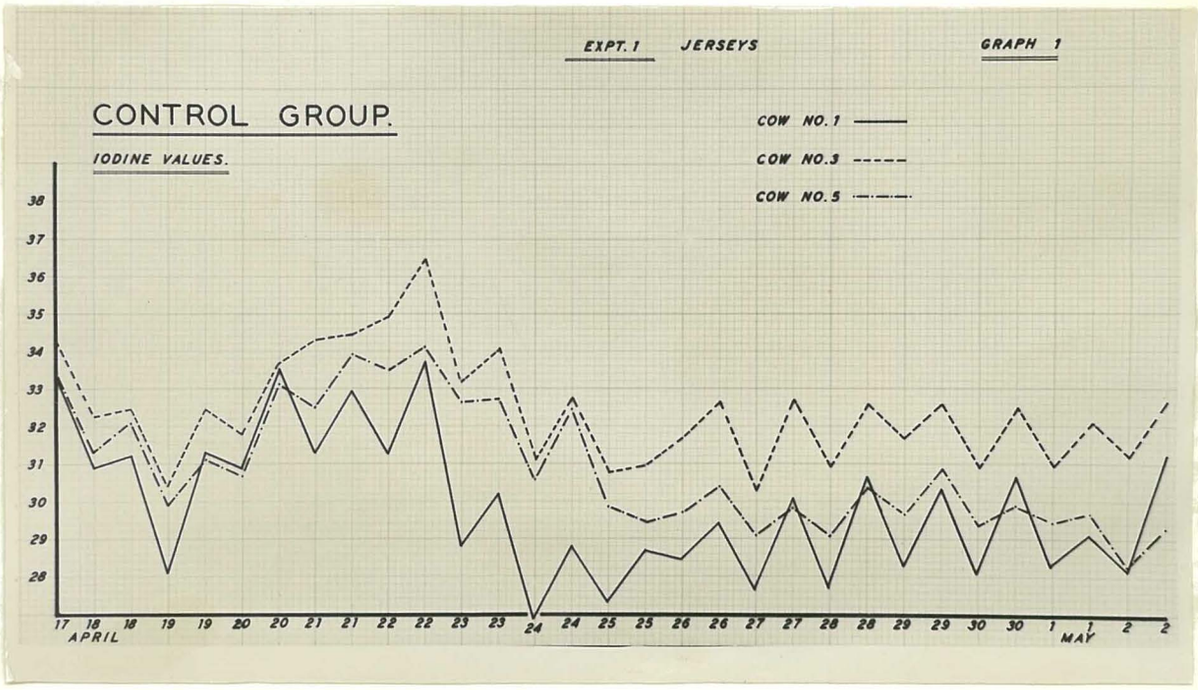
(In that this experiment was but a minor sideline to Experiment 1 only brief discussion will be devoted to it.)

Henderson and Roadhouse (87) observed that milk fat produced by cows on sub-maintainance rations shows a higher degree of unsaturation than normal and increased susceptibility, to oxidation.

Stebnitz and Sommer (88) following on this work found that the stability of butterfat toward oxidation is inversely related to the degree of unsaturation. It has been noted in the foregoing reviews of literature that dairy products are variable in composition and also that and "individuality of product" is apparent from different individuals. This is further emphasised by Stebnitz and Sommer (88) who found that with regard to stability of butterfat toward oxidation there is considerable variation between samples from different cows and between samples from the same cow at different times.

Brown, Dustman, and Weakley (83) in working on susceptibility to oxidised flavour in milk found that small changes in degree of unsaturation of butterfat had little influence on the intensity of metal-induced oxidised flavour when fat intake is low. When fat intake is high then the relationship shown by Stebnitz and Sommer tends to become evident.

In Experiment 1 it was noted that the degree of unsaturation of milk fat varied from morning to evening. (See graph 1 below and also graphs 2, and 3 later).



(The dates shown as 19,19,20,20, etc. refer respectively to morning and evening milkings on thos[E] dates.)

In view of the consistency of morning /evening differences for each individual cow and the suggestion from the literature that differences in keeping qualities of milk fats may have relation to degree of unsaturation it seemed possible that differences in keeping qualities might exist between milk fats from morning and evening milkings.

EXPERIMENT 5 was conducted simply as an exploratory check to see if any obvious differences in keeping quality do exist between morning and evening milk fats.

(Morning and evening fat samples from 6 individual cows on 4 separate days were used in the experiment i.e. 48 samples)

V

EXPERIMENTAL PROCEDURE.

V EXPERIMENTAL PROCEDURE.

EXPERIMENT 1

(April / May 1949).

The Effect of Adding Peanut Oil to the Daily Ration of Lactating Jersey Cows on the Degree of Unsaturation of the Milk Fat Secreted.

Material:-

The animals used in this study were six Jersey cows of the College herd; designated 1 - 6 in the ensuing data.

Numbers 1, 3, and 5 were "control" animals

Numbers 2, 4, and 6 were "experimental" animals.

Status of cows at time of experiment:-

Cow	Age yrs	In lactation	Pregnancy	Condition
1	7½	8 months.	Non-preg.	Good
3	6½	7 months.	"	Good
5	7½	5 months.	"	Fair
2	7½	8 months.	"	Average
4	6½	9 months.	"	V. Good
6	4½	7 months.	"	Average

Normal Feed:- During the course of the experiment all cows were pasturing with the college herd: receiving in addition to pasture a small amount of grass silage supplement after each milking.

Duration of experiment:

Pre-feeding period	1½ days
Oil-Feeding period	7 "
Post-Feeding period	7 "
Total	15½ days.

Routine:-

Milking was conducted at 5.30 a.m. and 4.00 p.m. daily. During the first three days of oil-feeding the experimental cows 2, 4 & 6 were milked at 11.00 a.m. and 11.00 p.m. in addition to the normal milkings. For sake of convenience, over these three days all six cows were isolated from the herd in a small field convenient to the milking shed.

Sampling was carried out at every milking for all cows for the 15½ days. The cows were milked individually by machine into 'testing' buckets, the milks weighed, and a quart sample of each taken.

Oil-feeding to the experimental cows 2, 4 & 6 was accomplished by 'drenching' in the milking bail immediately following each morning milking of the 7-day oil-feeding period. The amount administered each day was 8oz. of peanut oil (iodine value 96.2).

Efficiency of drenching:-

Owing to the amount of oil which tended to remain adhering to the sides of the drenching-bottle it was necessary to pour in more than the 8 oz. in order to ensure a drench of 8 oz.

Cow 2 proved subborn and difficult to manage and could not be considered to have received the full dose of oil at every drenching.

Cows 4 & 6 accepted the oil-drenching quite readily receiving the full amount at each administration without apparent loss.

Health of cows:- Throughout the experiment all cows appeared in normal health. No adverse effects were noted as resulting from the oil-feeding.

Laboratory Rouhine:-

The quart samples of milk taken at each evening

milking were stood overnight at room temperature to allow the cream to rise. In the forenoon of the following day the cream was drawn off with the aid of a Buchner flask, churned to butter with a Waring Blendor, the butter melted in an over at 65°C, filtered through No.1 Whatman paper in the oven into sample bottles, labelled and stored in a refrigerator for future analysis. (Photos in Appendix V)

The quart samples of milk taken at each morning milking were stood at room temperature until the afternoon or evening of the same day for the cream to rise, and then treated in the same manner.

Iodine Value determinations were conducted in duplicate on every fat sample by Wij's Method (detail in Appendix 1) Determinations were repeated on any samples of which, at the first estimation, duplicates differed in Iodine Value by greater than 0.40. Values were taken to two places of decimals.

(As a check on the accuracy of the work periodic iodine value determinations were conducted on a standard peanut oil supplied by the Fats Research Laboratory, D.S.I.R., Wellington. The standard oil was of Iodine Value 92.1 with a standard deviation of 0.44.
The mean of 16 determinations of Iodine Value on this oil was 90.62 and the standard deviation 0.24)

The actual Iodine Values found for the fat samples of Experiment 1. are set out in Appendix 11.

(One sample, viz: Cow 1, May 2nd, a.m. was accidently overturned in the laboratory and was irrecoverable for analysis.)

EXPERIMENT 1.

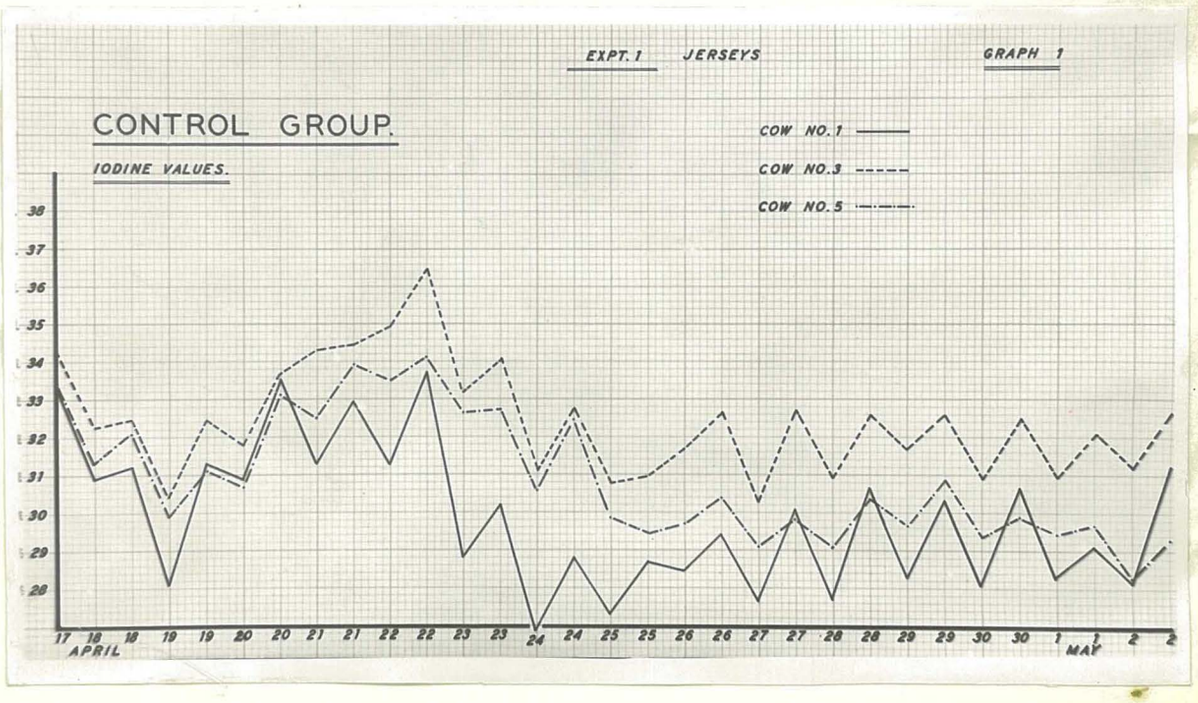
RESULTS:

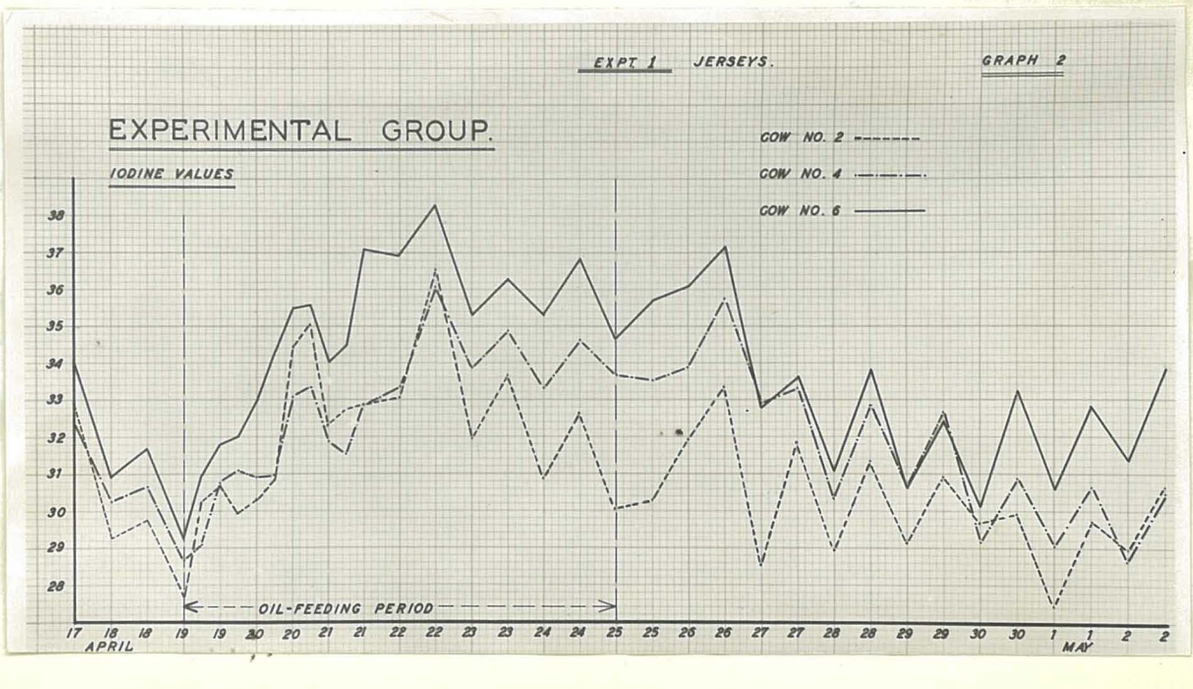
Graphs 1, and 2, below, depict the changes in Iodine Values of fat samples from individual cows throughout the experiment.

The figures 18, 18, 19, 19, etc. at the foot of each graph refer to morning and evening samplings respectively, on the date denoted by each number.

(In Graph 1 the iodine value of Cow 1, May 2nd. a.m. was estimated by averaging the Iodine Values of the three previous a.m. samplings from the same cow.)

In Graph 2 intermediate samplings at 11.00 a.m. and 11.00 p.m. on the 19th & 20th, and 11.00 a.m. on the 21st are included.



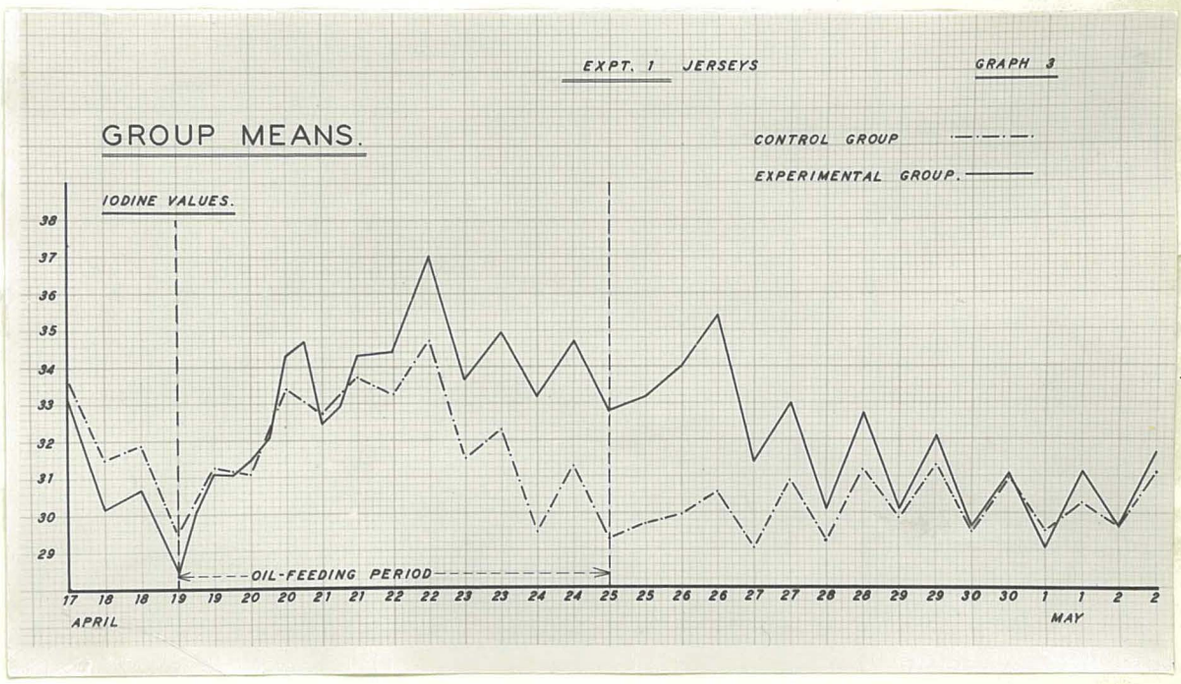


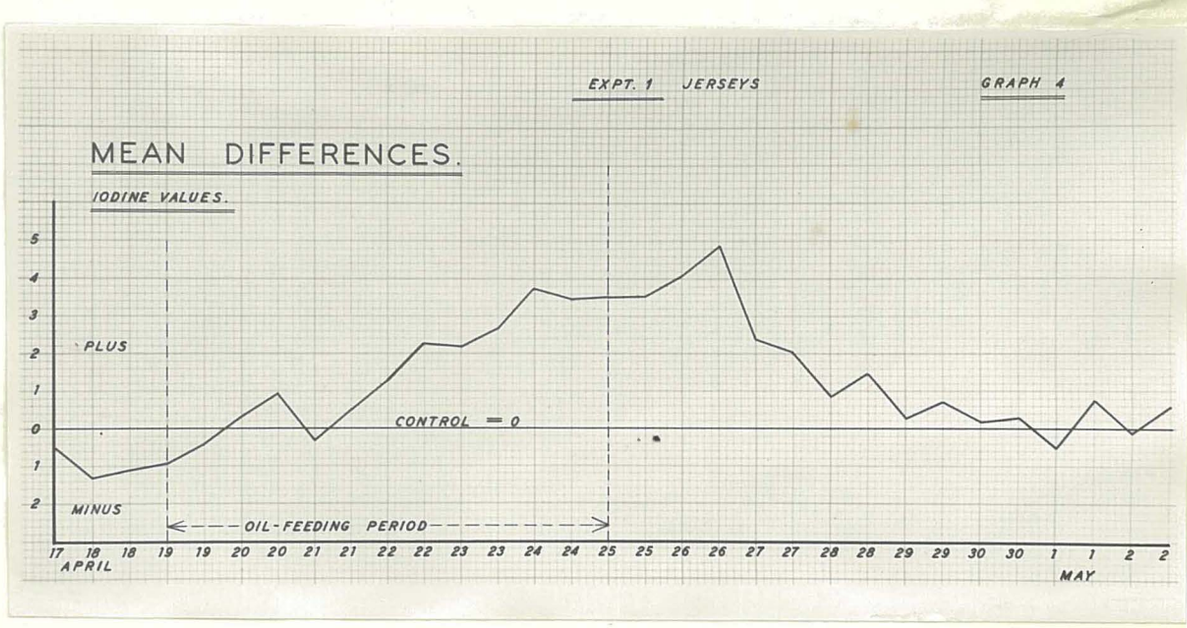
EXPERIMENT 1.

RESULTS (Continued)

Graph 3, below, illustrates the changes in mean Iodine Values of fat samples from cows in each group at each milking throughout the experiment.

Graph 4 shows the degree to which the mean Iodine Values of the fat samples at each milking from the oil-fed group varied above or below the mean Iodine Values of the fat samples at each milking of the control group.





EXPERIMENT 1

RESULTS (continued)

Analysis of Variance of Iodine Values of Milk Fat Samples over the period April 22nd. to April 28th. inclusive.

Source	d.f.	ss	ms	
Between cow-pairs	2	157.8559	78.9279	**
Between treatments	1	152.7931	152.7931	**
Error 1, (Treatments x pairs)	2	.2842	.1421	
Between days	6	145.2488	24.2081	**
Treatment x days	6	22.9447	3.8241	**
Error 2.	24	21.5861	.8994	
Periods (a.m./p.m.)	1	43.0144	43.0144	**
Periods x Treatments	1	.4651	.4651	NS
Periods x Days	6	7.4565	1.2427	**
Error 3.	34	9.3390	.2746	

** = Significant at the 1% level.

EXPERIMENT 1.

RESULTS (Continued)

In Graphs 1, and 2 the most outstanding feature is the fairly consistent morning / evening relationship of Iodine Values of samples from individual cows, an effect which tends to be superimposed on other longer-term effects which appear to affect all cows in a similar manner.

It may be noted also that there appears to be a marked "maintainance of individuality" regarding Iodine Values from individual cows.

The effects resulting from the oil-feeding are not so readily apparent except over the two days immediately following the cessation of oil-feeding, when the oil-fed group show in general a higher level of Iodine Values than the control group.

Graphs 3 and 4 illustrate the difference between the oil-fed and control groups to greater advantage. In Graph 3 the oil-fed group tends to differentiate itself from the control group at about 3 to 4 days following the commencement of oil-feeding and to return to approximately normal at 3 to 5 days after the cessation of oil-feeding. The marked morning / evening effect remains apparent even in terms of mean values.

Graph 4 shows even more plainly the "carry-over" effect of oil-feeding after oil-feeding has ceased.

.....

EXPERIMENT 1.

DISCUSSION OF RESULTS.

The analysis of Variance of Iodine Values was carried out on those values determined from morning and evening samples of the period April 22nd. to April 28th. inclusive, i.e. :- from three days following the commencement of oil-feeding to the third day after the cessation of oil-feeding .

From Graphs 3 and 4 it may be seen that the experimental group begins to be differentiated from the control group from 3 to 4 days following the commencement of oil-feeding and that upon the cessation of oil-feeding a "carry-over" effect of from 3 to 5 days is evidenced.

The "carry-over" effect coincides with the findings of Brown and Sutton (78) who found the iodine values returning to normal about 6 days after the cessation of feeding menhaden oil. Allen (7) had noted a time lag of 30 to 42 hours before fat percentage returned to normal after ceasing oil-feeding. Gibson and Huffman (9) had observed from 4 to 12 days lag following soybean oil feeding before butterfat percentage returned to normal. In the cod liver oil feeding experiments of Moore, Hoffman and Berry (84) marked increase in degree of unsaturation of milk fat was noted within 24 hours after the first feeding of 5 - 8 oz. of oil.

A. The highly significant difference between cow-pairs illustrates the validity of pairing of control and oil-fed cows on the basis of Iodine Values determined during the pre-feeding period.

By inspection of Graphs 1 and 2 it becomes apparent that the Iodine Values of samples from individual cows almost without exception maintained their relative positions throughout the experiment. This additionally emphasised the statement in the introduction to the Particular Review of Literature relating to Experiment 1 (p. 51)

> ".... it is necessary to take into account the well-recognised individual variability of animal product samples and to view with considerable reserve figures resulting from extremely critical analyses of small numbers of samples."

B. The difference between treatments (shown to be highly significant) is quite apparent from Graph 3 of Group Means and from Graph 4 of Mean Differences between the control and oil-fed groups. It appears definite that some fraction or fractions of the ingested peanut oil have passed into the milk without sufficient de-saturation during metabolism to prevent the degree of unsaturation of the milk fat from increasing. The peanut oil was of Iodine Value 96.2. The actual composition was not known but according to Jamieson (80) this oil would probably contain approximately

Oleic Acid	58%
Linoleic Acid	20%
Saturated Acids	16%

Since oleic acid has been shown (P.42) to be a major acid of ruminant depot fat and also that oleic acid is one of the most readily utilised acids during mobilisation from depot fat (in non-ruminants,(similar information regarding ruminants being lacking) it is a logical anticipation for a fed oil of high oleic acid content to be readily assimilated. As mentioned on P60 Hilditch and Jasperson (69) in feeding groundnut cake of oleic acid content 55% of the total fatty acids, noted an increase in the oleic acid content of the milk fat plus a decrease in the lower fatty acids. It would seem that the effects of feeding peanut oil in Experiment 1 may have paralleled those noted in greater detail by Hilditch and Jasperson.

C. Adding weight to the between cow-pairs discussion it may be pointed out that the differences between days have been shown to be highly significant.

From inspection of Graphs 1, 2, and 3 it is apparent that over the period 22nd. April to 28th April a longer term effect was functioning. There is a marked smoothing-out of the a.m./ p.m. effect over the 25th. and 26th. April where in all

individual samples except those from cow No.1, the Iodine Values of the morning of the 26th April were higher than the corresponding ones of the previous evening which is in marked contrast to the regularity of the a.m./p.m. effect over the ensuing 6 days to the end of the experiment.

D. The interaction between treatments and days which the analysis of variance has shown to be highly significant indicated that the effect of the treatment differed on different days and is probably due to the longer term effect mentioned in the discussion on differences between days.

E. The treatment x period interaction proved to be non-significant, showing that the treatment effect did not differ as between a.m. and p.m. milkings.

F. From the days x periods interaction proving highly significant it appears that the period (a.m./p.m.) effect was not the same on all days.

G. The striking evidence of period (a.m./p.m.) differences (shown to be highly significant) is the prinicipal result arising from the frequent sampling. It is an effect which would have been effectively masked by:-

composite sampling of morning and evening milk fats,
composite sampling over longer periods,
or by intermittent sampling.

The cause of the effect is not apparent. The cows were receiving pasture plus a small amount of silage both morning and evening so there were no obvious differences of diet between day and night. If the effect were to studied throughout a lactation period better indications as to the cause might accrue. Hancock (89) has shown that with dairy cows the time spent grazing over 24 hours is divided into 60% between morning and evening milking, and 40% between evening and morning milking. Hancock points out that most of the rumination is done during the night for during the day the majority of cows are too busy grazing to ruminate.

One possible cause of morning / evening variation in Iodine Values is here suggested. During the day when the cow is actively

engaged grazing, gathering a large bulk of feed into the rumen without previous chewing, there may be to a very minor extent an effect of partial starvation resulting from the mobilisation of body reserves to a slightly greater extent than during the ~~day~~ NIGHT. In Experiment 1, five of the cows almost invariably secreted a greater amount of milk at the morning milking than at the evening milking. This might at first sight appear to bear some relation to the diurnal variations in Iodine Values; but Cow 5 did not conform to the greater-secretion-in-morning principle. The milk weights for this cow were in opposite relation to those of the other five cows, viz: milk weights were greater at the evening milking that at the morning milking. In spite of this the trends of Iodine Values for the evening / morning fat samples from this cow followed those of the other cows, as readily may be observed in Graph 1. Moreover since all cows in the experiment were non-pregnant several came in-season during the course of the experiment. At these instances the milk weights of these particular cows declined markedly, usually for one milking only returning to normal or above at the succeeding milking. Regardless of the marked difference in milk weights recorded at these milkings the diurnal rythm of Iodine Value change continued uninterrupted. (milk weights are recorded in Appendix 111) It would appear from these observations that amount of milk secreted bears little relation to the observed regular changes in degree of unsaturation of the milk fat.

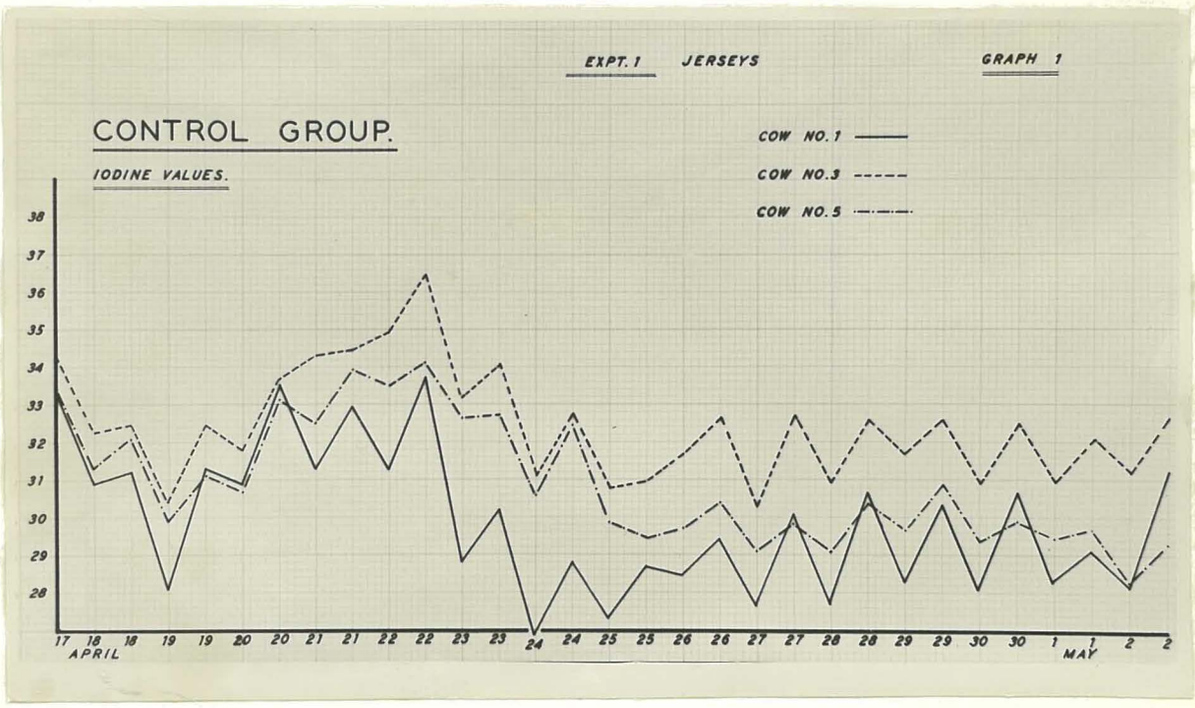
Brouwer and Jonker-Scheffener (91) (Holland 1947) found that grass feeding effected increased in the vaccenic acid content of the butterfat secreted by dairy cows and that a positive correlation may exist between the vaccenic acid content of the milk fat and its Iodine Value. No significant differences between morning and evening samples were found. The extent to which grass feeding was carried out in the trial was not noted in the abstract. Cannon, Espe, and Bird (92) in an experiment on soybean feeding to dairy cows found unexplained daily fluctuations in Iodine Values of the milk fats (not only from soybean-fed cows.) It was suggested that a

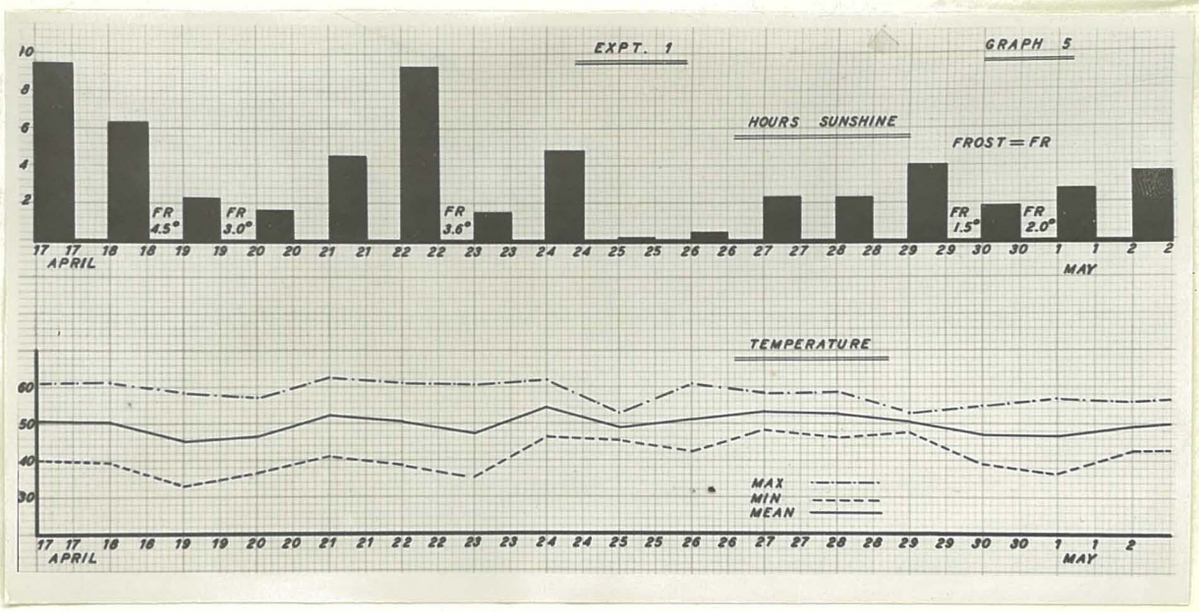
correlation might exist between temperature and Iodine Value but no data were furnished..

Regan and Richardson (93) had shown earlier with dairy cows that as external temperature increases respiration rate increases and pulse rate falls, but body temperature remains constant at 101°F until an external temperature of 70°F is reached, after which, body temperature does increase. In their experiments when the external temperature was raised above 80°F marked changes in milk composition and butterfat constants occurred.

At no time during Experiment 1 did the daily temperature rise above 63°F which would tend to throw doubt on the suggestion that temperature variations experienced during Experiment 1 may have been related to the changes in Iodine Values recorded.

In Experiment 1, weather data were inspected for possible relationships to the fluctuations in Iodine Values. Temperature and Hours of Sunshine were the only records that appeared to bear faint relation to trends in Iodine Values. For purposes of comparison Graph 1. is repeated below together with Graph 5 which illustrates variations in hours of sunshine and maximum, minimum, and mean temperatures throughout the experiment.





It may be seen that the major trends of Iodine Values appear to follow very approximately the recorded hours of sunshine. In respect to the temperature, if degree of unsaturation of butterfat and temperature are related in any way, marked fluctuations in temperature may feasibly be expected to be reflected in some marked alterations in Iodine Values. In Graph 5 the greatest daily temperature ranges occur between the 17th. and 23rd. April and to some extent tend to coincide with the period of marked fluctuations in Iodine Values in Graph 1, but on May 1st. the temperature range again extends towards previous values in the early part of the experiment, without apparent reflection in Iodine Value changes.

The 4.5° frost on the morning of the 19th April coincides with the marked depression of Iodine Values of Graph 1 on the same morning, and the 3.6° frost on the morning of the 23rd. April coincides also with a marked depression of Iodine Values. Although in the above cases some conformity does appear, none is evidenced on the 20th. and 30th April and 1st May when other frosts of lower degree were experienced.

Since in Experiment 1 there seemed to be a slight possiblity of relationship of weather to degree of unsaturation of butterfat, sunshine and temperature records were graphed with the results from Experiments 2, and 3, as well and in these cases no relations appear to exist. (See Graphs 6 and 10, P.91 and Graphs 11 and 13. P.99)

The evidence which has been presented indicating the existence of diurnal variation in degree of unsaturation of milk fat points to the desirability of ascertaining if this effect continues throughout lactation and is not a short term irregularity. If the effect does persist throughout lactation it emphasises the necessity, if daily composite samples for the purpose of determining butterfat constants are to be readily accurate, of taking equal quantities of milk fat from morning and evening milk.

Diurnal and longer-term variations such as clearly

illustrated in Experiment 1 could invalidate work founded on minor differences between composite samples.

EXPERIMENT 1.

SUMMARY.

The daily addition of 8 oz. of an unsaturated oil to the ration of lactating dairy cows was shown to lead to an increase in the degree of unsaturation of the milk fat secreted; the increase becoming manifest 3 to 4 days following the commencement of oil-feeding; the return to 'normal' requiring 3 to 5 days following the cessation of oil-feeding.

Lactating dairy cows were shown to exhibit marked individuality with regard to the Iodine Values of the milk fat secreted.

The existence of diurnal differences in degree of unsaturation of milk fat secreted by dairy cows was demonstrated, in addition to longer-term variations.

The importance of diurnal variations in milk fat constants with regard to the practice of composite sampling was considered.

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EXPERIMENT 2.

(June / July 1949)

The Effect of Short-term Inanition on the Degree of Unsaturation of Milk Fat Secreted by Dairy Cows.

Material:-

The animals used in this study were four Friesian cows of the College herd; designated A,B,C, and D in the ensuing data.

A and C were "control"animals.

B and D were "experimental" animals.

Status of cows at time of experiment:-

Cow	Age yrs.	In lactation	Pregnancy	Condition
A	5	11 months	6 mths. preg.	Fairly good.
C	8	20 "	non-preg.	Fat
B	8	12 "	"	V.Good
D	5	14 "	"	V. Good

Normal feed:- During the course of the experiment, with the exception of the period of inanition of cows B and D, all cows were pasturing with the College herd and receiving in the bail at each milking about 1lb of concentrate each (approx. S.E. 60)

Duration of experiment:-

Prior to inanition	8½ days
Period of inanition	1½ "
Post-inanition period	4 "
Total	14 days

Routine:- Milking was carried out at 5 a.m. and 4.30 p.m. daily.

Sampling:- was carried out at evening milkings only, for all cows for the 14 days of the experiment. The cows were

milked individually by machine into "testing" buckets, the milks weighed, and a quart sample of each taken, with the exception of cow B whose milk was of low test and necessitated taking a 2-quart sample in order to obtain an adequate amount of cream for churning.

Inanition was effected in the treatment period by withholding cows B and D from pasture on a concrete yard for a period of 36 hours. Adequate water was allowed.

Ration allowed each for cows B and D for each 12-hour period of the 36 hours inanition consisted of:-

2¼lbs concentrate supplying approx.	1.35 lbs.	S.E.
3½lbs hay supplying approx.	1.35 lbs.	S.E.
Total	2.70 lbs.	S.E.

The above ration was approximate only and was designed to be comparable on a Starch Equivalent basis to the ration employed in Experiment 3 during similar periods of inanition where peanut oil was included in the ration. The concentrate was supplied in the regular feed-boxes in the milking bails and the hay was fed outdoors on the concrete yard.

Laboratory Routine and Iodine Value determinations were exactly as for Experiment 1.

(The Friesian cream proved to be slightly more difficult to churn than the Jersey cream of Experiment 1)

The actual Iodine Values found for the fat samples of Experiment 2 are set out in Appendix V.

(One sample from cow D on July 3rd was missed owing to a shed-worker inadvertently placing the "regular" machines on the cow without the usual testing bucket for collecting the sample)

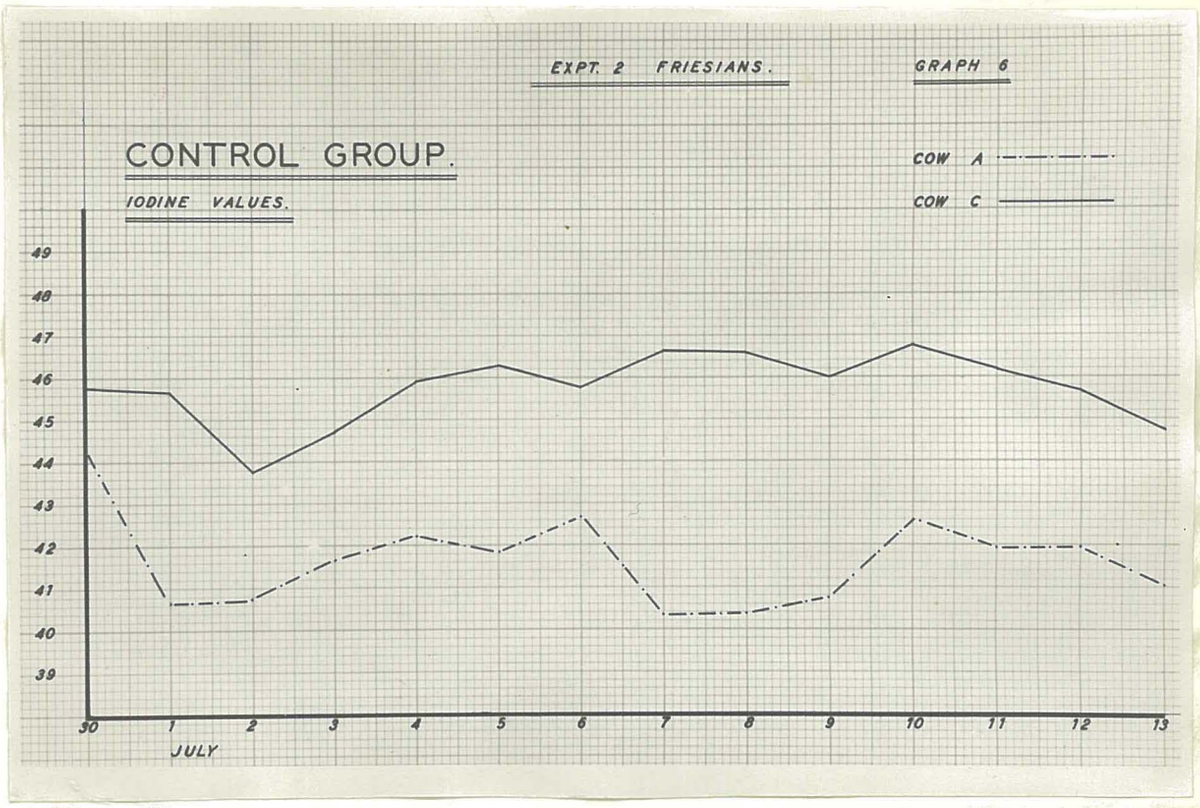
EXPERIMENT 2.

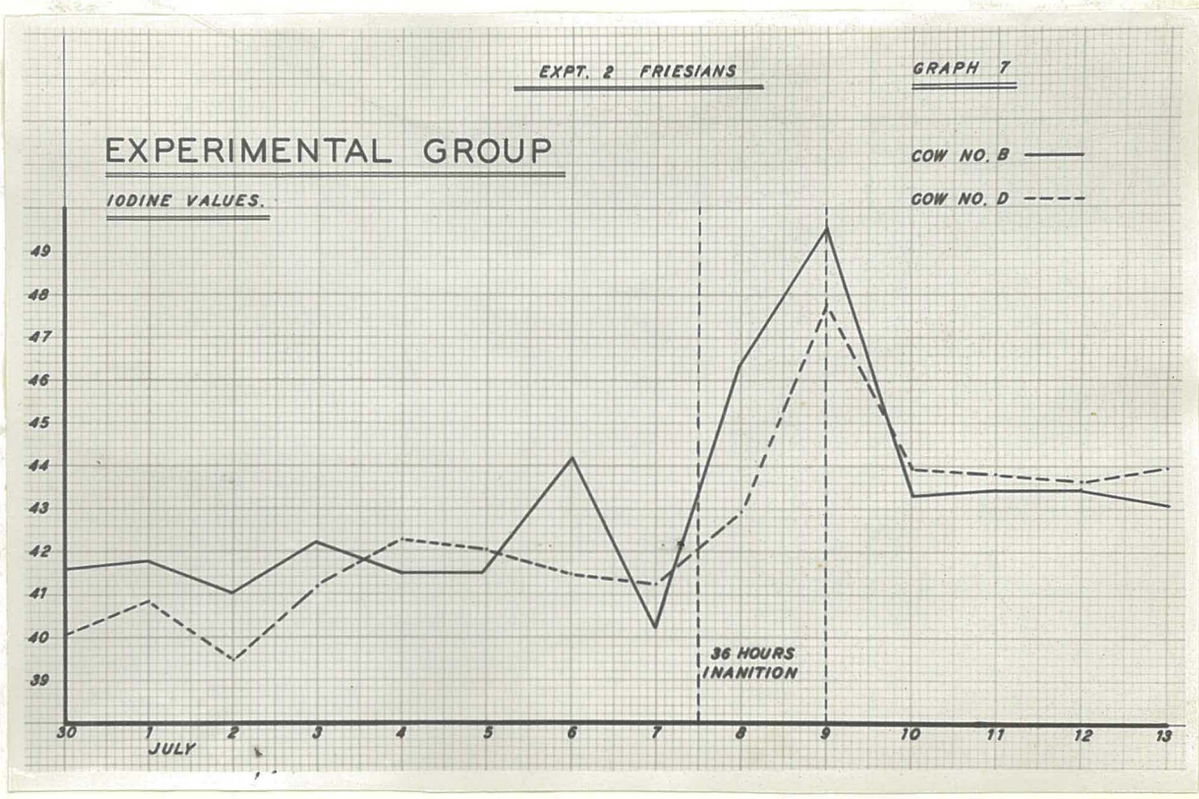
RESULTS

Graphs 6 and 7 below, show the changes in Iodine Values of fat samples from individual cows throughout the experiment.

(In Graph 7 the missing Iodine Value for cow D on July 3rd was estimated at a value that seemed reasonable in order to facilitate drawing an unbroken graph.)

The immediate and marked effect of inanition may be readily noted from Graph 7.





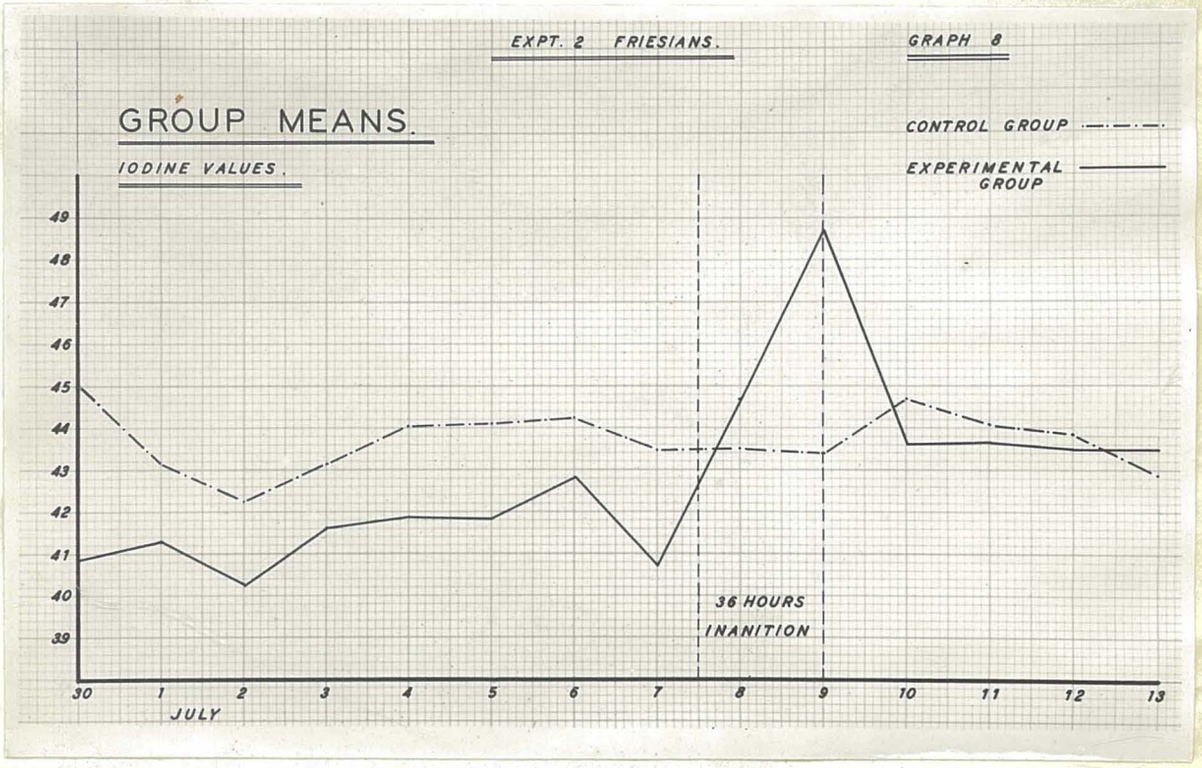
EXPERIMENT 2.

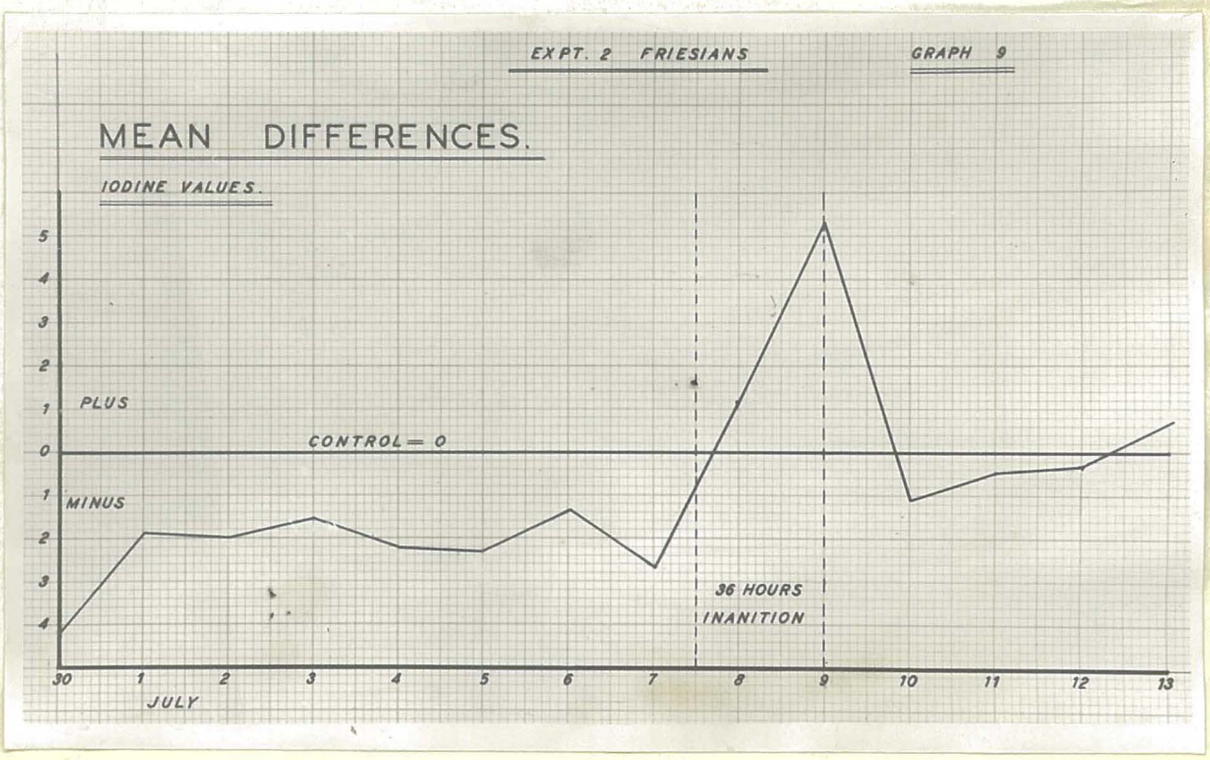
RESULTS (continued)

Graph 8, below, demonstrates the changes in mean Iodine Value of fat samples from cows in each group at each sampling throughout the experiment.

Graph 9 shows the degree to which the mean Iodine Values of the fat samples at each sampling from the starved group varied above or below the mean Iodine Values of the fat samples at the corresponding samplings of the Control group.
The immediate effect of inanition is again emphasised in these graphs.

(The fact that the mean Iodine Values of the starved group amount to a straight line between July 7th. and 9th. is purely co-incidental.)





EXPERIMENT 2.

DISCUSSION OF RESULTS.

Experiment 1 demonstrated clearly the importance of considering diurnal variations in degree of unsaturation of milk fats from individual cows when carrying out composite sampling, in cases where the samples obtained are to be subjected to critical analysis.

In view of the fact that both in Experiments 2 and 3 only major effects were to be investigated, and in order to lessen the amount of work involved, sampling was carried out at evening milkings only.

It may be seen from Graphs 6 and 7 that, as in Experiment 1, the Iodine Values of fat samples from individual cows demonstrate marked individuality. Of the control cows A and C, Cow A exhibited a degree of unsaturation of milk fat consistently lower than cow C. Cow A was in normal lactation and pregnant whereas Cow C was non-pregnant, had been in lactation 20 months, and was extremely fat.

Although the variations in Iodine Values of fat samples from milk of cow A show an opposite trend from those of cow C over the period 6th to 9th July, certain long-term influences seem, in general, to affect the degree of unsaturation of the milk-fat from both cows similarly. This parallels some of the observations in Experiment 1.

In like manner cows B and D show similarity of trends in the period prior to the imposition of inanition. Diurnal variation is not, of course, illustrated as only evening samples were being taken.

The immediate effects of inanition are strikingly shown in Graphs 7, 8, and 9. Also it is seen that on resumption of normal diet there is a prompt return toward normal in the degree of unsaturation of milk fat secreted but that in the particular case of the two cows under experiment normal values were not immediately regained. Experiment 3 was commenced a few days later and from Grap 12 it may be seen that from the 19th July onward the Iodine Values of milk fat samples from cow D were at a 'normal' level similar to those evidenced

in the period prior to inanition in Experiment 2, i.e. :- from Graph 7 cow D had not regained normal values up to 4 days following resumption of full rations after inanition, but 10 days later when Experiment 3 commenced the Iodine Values of samples from cow D appeared to be normal. Returning to the idea of competitive balance of supply of milk-fat precursors put forward in the concluding section of the General Review of Literature, if body fat is utilised fairly directly during inanition it would appear from Experiment 2 that the normal balance may be a delicate one and rapidly responsive in a compensatory direction when the supply of precursors of milk fat from any one source is diminished. If utilisation of depot fat for participation in milk-fat secretion is not a normal mechanism it could be expected that the response to "fill the gap" in supply would not be quite so rapidly evidenced as in Experiment 2. The need for greater frequency of sampling is indicated in order to follow better the rapidity of response stimulated by inanition.

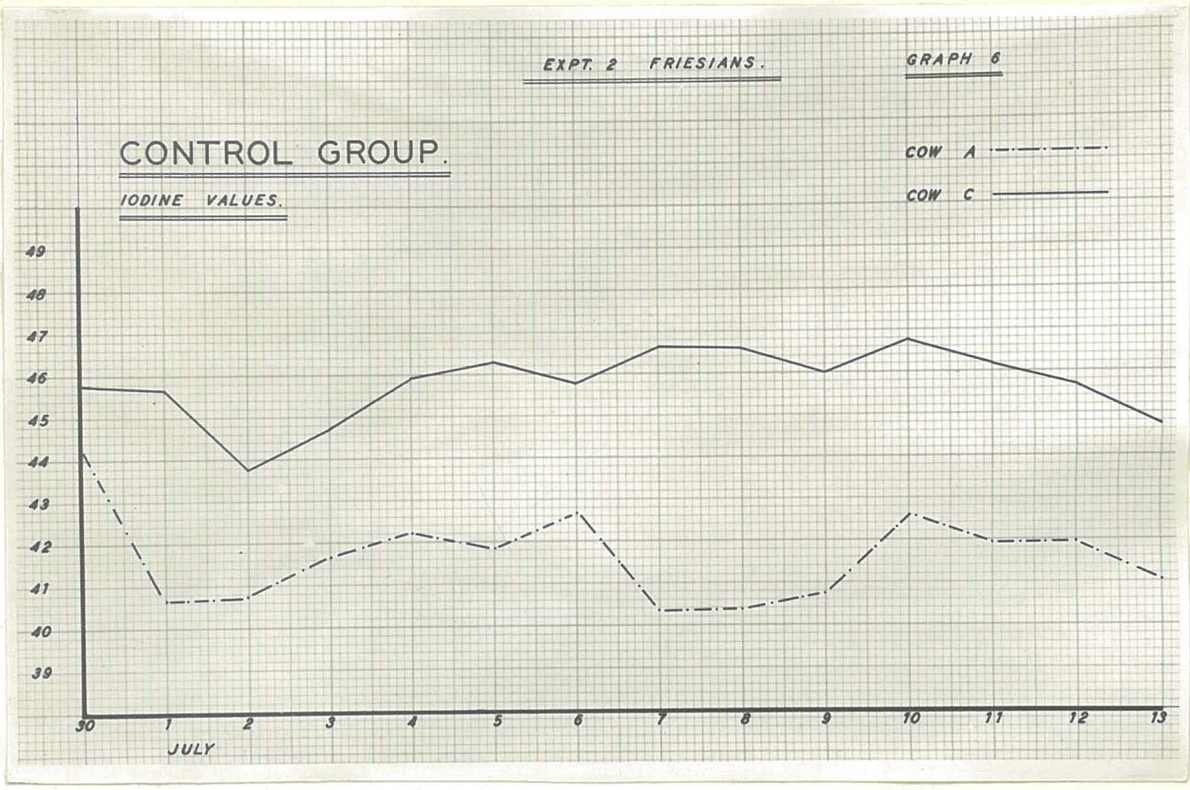
In addition to a simple effect resulting from drastic underfeeding in Experiment 2, there was the "fretting" aspect of the two experimental cows being shut away from the rest of the herd for the 36-hour underfeeding period, an entirely unaccustomed procedure quite apart from the imposition of inanition. Further, there was the additional upset of rejoining the herd for each milking and the repeated frustration of not being able to return to pasture with the rest of the herd following the milking. It is possible that this factor may have accelerated and magnified the changes resulting directly from inanition.

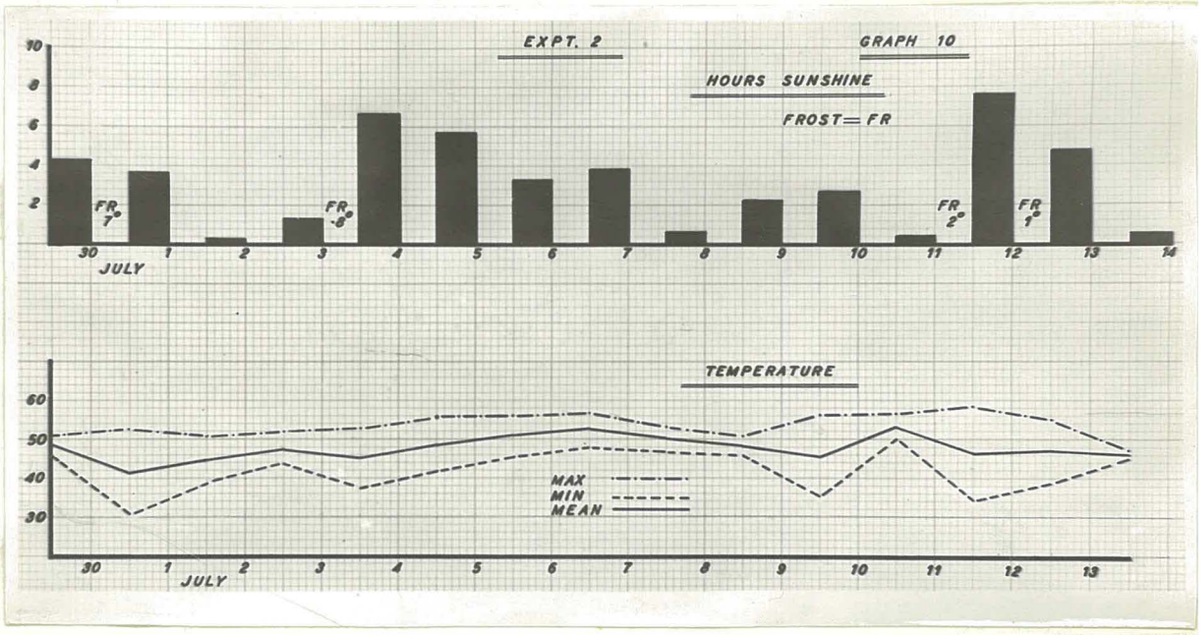
If Friesian milk fat samples exhibit the same type of diurnal variation of degree of unsaturation as shown for Jersey milk fat in Experiment 1, morning samples would have lower Iodine Values than the evening's. Applying this to Graph 7: the 36-hour period of inanition was commenced after the morning milking on the 8th July and the iodine value of the milk fat of that milking would be expected

to be lower than that shown on the graph for the evening milking of the 7th July. The first Iodine Values determined during inanition were of samples collected on the evening of the 8th 12 hours after the period of inanition commenced. If the degrees of unsaturation of the milk fats on the morning of the 8th were lower than those shown for the evening of the 7th. the resulting graph if plotted for the first 12 hours inanition would prove considerably steeper and of greater range than shown by Graph 7. Likewise the return toward normal may be more rapid than Graph 7 indicates.

As intimated in the discussion of results of Experiment 1 weather data were inspected during the course of Experiment 2 for possible effects on the degree of unsaturation of milk fat of the cows under experiment.

For purposes of comparison Graph 6 showing the Iodine Value trends of the milk fats of the control cows in Experiment 2, is repeated below together with temperature and hours-of-sunshine graphs. Although in Experiment 1, a possible relation appeared in one or two places, they do not appear to be paralleled in Experiment 2, except perhaps for the downward trend in Iodine Values from the 1st to 2nd July coinciding with a marked decrease in the hours of sunshine over the same two days.





In the absence of data from greater frequency of sampling but utilising the inferences from Experiment 1 it may be expected that the witholding of dairy cows from pasture for even brief periods of a few hours may result in marked changes in milk fat constants,

EXPERIMENT 2.

S U M M A R Y.

It was demonstrated that by witholding feed from dairy cows for 36-hour periods marked increases in degree of unsaturation of the milk fat secreted may be effected, and that this may become evident within the first 12 hours.

The marked 'individuality' of dairy cows with regard to the nature of the milk fat secreted was observed as in Experiment 1.

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EXPERIMENT 3.

(July / August 1949)

THE EFFECT OF INANITION ON THE DEGREE OF UNSATURATION OF MILK FAT SECRETED BY DAIRY COWS WHEN 1 LB. OF PEANUT OIL (Iodine Value 96.2) IS SUPPLIED DAILY IN THE RATION.

Material:- The cows used in Experiment 3 were three Friesian cows from the College herd, being cows A, B, and D which were employed in Experiment 2.

Cow D was used as a control animal.

Cows A and B were experimental animals.

Status of cows at time of experiment:-
As for Experiment 2.

Normal feed:-
As for Experiment 2.

Duration of experiment	Cow A	Cow B	Cow D
Prior to inanition	11½ days	7½ days	
Period of inanition	1½ "	1½ "	Control
Post-inanition period	4 "	8 "	
Total	17 days	17 days	17 day

Routine:- Milking was carried out at 5 a.m. and 4.30. p.m. daily. Sampling was carried out at evening milkings only for all cows for the 17 days of the experiment. The cows were milked individually into 'testing' buckets, the milks weighed, and a quart sample taken from the milk of Cow D, and two-quart samples taken from Cows A and B.

Ration:- In addition to normal pasturing with the herd and concentrate ration as described in Experiment 2 (1lb. concentrate at each milking), cows A and B received 1lb of peanut oil (Iodine Value 96.2) mixed in each evening's concentrate ration. This resulted in quite an oily mash but was accepted quite well little residue being left.

Inanition was effected, as in Experiment 2, in the respective treatment period allotted Cows A and B, by withholding these cows from pasture on a concrete yard for periods of 36 hours. Adequate water was allowed.

Ration allowed each for Cows A and B during the allocated periods of inanition amounted to a total daily allowance of:-

1lb. of peanut oil supplying approx.	2.41lbs.	S.E.
3lbs. of hay supplying approx.	1.20lbs.	S.E.
3lbs. of concentrate supplying approx.	1.80lbs.	S.E.
Total	5.41lbs.	S.E.

i.e. equivalent to about 2.70 lbs of S.E. for each 12 hour period. As mentioned in Experiment 2 the ration used in that Experiment was approximate only and designed to be comparable on a Starch Equivalent basis to the ration shown above; but as the food values may only be estimated and the losses in attempting to feed such small quantities of hay under the conditions of the experiment, difficult to assess, (rain, wind.) the rations are only roughly comparable.

Laboratory Routine and Iodine Value determinations were exactlyas for Experiment 1 and 2.

(As in Experiment 2 the Friesian cream proved to be slightly more difficult to churn than Jersey cream but in addition, cream from the oil-fed cows A and B proved more difficult than from Friesian cows on normal rations. Accordingly as has been noted under Sampling (above) two-quart milk samples were taken from Cows A and B throughout the experiment.)

The actual Iodine Values found for the fat samples of Experiment 3 are set out in Appendix VIII.

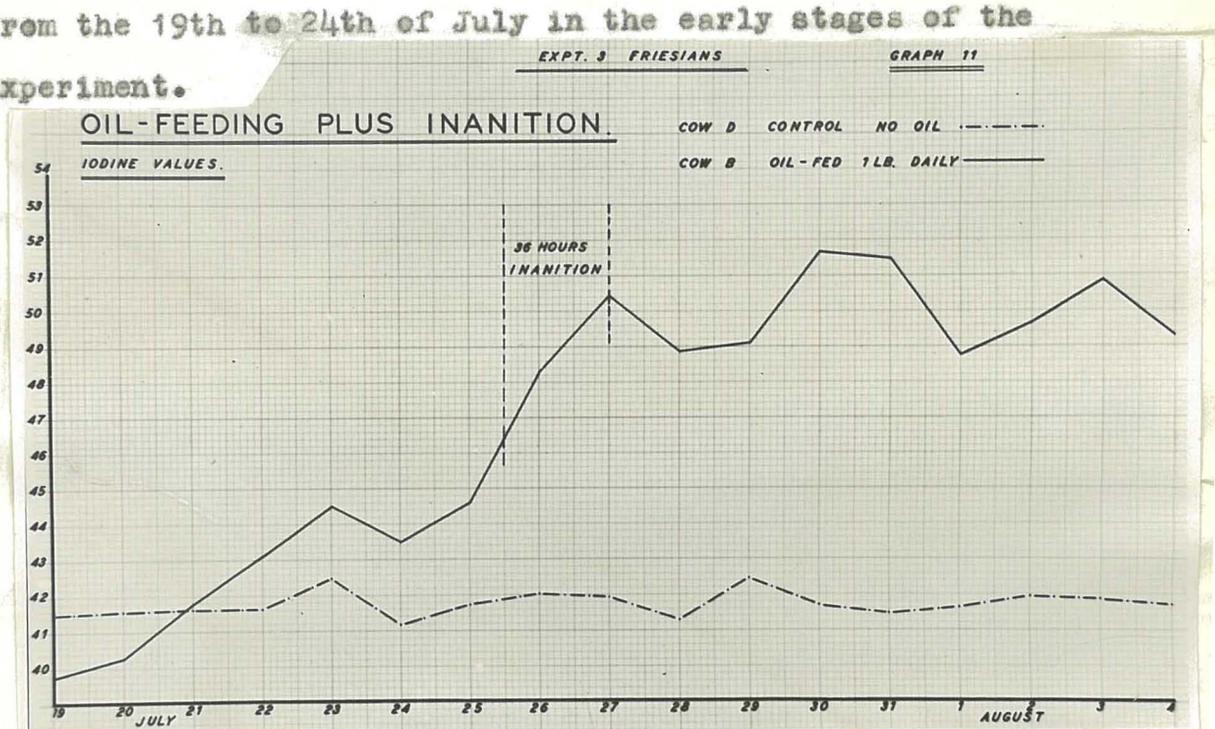
EXPERIMENT 3.

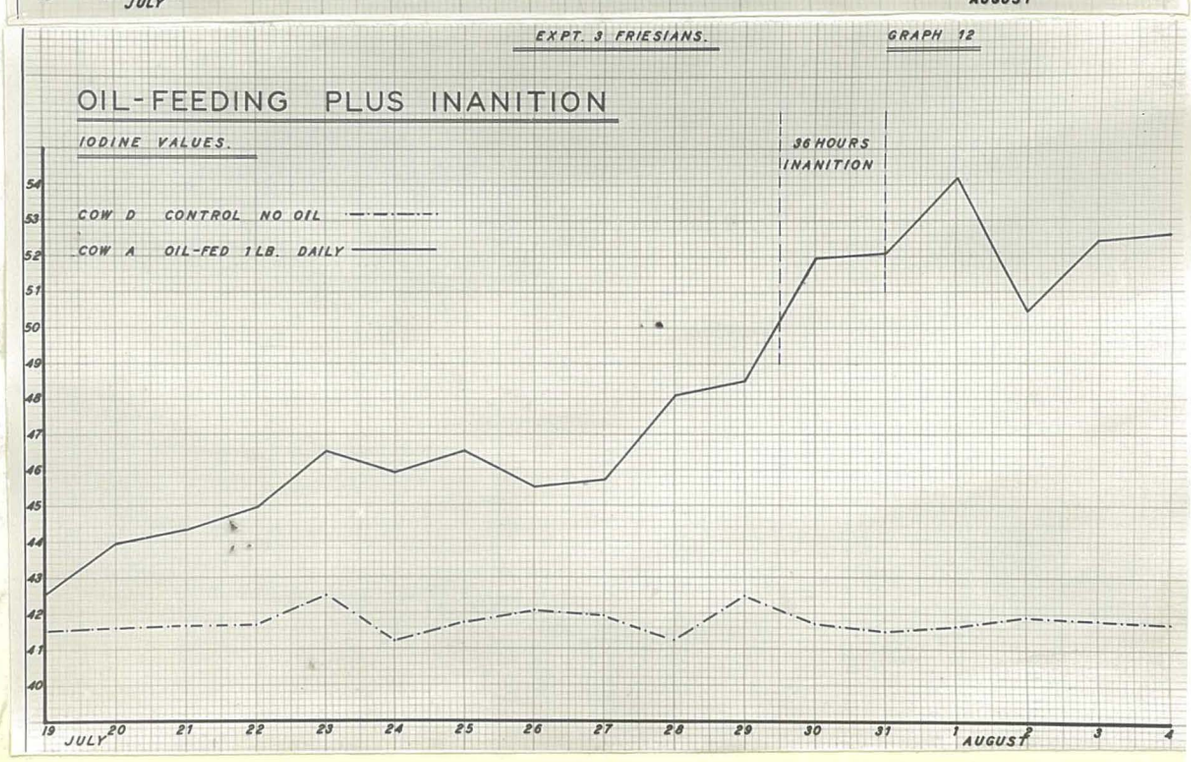
RESULTS

Graph 11 and 12 below, illustrate the trends in degree of unsaturation of milk fat from individual cows throughout the experiment.

The Iodine Values for control Cow D appear in both graphs.

The trends in Iodine Values for Cows A and B show plainly the increasing degree of unsaturation of milk fat apparently induced by the oil-feeding. Also the periods of inanition appear to have been imposed while the Iodine Values of the milk fat were still increasing. In spite of the oil-feeding to Cows A and B there appears to be some parallelism between the degrees of unsaturation of milk fat from all three cows from the 19th to 24th of July in the early stages of the experiment.





EXPERIMENT 3.

DISCUSSION OF RESULTS.

In Experiment 1 it had been noted that as judged by the Iodine Values the oil-fed group differentiated itself from the control group by the third day at an oil-feeding level of 8 oz. per head per day.

In Experiment 3 it was anticipated that the Iodine Values of the oil-fed cows would probably reach a maximum levels by the sixth day after commencement of oil-feeding. Then by imposing inanition it was expected that blood lipids resulting from the ingested oil (to which the cows had become accustomed) might be freed from the competition of lipoids normally resulting from carbohydrate of the food, and be utilised to a greater extent by the mammary gland, becoming manifest by extremely high degrees of unsaturation of secreted milk fat.

By inspection of Graph 11 it appears that Cow B was subjected to inanition while the degree of unsaturation of the milk fat was still increasing as a result of the oil-feeding. It seems likely that the inanition accelerated the increase since the Iodine Value of the milk fat was lowered slightly on return to normal feeding plus oil. With only one cow being subjected to inanition at this time, and the results not very marked it is not possible to draw any definite conclusions. It may be noted that the drop in Iodine Value of the milk fat of Cow B following inanition coincides with a fall of lesser degree in the Iodine Value of the milk fat of the control cow C, so it is possible that the slight fall in Iodine Value of milk fat from Cow B following inanition may not be related entirely to the effect of returning to normal rations.

As emphasised by Experiment 1, with "individuality" of cows involved and so few samples, no outstanding effect attributable to inanition being noted, positive conclusions may not be drawn.

Cow A (Graph 12) was subjected to inanition at a later stage of oil-feeding but it was still not clear

whether a maximum degree of unsaturation of milk fat as a result of oil-feeding had been achieved at that time.

From Graph 12 the steady increase in Iodine Values of milk fat of Cow A appears to continue undisturbed by the imposition of inanition with no marked fall occurring immediately following the relaxing of inanition, although an irregularity does appear on the second day.

The fall in milk yield of Cows A and B resulting from inanition during oil-feeding was not as marked as in the corresponding periods of inanition in Experiment 2. (For detail of milk yields see Appendix IX)

It is apparent, in spite of having so few cows in the experiment, that oil-feeding affected the degree of unsaturation of the milk fat in a similar manner to that shown in Experiment 1 but it seems that with the heavier daily ingestion of oil (1 lb. as compared with 8 oz. in Exp.1) the "build-up" effect of the oil-feeding extends over a longer period than expected. Brown, Dustman, and Weakley (83) had observed that under the conditions of their experiments a period of three weeks was required before the degree of unsaturation of milk fat reached its maximum as a result of the feeding of 1lb of soybean oil daily. The highest value obtained was 52. From this it would appear that had Experiment 3 been continued the Iodine Values of the milk fat would probably shown little increase on the v alues obtained within the period of the experiment.

The fact that the experiment was conducted on such a small scale and that only one type of oil was fed renders interpretation difficult, but a possible explanation is suggested:-

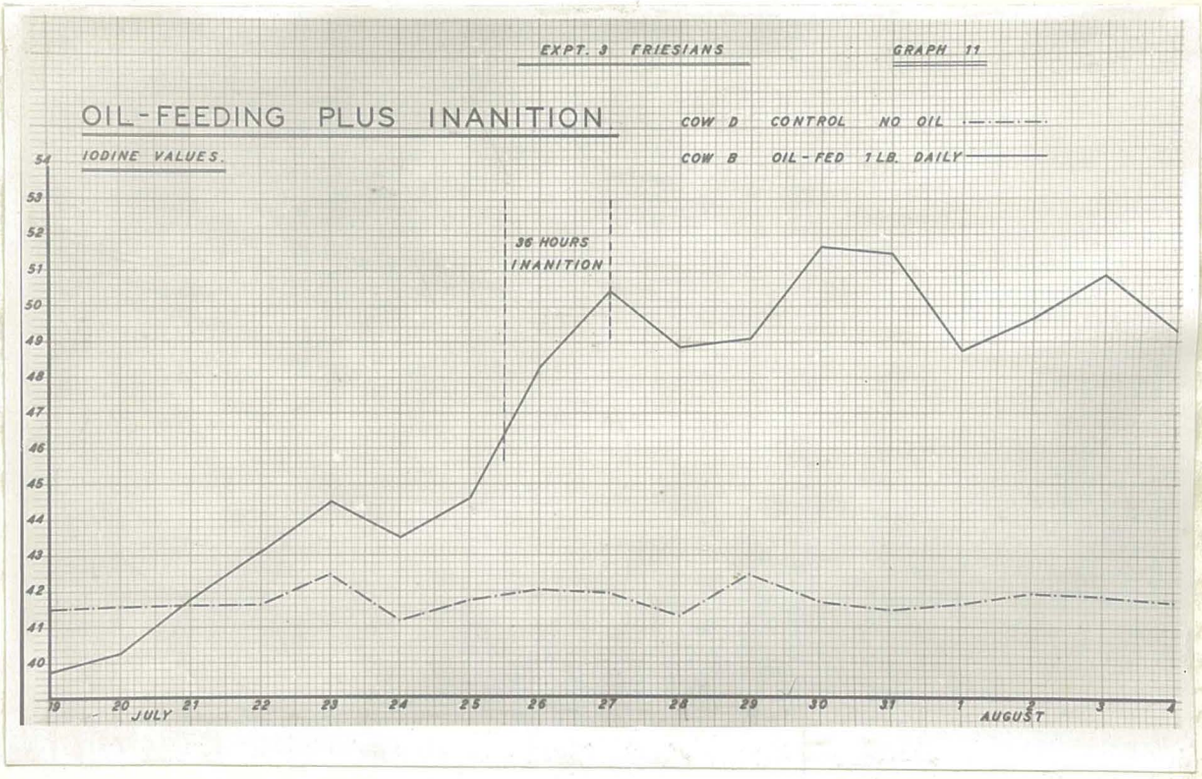
If body depot fat may be drawn upon readily to supply milk fat precursors, and if the theory that oleoglycerides are the "first preference" glycerides to be withdrawn from depot fat is correct, it would be possible for theoretical purposes to postulate a hypothetically perfect instance where, in the first stage of starvation, pure

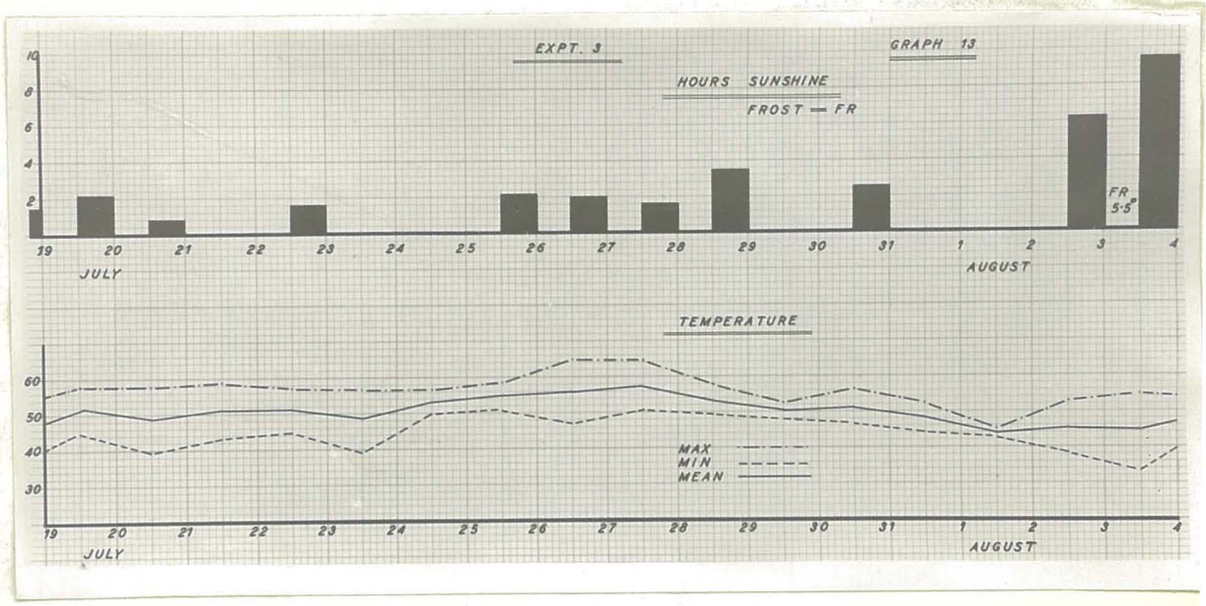
triolein was being withdrawn from adipose tissue. This would be expected to have an Iodine Value of 86.2 In the case of Experiment 3 the oil being fed was not very different in degree of unsaturation, being of Iodine Value 96.2. This latter oil would be expected, in addition, to be somewhat altered during the processess of digestion and absorption. In effect blood lipoids resulting from the oil being fed may have differed very little in degree of unsaturation from those being offered the mammary gland from the fat depots. If such a situation may exist it is feasible that it may have been in operation in Experiment 3. It would be of interest to duplicate the experiment using greater numbers of cows and to feed oil of low degree of unsaturation in comparison with oils of similar degree of unsaturation to ruminant depot fat, of similar degree of unsaturation to triolein, and of markedly higher degrees of unsaturation than triolein, and in comparison with simple inanition. It might logically be anticipated that the feeding of oils of low unsaturation such as coconut oil, or hydrogenated oils, during inanition, might lessen the extent of the normally manifested increases in Iodine Value of milk fat if these ingested oils were to be utilised more avidly during the period of drastic underfeeding.

The fact that superimposing inanition on oil-feeding to dairy cows did not appreciably modify the normally observed effects of oil-feeding, together with the fact that the oil being fed was not markedly dissimilar in degree of unsaturation from triolein, adds further circumstantial evidence to the theory that the oleoglycerides, known to be predominant glycerides in ruminant depot fat, may be readily available to furnish milk fat precursors "on demand" i.e. :- that depot fat may be mobilised as required to participate in milk fat synthesis.

For comparative purposes Graph 11 is repeated below together with Graph 13 which shows the temperature and hours of sunshine during the period of the experiment.

As in Experiment 2 there does not appear to be any relation obvious between the weather data shown and degree of unsaturation of milk fat secreted throughout the same period.





EXPERIMENT 3.

S U M M A R Y.

As in Experiment 1, the addition of 1lb of peanut oil to the daily ration of lactating dairy cows was shown to lead to marked increases in the degree of unsaturation of the milk fat secreted.

When periods of 36 hours inanition were imposed on lactating dairy cows, which were receiving 1lb of peanut oil daily in addition to the normal ration, no marked effects attributable to greater utilisation of blood lipids and lipoids resulting from the ingested oil were apparent.

It was suggested that the blood lipids and lipoids resulting from the ingested oil (Iodine Value 96.2) nearly may have matched in degree of unsaturation the blood lipids and lipoids resulting from mobilisation of the most readily available glycerides of depot fat; presumably oleoglycerides.

.....

EXPERIMENT 4.

THE EFFECTS OF FEEDING A STAINED FAT TO A LACTATING GUINEA PIG.

Procedure:-

Compressed-meal "nuts" on which experimental guinea pigs are normally fed were dipped in melted butter-fat deeply stained with Sudan IV.

These were fed ad lib to a lactating guinea pig and her two young one week following parturition.

Seven hours after placing the stained food in the cage it was observed that an appreciable amount of the food had been eaten. The adult guinea pig was killed by chloro-forming and then dissected. The mammary glands were carefully removed and about 0.25 cc. of milk expressed via the teats by squeezing the glands. The young were also killed and examined.

The milk was then centrifuged to separate the milk fat as a concentrated layer.

Another guinea pig which had not received stained fat was also killed and dissected for comparison of depot fats.

RESULTS:

The milk on being expressed from the glands was faintly but positively pink. On being centrifuged for 20 minutes in a small phial the fat layer separated to the top satisfactorily. This fat layer was markedly pink in colour.

The depot fats of both the adult guinea pig and her young were faintly pink in colour in comparison with the depot fats of the guinea pig which had not received stained food. The full length of the intestines of the guinea pigs fed stained fat were of definite pink colour in comparison with the grey of the intestines of the normally-fed animal.

EXPERIMENT 4.

DISCUSSION OF RESULTS.

The observations in this experiment in general corroborate those of parallel cases mentioned in the literature.

Mendel and Daniels (11) had found difficulty in getting guinea pigs to evidence stained depot fat resulting from ingesting of stained food fat but in Experiment 4 seven hours after the ingestion of heavily stained food fat there was positive evidence of colouration of depot fats in both adult and young guinea pigs. Although the young were daily eating a certain amount of the normal adult ration they were also receiving the milk of the mother.

The appearance of stained milk fat in the milk of the adult was positive and it may be presumed that this stained milk fat would have contributed to the staining of the depot fats of the young.

The positive colouring of the intestines of the adult and young in comparison with that of a normally-fed guinea pig was not due to colouration of ingesta showing through. A washed section of the intestine of the coloured-fat-fed adult was still markedly pink in contrast to that of the normally fed animal. This would seem to confirm Frazer's (4) observation that fat absorption occurs in the intestine. It was only from the entry of the bile duct onward that the walls of the alimentary canal were pink in colour which would also appear to be evidence favouring the observations in the literature that bile salts are major factors in fat absorption.

.....

S U M M A R Y.

It was shown that the ingestion of fat stained with the fat soluble dye Sudan IV induces secretion of stained milk fat and appearance of slightly stained depot fat in guinea pigs.

The absorption of ingested fat appeared to occur principally in the small intestine.

EXPERIMENT 5.

THE RELATION OF THE DEGREE OF UNSATURATION OF BUTTERFAT TO ITS KEEPING QUALITIES.

This experiment was carried out on morning and evening samples from individual cows during the last week of Experiment 1.

Method:-

The milk fat samples used in this experiment were from Cows 1 to 6 in Experiment 1, taken at the a.m. and p.m. milkings of the 27th, 28th, and 29th April, and 1st May. These samples were originally taken for the purpose of carrying out Iodine Value determinations, and in the course of these the samples had not been heated for exactly similar periods of time so that the comparisons shown in Experiment 5 may be by no means critical.

2 ccs. of each sample were placed separately in small square-bottomed test tubes. (See plate in Appendix X)

The racks of samples were placed in a constant temperature cupboard maintained at 38° C.

A dilute solution of potassium dichromate was prepared to match in colour the first sample to discolour visibly on oxidation. Thereafter the number of days taken for each sample to discolour sufficiently to match the potassium dichromate solution was noted. In all cases the discolouration was sudden and not gradual.

The next page shows a table in which are set out the number of days required for each Sample to discolour.

DAYS TAKEN FOR SAMPLES TO OXIDISE.

	A. M.				P.M.				
Date / Cow	27th	28th	29th	1st	27th	28th	29th	1st.	
1	58	13	36	46	73	53	48	67	412
2	74	57	19	34	74	71	32	49	410
3	45	2	Unfin,	49	57	49	Unfin.	55	257
4	43	15	6	44	68	57	30	Unfin.	263
5	45	2	28	75	67	62	49	75	403
6	54	2	11	Unfin.	Unfin.	64	48	73	252
Sum.	319	109	100	248	339	356	207	319	1997
Avg.	53.1	18.1	20.0	49.6	67.8	59.3	41.4	63.8	

A.M. Average = 35.27 days

(Exluding low values of 2,2,2, of the 28th and 6, and 11 of the 29th

a.m. average becomes 44.3 days.)

P.M. Average = 58.1 days.

EXPERIMENT 5

DISCUSSION OF RESULTS.

No detailed analysis of the results has been made as the initial samples taken could not be considered perfectly standard, on account of having been heated previously for the purpose of taking samples for Iodine Value determinations. In general all samples would have had approximately the same amount of heating prior to Experiment 5.

It may be seen from the table of results that the average number of days taken for the A. M. samples to oxidise was 35.3 days but that several very low values occurred. If these are assumed to be the result of previous overheating and left out of the table the A.M. average becomes 44.3

Even this does not raise the average as high as that of the P.M. samples.

It would appear from this experiment that the A.M. samples which were of lower Iodine Value than the P.M. samples were more susceptible toward oxidation than the P.M.

This would not be in accordance with the observations of Henderson and Roadhouse (87) who found that "any condition or feed that will greatly increase the unsaturation of milk fat will increase the susceptibility of the fat to become oxidised."

The experiments of Henderson and Roadhouse were conducted on samples of extreme Iodine Values, low ones being of the order of 30.0 to 36.0 and the higher values 48.0 to 54.0.

The average Iodine Value of the A.M. samples in Experiment 5 was 29.8 while the average of the P.M. samples was 31.5. It is apparent that a much more critical experiment than Experiment 5 would be necessary in order to establish definitely whether or not differences in keeping qualities do exist between morning and evening samples of milk fat.

EXPERIMENT 5

S U M M A R Y

In an experiment carried out as a rough exploratory check it was found that samples of milk fat from A.M milkings, of average Iodine Value 29.8 tended to be more susceptible toward oxidation than samples of milk fat from evening milkings, of average Iodine Value 31.5.

It was emphasised that a far more critical experiment than Experiment 5 would be necessary in order to distinguish differences in keeping qualities between samples of milk fat from A.M. and P.M. milkings.

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VII.

APPENDICES.

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APPENDIX 1.

THE DETERMINATION OF IODINE VALUE.

Preparation of Wijs Reagent:-

Made up by dissolving crystals and iodine trichloride in acetic acid in the ratio of :-

8.5 gms. Iodine to 7.8 gms. of Iodine trichloride per litre of acetic acid.

Scrupulous dryness of containers is essential.

The total volume of acetic acid is halved and the iodine crystals and the iodine trichloride dissolved in the acetic acid. Occasional agitation aids the solution of the iodine but the iodine trichloride dissolves readily.

Once the iodine crystals have dissolved the two solutions are mixed to form Wijs Reagent.

(In Experiment 1, 2, and 3, Wijs Solution was prepared 5 litres at a time.)

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Determination of the Iodine Value of Butterfat:-

0.20-0.25 gms. of melted and filtered sample is weighed into a glass-stoppered 16 oz. bottle.

5 cc. of chloroform is added and the fat sample dissolved.

10 cc. of the Wijs Solution is added by pipette, allowing the same draining time at each determination.

The stopper is moistened slightly with 10% potassium iodine solution taking care to allow none to run down the inside of the bottle on replacing the stopper.

The stoppered bottle is then set aside in the dark for 30 mins.

10 cc. of 10% potassium iodine solution is added plus 100-150 cc. of distilled water. The potassium iodine solution is added in such a manner that it rinses the

stopper and neck of the bottle in entering. A wash-bottle may be used in addition.

The resulting light-brown solution is next titrated with N/10 sodium thiosulphate solution, the latter being added until the solution in the bottle is almost colourless. Violent shaking is necessary to enable the potassium iodine to take up any iodine remaining in the chloroform.

At this point a few drops of starch indicator solution is introduced and titration continued cautiously until the last trace of blue colour vanishes.

Blank determinations (all above reagents but no fat) are carried out at the same time as the actual determinations.

Iodine Value of the butterfat is given by

$$1.27 \times \frac{\text{(No. ccs. N/10 } Na_2S_2O_3 \text{ required for blank, minus No. ccs. N/10 } Na_2S_2O_3 \text{ required for sample.)}}{\text{Gms. of fat sample initially taken.}}$$

i.e.- the number of centigrams of iodine absorbed by 1 gm. of fat.

APPENDIX II

EXPERIMENT 1.

(April/May 1949)

IODINE VALUES OF INDIVIDUAL MILK FAT SAMPLES

Peanut oil (8 oz.) was given by drench to each of Cows 2, 4, and 6 following the morning milkings of April 19th. to April 25th, inclusive.

Analysis of Variance of Iodine Values (P.73A) was carried out on those values between the two dotted lines in the table.

(i.e.:- from April 22nd. to April 28th. inclusive.)

Date		Control 1	Control 3	Control 5	Experimental 2	Experimental 4	Experimental 6
April	17	33.33	34.26	33.37	32.90	32.40	34.19
am	18	30.87	32.22	31.34	29.26	30.26	30.96
pm	18	31.18	32.45	32.02	29.75	30.64	31.67
am	19	28.10	30.40	29.86	27.62	28.67	29.21
11 am	19	-	-	-	30.24	29.04	30.97
pm	19	31.34	32.43	31.02	30.64	30.75	31.80
11 pm	19	-	-	-	29.96	31.09	32.00
am	20	30.87	31.76	30.66	30.37	30.92	32.99
11 am	20	-	-	-	30.82	30.97	34.30
pm	20	33.46	33.59	33.12	34.41	33.07	35.46
11 pm	20	-	-	-	35.02	33.34	35.58
am	21	31.25	34.27	32.54	32.38	31.86	34.03
11 am	21	-	-	-	32.79	31.55	34.44
pm	21	32.90	34.44	33.90	32.84	32.84	37.09
am	22	31.25	34.93	33.47	33.05	33.38	36.95
pm	22	33.69	36.46	34.11	36.58	36.09	38.33
am	23	28.82	33.18	32.66	31.96	33.87	35.32
pm	23	30.19	34.04	32.69	33.66	34.85	36.24
am	24	26.92	31.08	30.60	30.85	33.36	35.37
pm	24	28.79	32.75	32.25	32.61	34.61	36.81
am	25	27.34	30.76	29.86	30.04	33.67	34.67
pm	25	28.68	30.93	29.49	30.33	33.56	35.68
am	26	28.44	31.65	29.77	31.96	33.85	36.12
pm	26	29.38	32.61	30.20	33.39	35.74	37.13
am	27	27.75	30.28	29.09	28.55	32.96	32.78
pm	27	30.09	32.74	29.87	31.83	33.34	33.58
am	28	27.72	30.91	29.13	28.95	30.39	31.08
pm	28	30.62	32.57	30.39	31.38	32.81	33.76
am	29	28.22	31.72	29.61	29.16	30.66	30.64
pm	29	30.36	32.62	30.82	30.93	32.66	32.48
am	30	28.08	30.91	29.37	29.69	29.17	30.07
pm	30	30.62	32.50	29.88	29.88	30.85	33.18
May	1	28.32	30.94	29.41	27.43	29.04	30.58
pm	1	29.08	32.03	29.69	29.73	30.63	32.77
am	2	Spilt	31.20	28.27	28.95	28.63	31.39
pm	2	31.19	32.71	29.28	30.65	30.48	33.82

APPENDIX III

EXPERIMENT 1

(April/May 1949)

MILK WEIGHTS RECORDED AT EACH MILKING FOR INDIVIDUAL COWS.

(Weight given in lbs.)

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Date	Cow	CONTROL 1	3	5	EXPERIMENTAL 2	4	6
April	17						
am	18	9.6	9.5	7.1	11.4	9.9	8.0
pm	18	8.0	7.9	9.3	10.1	8.5	7.8
am	19	10.6	8.8	8.0	12.1	8.9	8.2
11 am	19	-	-	-	5.6	2.1	3.5
pm	19	8.4	6.9	7.7	5.0	5.7	4.0
11 pm	19	-	-	-	5.5	3.2	4.9
am	20	11.5	NN5.0	9.4	7.6	6.9	4.8
11 am	20	-	-	-	1.4	2.5	3.9
pm	20	9.5	11.3	6.6	7.5	6.0	3.6
11 pm	20	-	-	-	10.1	4.7	6.0
am	21	11.1	8.9	10.0	5.8	5.2	4.7
11 am	21	-	-	-	6.9	4.5	5.4
pm	21	10.4	8.8	7.9	5.2	4.2	4.8
am	22	11.1	9.6	7.6	14.1	7.9	9.6
pm	22	9.2	7.7	8.4	10.1	9.4	9.1
am	23	11.2	8.8	7.1	12.8	10.0	10.1
pm	23	8.0	7.9	8.6	9.6	7.1	8.4
am	24	10.7	9.3	5.1	11.9	9.3	NN2.9
pm	24	8.6	6.9	9.6	9.9	7.6	13.5
am	25	10.4	8.3	5.3	11.5	10.5	9.7
pm	25	9.8	7.5	7.9	10.5	8.4	7.5
am	26	10.4	8.8	6.6	11.3	NN2.9	9.5
pm	26	8.5	6.3	6.8	9.1	13.3	7.0
am	27	11.0	9.2	7.1	12.5	10.3	8.9
pm	27	9.2	8.0	8.6	10.1	8.4	8.6
am	28	10.8	8.3	5.7	12.5	9.5	8.9
pm	28	9.4	9.1	8.1	9.7	8.1	8.2
am	29	10.7	8.4	6.5	12.0	9.8	9.4
pm	29	7.1	7.4	6.1	9.8	7.6	7.9
am	30	10.3	9.5	6.8	11.8	9.5	7.9
pm	30	7.6	7.4	7.0	8.3	7.5	8.5
May am	1	10.8	9.4	5.7	11.9	9.4	8.2
pm	1	7.9	6.8	8.9	9.3	7.9	8.2
am	2	11.1	10.0	5.8	10.9	9.2	9.2
pm	2	9.3	7.7	7.8	10.8	7.9	7.7

.....

NN = Not normal; in season.

APPENDIX IV

EXPERIMENT 1

Analysis of Variance of Iodine Values of Milk Fat Samples over the period April 22nd. to April 28th. inclusive.

Source	d.f.	ss	ms	
Between cow-pairs	2	157.8559	78.9279	**
Between treatments	1	152.7931	152.7931	**
Error 1,(Treatments x pairs)	2	.2842	.1421	
Between days	6	145.2488	24.2081	**
Treatment x days	6	22.9447	3.8241	**
Error 2.	24	21.5862	.8994	
Periods (a.m./p.m.)	1	43.0144	43.0144	**
Periods x Treatments	1	.4651	.4651	NS
Periods x Days	6	7.4565	1.2427	**
Error 3.	34	9.3390	.2746	

** = Significant at the 1% level.

	CONTROL						TREATMENT						
Periods.	A.M.			P.M.			A.M.			P.M.			
Cows / Days	1	3	5	1	3	5	2	4	6	2	4	6	
1	31.25	34.93	33.47	33.69	36.46	34.11	33.05	33.38	36.95	36.58	36.09	38.33	418.29
2	28.82	33.18	32.66	30.19	34.04	32.69	31.96	33.87	35.32	33.66	34.85	36.24	397.48
3	26.92	31.08	30.60	28.79	32.75	32.25	30.85	33.36	35.37	32.61	34.61	36.81	386.00
4	27.34	30.76	29.86	28.68	30.93	29.49	30.04	33.67	34.67	30.33	33.56	35.68	375.01
5	28.44	31.65	29.77	29.38	32.61	30.20	31.96	33.85	36.12	33.39	35.74	37.13	390.24
6	27.75	30.28	29.09	30.09	32.74	29.87	28.55	32.96	32.78	31.83	33.34	33.58	372.86
7	27.72	30.91	29.13	30.62	32.57	30.39	28.95	30.39	31.08	31.38	32.81	33.76	369.71
SX	198.24	222.79	214.58	211.44	232.10	219.00	215.36	231.48	242.29	229.78	241.00	251.53	2709.59
SX^2	5626.6114	7107.1903	6595.9340	6404.0356	7713.4052	6869.6598	6642.5148	7663.6960	8410.9999	7567.1584	8306.5356	9056.5559	87964.2969

$$C = \frac{7341877.9681}{84}$$

$$= 87403.3091$$

$$\text{Total SS} = 87964.2969 - C$$

$$= 560.9878$$

Between Pairs.

	Pairs	Totals for Pairs
Cows	1 + 2	948.71
Cows	3 + 6	854.82
Cows	5 + 4	906.06

SS for Pairs = 157.8559

Between Treatments.

Treatments	Totals for Treatments
Control	1298.15
Treatment (Oil-fed)	1411.44

SS for Treatments = 152.7931

Treatment x Pairs.

Cow	Control		Cow	Treatment	
1	409.68		2	445.14	854.82
3	454.89		6	493.82	948.71
5	433.58		4	472.48	906.06
	1298.15			1411.44	2709.59

Subclass SS = 310.9332
Total of SS's for Treatments & Pairs = 310.6490
SS for T x Pr. (Error 1) = .2842

Between Days.

	Days.	Totals for Days.
April 22	1	418.29
23	2	397.48
24	3	386.00
25	4	375.01
26	5	390.24
27	6	372.86
28	7	369.71

SS for Days = 145.2488

Treatment x Days

T \ D	1	2	3	4	5	6	7	
Control	203.91	191.58	182.39	177.06	182.05	179.82	181.34	1298.15
Treatment	214.38	205.90	203.61	197.95	208.19	193.04	188.37	1411.44
	418.29	397.48	386.00	375.01	390.24	372.86	369.71	2709.59

Subclass SS = 320.9866

Total SS's for

Tratments & Days = 298.0419

T x D = 22.9447

PAIRS X TREATMENTS X DAYS

	CONTROL			TREATMENTS			
Cows / Days	1	3	5	2	4	6	
1	64.94	71.39	67.58	69.63	69.47	75.28	418.29
2	59.01	67.22	65.35	65.62	68.72	71.56	397.48
3	55.71	63.83	62.85	63.46	67.97	72.18	386.00
4	56.02	61.69	59.35	60.37	67.23	70.35	375.01
5	57.82	64.26	59.97	65.35	69.59	73.25	390.24
6	57.84	63.02	58.96	60.38	66.30	66.36	372.86
7	58.34	63.48	59.52	60.33	63.20	64.84	369.71
	409.68	454.89	433.58	445.14	472.48	493.82	2709.59

Subclass SS = 500.7128

Total SS's for Treatments, Days, Pairs, T X D and T X Pr. = 479.1267

SS for T X D X Pr. (Error 2) = 21.5861

Between Periods

Periods	Totals for Periods
A. M.	1324.74
P. M.	1384.85

SS for Periods = 43.0144

Periods x Treatments

P \ T	Control	Treatment	
A, M.	635.61	689.13	1324.74
P. M.	662.54	722.31	1384.85
	1298.15	1411.44	2709.59

Subclass SS = 196.2726

Total SS's for P and T = 195.8075

P x T = .4651

Periods x Days

P \ D	1	2	3	4	5	6	7	
A. M.	203.03	195.81	188.18	186.34	191.79	181.41	178.18	1324.74
P. M.	215.26	201.67	197.82	188.67	198.45	191.45	191.53	1384.85
	418.29	397.48	386.00	375.01	390.24	372.86	369.71	2709.59

Subclass SS = 195.7197

Total SS for P & D = 188.2632

P x D = 7.4565

TOTAL SS	=	560.9878
Total SS's for Pairs, Treatments, T x Pr, Days, T x D, Pr x T x D, Periods, P x T, and P x D	=	551.6488
Error 3	=	9.3309

.....

APPENDIX V

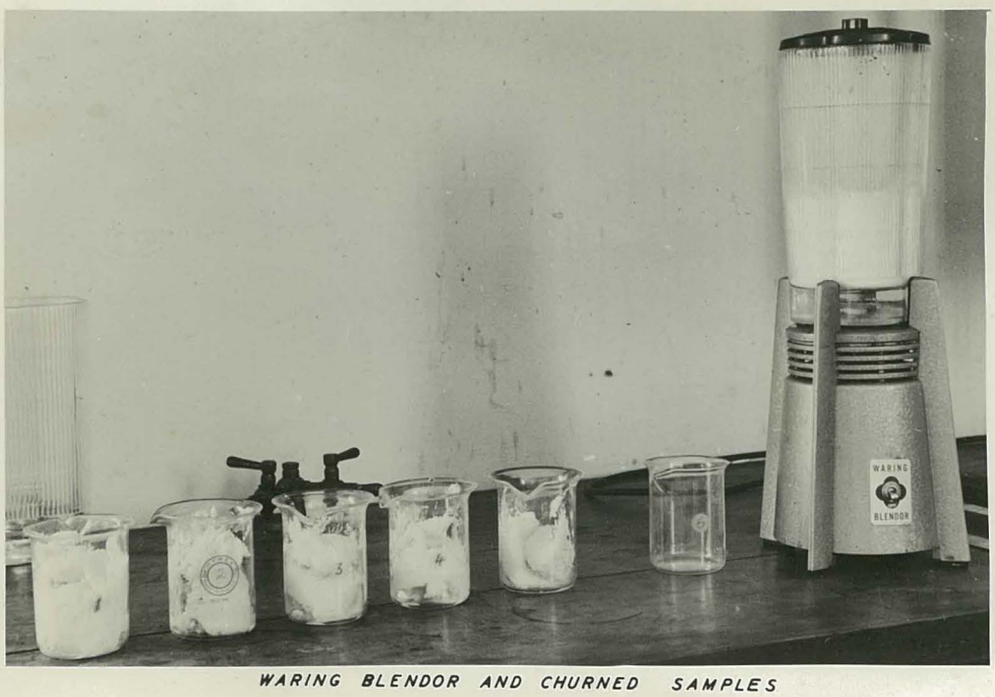
EXPERIMENT 1.

PHOTOGRAPHS ILLUSTRATING LABORATORY PROCEDURE AND EQUIPMENT.

.....

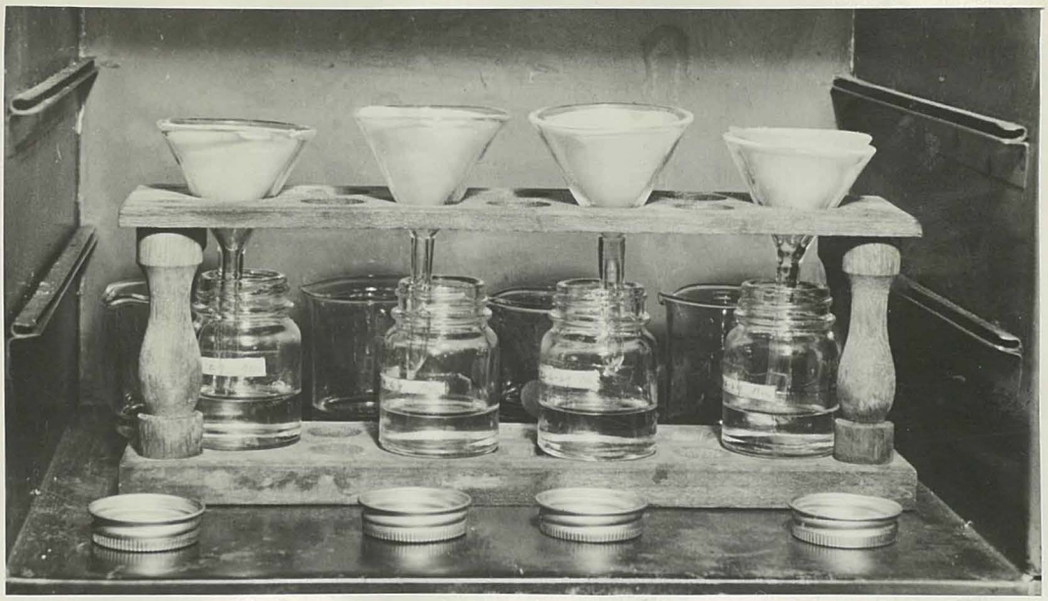
EXPERIMENT 1.

ILLUSTRATION ABOVE SHOWS CREAM BEING DRAWN OFF PREPARATORY TO CHURNING IN THE WARING BLENDOR (below)/.



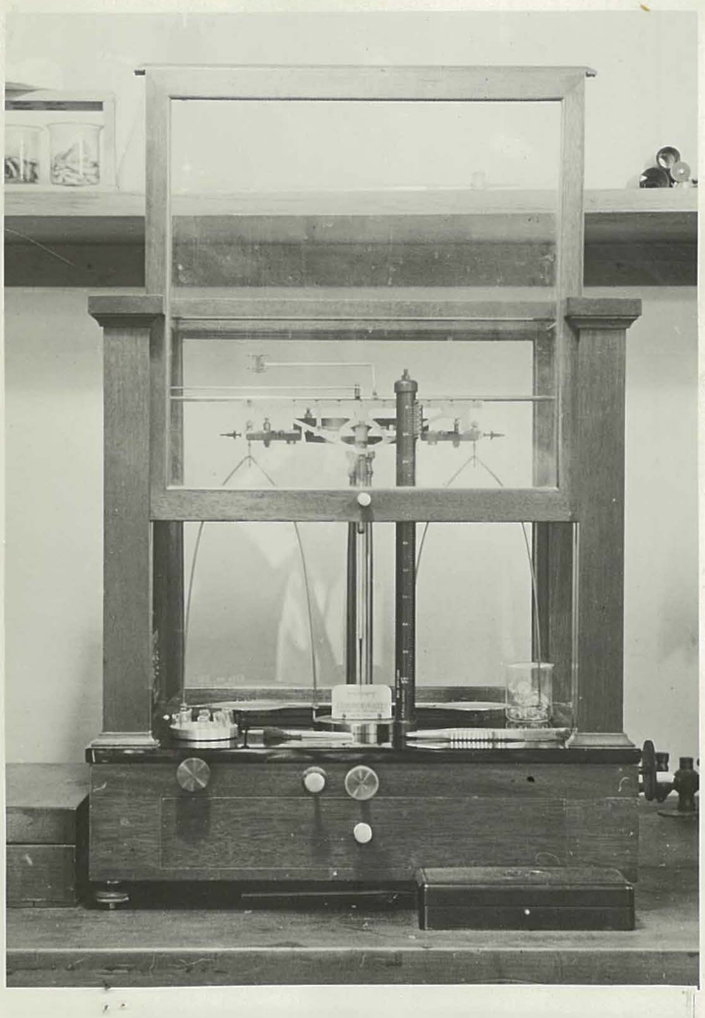
WARING BLENDOR AND CHURNED SAMPLES

EXPERIMENT 1.

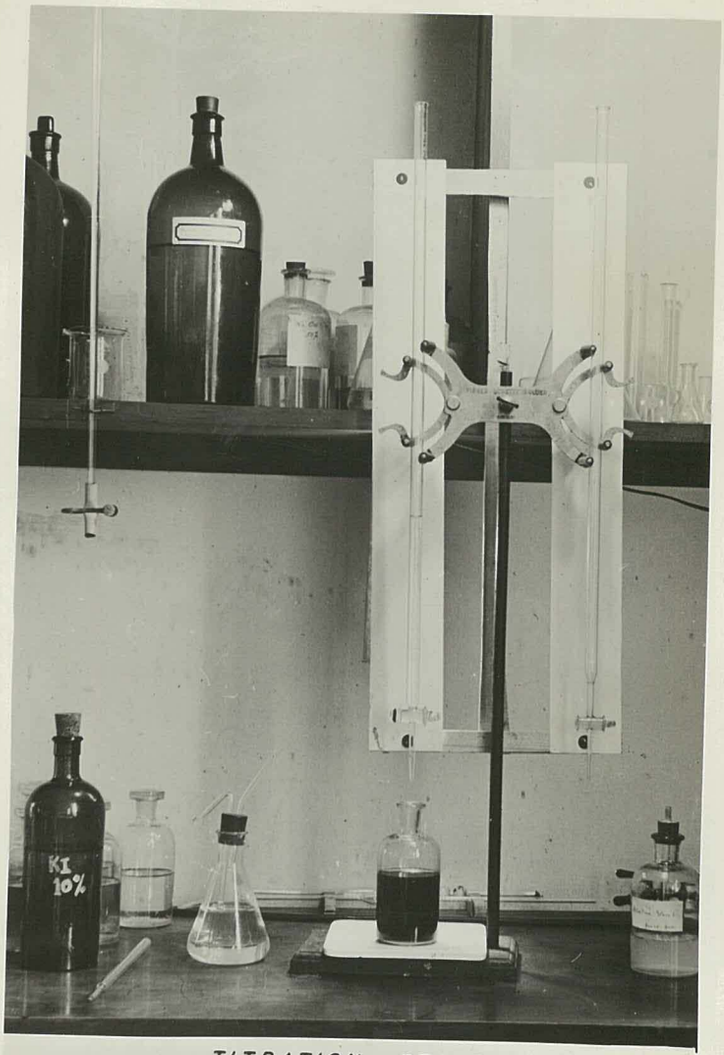


FILTERING MELTED BUTTER SAMPLES IN OVEN

INDIVIDUAL SAMPLES FOR IODINE VALUE DETERMINATIONS WERE WEIGHED ON BALANCE BELOW.



CHAINOMATIC BALANCE



TITRATION STAND

APPENDIX VI.

EXPERIMENT 2. (June/July 1949)

THE EFFECT OF INANITION ON THE IODINE VALUE OF BUTTERFAT.

Iodine Values of evening sampling:-

Date	Cow A	Cow B	Cow C	Cow D
June 30	44.34	41.59	45.77	40.14
July 1	40.70	41.77	45.66	40.86
2	40.73	41.04	43.78	39.48
3	41.64	42.22	44.68	-----
4	42.22	42.52	45.95	42.27
5	41.85	41.59	46.34	42.07
6	42.66	44.17	45.77	41.51
7	40.28	40.25	46.61	41.22
8	40.39	46.39	46.60	42.91
9	40.77	49.54	45.98	47.77
10	42.61	43.27	46.80	43.94
11	41.93	43.42	46.20	43.83
12	41.97	43.42	45.70	43.62
13	41.06	43.09	44.72	43.98

Iodine values of fat actually secreted during inanition are underlined in above table.

The sample from cow D on July 3rd. was missed owing to a shed-worker inadvertantly placing the standard machines on the cow without the testing bucket for collecting the sample.

APPENDIX VII

EXPERIMENT 2.

MILK WEIGHTS RECORDED AT EACH MILKING FOR INDIVIDUAL COWS.

(Weight given in lbs.)

Cow / Date	A (Control)	B	C (Control)	D
June 30	8.3	14.6	6.6	9.9
July 1	8.8	14.0	6.7	7.8
" 2	8.8	15.4	8.0	8.0
" 3	8.7	12.2	7.4	-
" 4	9.1	12.9	5.4	11.0
" 5	8.5	15.8	5.3	9.0
" 6	8.0	11.4	6.7	15.1
" 7	8.2	13.4	6.3	9.4
" 8	7.5	8.8	5.7	4.3
" 9	8.1	8.1.	5.7	7.8
" 10	7.7	11.9	6.8	7.8
" 11	9.1	12.6	6.5	8.2
" 12	8.0	12.6	6.0	10.0
" 13	9.2	13.7	4.7	8.0

Milk weights recorded at evening milkings for Cows B and D during inanition underlined in above table.

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APPENDIX VIII

EXPERIMENT 3. (July/August 1949)

THE EFFECT OF INANITION ON THE IODINE VALUE OF BUTTERFAT WHEN 1 lb. OF PEANUT OIL (Iodine Value 96.2) IS SUPPLIED DAILY IN THE RATION.

Samples were taken at evening milkings only, over a period of 17 days.

Iodine Values of fat actually secreted during inanition are underlined in the table following:-

Date	Experimental Cow A	Experimental Cow B	Control Cow D
July 19	42.56	39.75	41.51
20	43.94	40.24	41.58
21	44.36	41.79	41.64
22	44.92	43.10	41.64
23	46.53	44.44.53	42.49
24	45.91	43.46	41.18
25	46.55	44.59	41.75
26	45.53	<u>48.30</u>	42.04
27	45.71	<u>50.41</u>	41.95
28	48.08	48.84	41.25
29	48.50	49.13	42.49
30	<u>51.93</u>	51.69	41.68
31	<u>52.03</u>	51.42	41.50
Aug. 1	54.17	48.80	41.63
2	50.44	49.66	41.95
3	52.39	50.86	41.81
4	52.60	49.23	41.74

APPENDIX IX

EXPERIMENT 3.

MILK WEIGHTS RECORDED AT EACH MILKING FOR INDIVIDUAL COWS.

(Weight given in lbs.)

Date \ Cow	A	B	D
July 19	8.1	13.5	12.0
" 20	7.1	15.0	5.8
" 21	7.7	12.6	6.3
" 22	8.1	12.3	6.4
" 23	7.5	14.2	6.8
" 24	7.4	13.6	8.4
" 25	7.5	12.2	9.8
" 26	6.6	10.6	NN6.5
" 27	8.2	8.9	8.3
" 28	7.1	9.5	8.5
" 29	7.3	12.5	8.7
" 30	6.5	11.8	8.5
" 31	2.5	11.8	8.8
Aug. 1	6.2	12.7	9.3
" 2	7.3	14.9	12.5
" 3	6.0	11.0	10.0

Milk weights recorded for Cows A and B at P.M. milkings during inanition underlined in above table.

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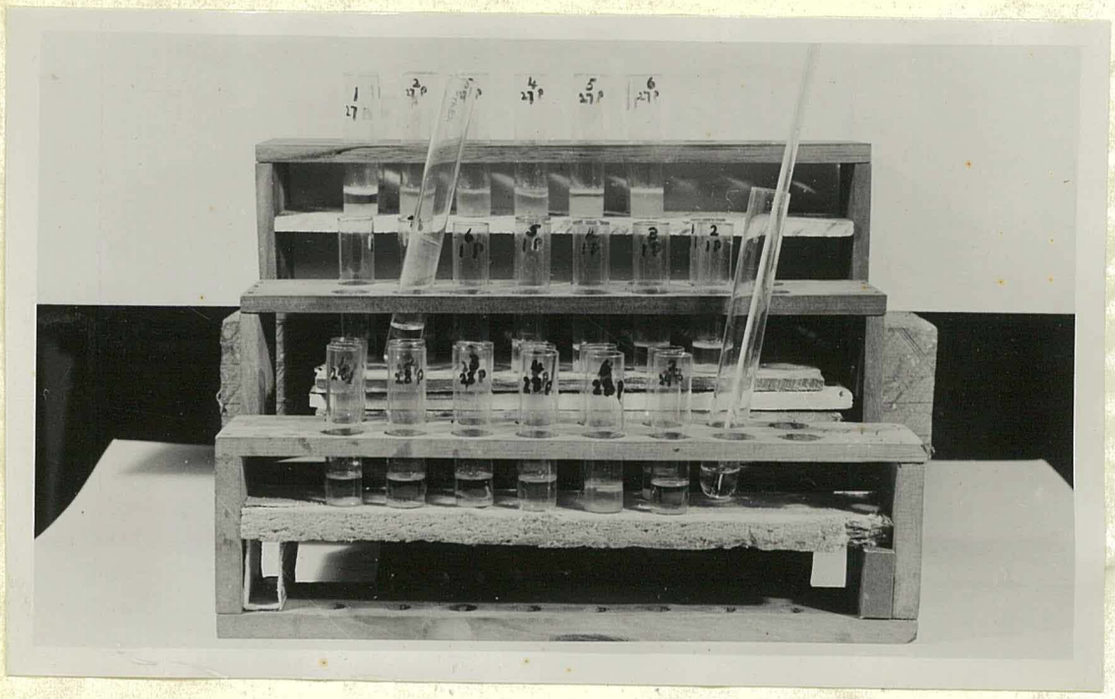
APPENDIX X

EXPERIMENT 5

ILLUSTRATION SHOWING RACKS OF FAT SAMPLES AS PLACED IN CONSTANT TEMPERATURE CUPBOARD TO AWAIT OXIDATION .



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