THE ABSORPTION OF MILK PRECURSORS BY THE MAMMARY GLAND.

with an Appendix on the Estimation of Iron in Small Quantities of Blood.

A Thesis submitted in accordance with the Regulations of the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

by

Janet Henderson Blackwood, B.Sc.,

Carnegie Research Scholar,

The Institute of Physiology,

The University of Glasgow.

------oOo------

April, 1932.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS.

ACKNOWLEDGEMENTS.

I have to thank the Carnegie Trust for a Research Scholarship during the tenure of which this work was carried out.

It is a pleasure to acknowledge my endebtedness to Professor E. P. Cathcart and to Dr N. C. Wright for their interest in this work and for the facilities which they have provided in the Institute of Physiology of the University of Glasgow and the Hannah Dairy Research Institute.

Owing to the technical complexities of this investigation I have worked in association with Dr, John D. Stirling Biochemist of The Hannah Dairy Research Institute to whom it is a pleasure to acknowledge my indebtedness.

INTRODUCTION.

The study of milk secretion is not only of interest in the more restricted domain of mammalian physiology but offers results capable of immediate application in the wider field of industry. At the present time it is generally agreed that one of the great social necessities is an adequate supply of milk of a certain standard quality and it will be appreciated that this requirement calls for the fullest information on the series of transformations which take place between the food of the dairy cow - the raw material of the dairy industry - and the milk which she produces. Our knowledge of mammary secretion is most highly developed on the functional side and a survey of the literature reveals the need for a more detailed and exact treatment of the subject from the biochemical point of view than has yet been attempted.

The problems involved are many and there is no method of approach which is at once completely satisfactory and easily applied. They may be summarised under two headings:

(1) the study of the substances utilised by the

mammary glands in the performance of their secretory function,

(2) the study of the reactions by which the milk constituents are actually synthesised in the cells of the mammary glands.

Investigations have so far been limited to the first study, i.e. to attempts to identify the constituents of the blood (the precursors) from which the specific milk proteins, fat and sugar are derived. The reactions whereby these precursors are utilised in the synthesis of milk products are, as yet, entirely unknown, but since this latter study is in large measure dependent on the former it has seemed advisable to make this survey of the methods used and of the conclusions which have been drawn.

The various constituents of milk-caseinogen, lactose and fat, are specific to the mammary secretion, i.e. they are not encountered as the products of the activity of any tissue of the mammalian organism other than the mammary gland. It must be remembered that the mammary gland is not supplied with the food materials in the form in which they are ingested. These are subjected to extensive breakdown in the intestinal tract and appear as metabolic units in the blood

stream - the common medium of metabolic exchange in the mammalian body. There is thus forced on the mammary gland the necessity of selecting from the circulating blood those constituents which may serve as the precursors of milk protein, fat and carbohydrate. If the ideal of complete correlation between the finished product and the raw material is to be approached in the field of milk production it is evident that two distinct relationships must be investigated:

(1) The relationship between the diet and the composition of the blood,

(2) The exchanges between the blood-stream and the mammary gland.

Our knowledge of the first of these relationships is to-day fairly extensive and may, without presumption, be considered applicable to the problems of milk production, provided our knowledge of the second field were equally developed. Unfortunately this is by no means the case and the present study is an attempt to initiate the treatment of milk production problems from the point of view of the relationship existing between the mammary gland and the blood stream.

In such a study it is apparent that there are two main questions upon which data are urgently required. Firstly, it must be determined which blood constituents

act as precursors of milk protein, fat and carbohydrate, and secondly, exact quantitative data must be obtained on the relationship of the amounts of precursors absorbed from the blood-stream and the amounts of milk constituents secreted. Sporadic work has been carried out by previous workers and before any description of the present experiments can be considered a critical review of previous findings must be made.

It will be convenient first to give some account of the study of the precursors of milk sugar, protein and fat in turn by all the methods which have been employed, and secondly to discuss the technique of the methods themselves.

The Precursor of Lactose.

Since the lactose of milk is peculiar to the mammary secretion and does not occur in the blood or lymph, it must be formed in the mammary glands themselves from material brought thither by the blood-stream. Lactose is a disaccharide composed of glucose and galactose. It was at one time believed that all sugars were converted into glucose in the course of their passage from the intestinal tract to the portal circulation. The work of Cori and Cori (1928) and Corley (1928) has shown that monosaccharides

of all kinds are absorbed as such into the blood stream and that only on passing through the liver are they converted into glucose. The blood supply to the mammary gland has then glucose as one of the necessary components of milk sugar.

The first work on the utilisation of blood sugar to be considered is that of Chauveau (1856) who found that the concentration of sugar in venous blood was always lower than that in arterial blood, the difference being accounted for by the uptake of sugar during the passage of the blood through the tissue. Bert (1884) removed the mammary glands of young goats, and had them bred. Pregnancy was normal and they gave birth to their young. Bert examined the urine immediately after parturition and found that it contained sugar, whereas the urine of intact goats examined after the birth of young did not contain sugar. From the results of such experiments Bert formed the opinion that immediately following parturition the blood receives an additional supply of sugar from some source - presumably the liver - and that this sugar is utilised for the synthesis of milk sugar. When there are no mammary glands and consequently no mammary secretion the excess sugar in the blood is excreted in the urine.

Porcher (1909) performed similar experiments which

confirmed and extended the work reported by Bert. He found that post-partum glycosuria in goats from whom the mammary glands had been removed was accompanied by hyperglycaemia, and also that the sugar excreted in the urine was glucose and not lactose. The post-partum glycosuria was only of short duration.

Removing the mammary glands from lactating goats, Porcher found hyperglycaemia and glycosuria to exist for a few hours after the operation. In both types of experiment the effects mentioned were marked for less than twenty-four hours and disappeared within three or four days.

From this work the theory was again formulated that during lactation dextrose is thrown into the blood by some organ - probably the liver - and that this dextrose may be the precursor of milk sugar.

Contradictory evidence was put forward however. Neither Bert nor Porcher were able to confirm their results using guinea-pigs as experimental subjects. Marshall and Kirkhess (1907) working on guinea-pigs also obtained results which negatived the theory.

Moore and Parker (1900 - 01) performed experiments similar to those of Bert on two goats. The urine of one of them after parturition showed a slight increase in reducing power. The exact interval between parturi-

tion and urinary collection and examination was however not stated by these workers.

Foà (1907 - '08) removed the mammary glands from two lactating goats but found that each operation lasted seven hours. He did not examine the urine until three hours after operation. Anaesthesia and operation are likely to have an effect on blood sugar values so that this method is not so satisfactory as the one first used by Bert and afterwards by Porcher.

Kaufmann and Magne (1906) wished to examine the consumption of blood glucose in the mammary gland under varying conditions of activity. Taking account of the findings of Chauveau, these workers considered that jugular blood might be taken to represent mammary arterial blood minus the sugar actually oxidised in the secreting cells of the mammary gland. Accordingly they took samples simultaneously from the jugular and abdominal subcutaneous veins of four cows, one of which was not lactating. They found that the blood coming from the mammary glands of lactating animals had a lower concentration of sugar than the blood of the jugular vein. The percentage difference in sugar content of the two bloods varied in the several experiments from 7 to 30 per cent. The sugar contents of the two bloods from the one non-lactating cow examined were the same. Kaufmann

and Magne drew the following conclusions from these experiments.

1. In the mammary gland at rest the consumption of glucose is the same as in the tissues of the head.

2. When the mammary gland is in active secretion, the blood traversing it loses more glucose than that draining head tissues.

3. Immediately before parturition the glucose consumption of the gland is increased and there is lactosuria which persists until several days after parturition.

Taking the blood samples at various stages of lactation, they found that the consumption of glucose in the gland was variable.

These conclusions have been taken as strong support of the view that dextrose is the precursor of lactose.

Foà (1907, 1911) removed the mammary glands from sheep and preserved them in Ringer's solution at body temperature. Perfusing the glands with Ringer's solution, with mixtures of blood and Ringer's solution, and with Ringer's solution to which various substances had been added, he determined the quantity of dextrose in the various perfusion mixtures before and after circulation through the gland and also the nature of the secretion in each case. Perfusing with Ringer's solution

alone the fluid secreted by the gland contained no lactose. Perfusing with a mixture of blood and Ringer's solution, milk containing lactose was secreted and the dextrose content of the perfusion liquid was found to have diminished. Foà found it possible to increase the lactose concentration of the milk secreted by adding dextrose to the perfusion fluid. Using Ringer's solution with added dextrose as perfusion mixture, the gland secreted a watery fluid containing lactose. When galactose took the place of dextrose, no lactose was secreted. These results again furnished strong evidence in favour of the view that the lactose of milk might be formed from the dextrose of the blood. Morgen and others (1904 - 1906) carried out an extensive survey on the effect of diet on the composition and yield of milk. They found that the concentration of lactose did not vary appreciably with changes in the ration.

Eckles and Palmer (1916) found that feeding cows on rations inadequate for maintenance and milk production produced no change in the lactose content of the milk secreted. From the results of these experiments there is no doubt that the lactating animal can draw upon reserves of carbohydrate in the body for the maintenance of the lactose content of the milk.

Cary, reported by Meigs (1922) repeating Kaufmann

and Magne's experiments, found that the mammary venous blood contained about 24% less sugar than the jugular blood of lactating cows; the mammary plasma about 32% less than the jugular plasma.

Summarising the foregoing evidence, it appears that the majority of the experiments performed support the view that lactose is derived from the glucose of the blood, the supply of which can be adequately maintained by the liver from its glycogen stores.

The Precursor of Milk Protein.

Using his perfusion method, Foà (1911 - 12) investigated the problem of the secretion of milk protein. His results were mainly negative. Perfusing the excised mammary glands of sheep with Ringer's solution and various forms of protein he was unable to demonstrate the presence of protein in the fluid secreted, and concluded that milk protein was derived from some constituent of the blood other than protein.

The knowledge of protein metabolism formulated by Delaunay (1913), Folin and others (1912) and Van Slyke and Meyer (1912- 13) has shown that protein metabolism is essentially the metabolism of the amino acids.

Delaunay, using the formol titration method, and Van Slyke and Meyer, the nitrous acid method, were able to demonstrate measureable amounts of amino acid nitrogen

in protein-free blood filtrates. Several amino acids were then isolated from protein free filtrates by Aberhalden (1913) and by Abel, Rowntree and Turner (1914) from filtrates obtained by vividiffusion of the circulating blood. That these amino acids are the products of protein digestion was shown finally by Delaunay (1913), Van Slyke, Cullen and McLean (1915), Folin and Berglund (1922) and by Witts (1929).

In 1920, Cary, investigating the possible role of these amino acids in the synthetic activities of the mammary gland, applied the technique of Kaufmann and Magne to the study of the precursor of milk protein. He found that the jugular blood and blood plasma of lactating animals had a higher content of amino acid nitrogen than the mammary venous blood and blood plasma, the two samples being taken simultaneously. No such differences were found in the blood of non-lactating cows. Cary concluded that this apparent loss of amino acid nitrogen in the lactating state could be accounted for by the uptake of blood constituents by the mammary gland, and that the differences observed were of sufficient magnitude to establish the amino acids of the blood plasma as the precursors of milk protein.

The results of Van Slyke (1917) suggest that the liver, immediately it receives the amino acids of the

blood coming from the intestinal tract, begins to deaminise them. It may well be that this hepatic action is selective - if so, it may change the quality of the amino acid mixture of the blood to bring it more closely in accord with the proportions of the various amino acids composing milk protein. If, for any reason, an animal is in negative nitrogen balance it may maintain a constant level of amino acid nitrogen by utilising the amino acids of the tissues. In this case, instead of passing through the liver to enter the systemic circulation, the amino acids may circulate several times before undergoing the deaminising and selective action of that organ.

Again, the liver may modify its deaminising activity according to the balance of nutritive materials other than protein. Carbohydrate shortage in the animal body may be made up from protein sources in the diet if the deaminising activity of the liver is stimulated. It appears then that the quality of the amino acid mixture circulating in the blood may change in addition to or without change in the quantity of amino acid nitrogen. In this connection it is interesting to note that Folin and Denis (1912) found that when single amino acids were injected into the intestinal tract, there followed a rise in amino acid nitrogen in

the blood (estimated as non-protein nitrogen - urea nitrogen).

Cary (1921 - 1922) carried out three experiments designed to correlate changes in the quality and quantity of the amino acid mixture of the blood of lactating animals with dietary changes and milk yields. The general conclusions of these experiments were:

1. that the changes in diet produced changes in the quality of the blood amino acid mixture as well as in the quantity of amino acid nitrogen per 100 cc. of blood;
2. that milk secretion is markedly affected by these changes;
3. that protein metabolism is intimately related with the metabolism of carbohydrate and fat.

In later investigations Cary claims to have shown experimentally that the specific amino acids, tyrosine and tryptophane are utilised by the mammary gland for the secretion of milk protein.

The Precursor of Milk Fat.

Investigations on the origin of milk fat were made by Foà (1911 - 12) using his mammary gland perfusion method. He found that the watery fluid secreted by an excised mammary gland perfused with Ringer's

solution contained no fat. Foà added, firstly, olive oil, and, secondly, tri-olein to Ringer's solution and perfused the excised gland with the emulsions. The fluid secreted by the gland contained fat in globules resembling microscopically the globules of milk fat. The iodine number of these fat globules was lower than that of the original fat of the perfusion mixture. Thus he claimed to have shown that the gland had utilised fat in the form of glycerides and suggested that the triglycerides of the blood were the precursors of milk fat.

Meigs, Blatherwick and Cary (1919) published results leading to conclusions at variance with those of Foà. In a study of the phosphorous metabolism of the mammary gland using the experimental method of Kaufmann and Magne, they analysed the plasma of jugular and mammary venous blood of milking cows for phosphatid and inorganic phosphate. They regarded the total phosphorus of the plasma as divided between these two classes of compounds only. It should be noted however that Feigl (1917) and Bloor (1918) had previously reported a third kind of phosphorus in plasma. Meigs, Blatherwick and Cary found that the mammary venous blood invariably contained less phosphatid than jugular blood, but that it had a higher content of inorganic phosphate. These

changes in the distribution of phosphorus between the two types of compound on passage through the mammary glands appeared to compensate one another, and on these facts Meigs, Blatherwick and Cary based the opinion that milk phosphorus and milk fat are derived from the phosphatid of the blood. They concluded that since the proportion of phosphorus to fatty acids in the phosphatids of the blood is probably about 1 to 20 as compared with 1 part of phosphorus to 50 parts of fat in milk, that the mammary gland receives more phosphorus with a given amount of lipoid than it requires to maintain the normal phosphorus fat ratio of the milk, and that the excess phosphorus is returned by the gland to the efferent blood as inorganic phosphorus, thus accounting for the compensating effects observed. They stated however, that this phosphorus work was greatly affected by the degree of disturbance to the animal in the course of blood sampling.

If the amount of fat fed to a milking cow is reduced to below 1 gm. per kgm. of body weight, the concentration of fat in the milk secreted is diminished together with the total yield (Morgen, Beger, und Fingerling, 1904 - 1906, Morgen, Beger und Westhausser, 1907). This should be accompanied by changes in the phosphatid content of the blood if Meigs, Blatherwick

and Cary's conclusions are justified, but, as yet, this has not been shown.

Methods.

The work summarised in the foregoing section may also be classified under the headings of three experimental methods. These I now propose to outline with some critical discussion of each, leading up to the choice of an experimental method to further the study of the absorption of milk precursors by the mammary gland.

1. The perfusion of the blood vessels of excised mammary glands with solutions of known composition and the examination of the resulting secretion.

This method, originated by Foà (1907) has yielded some very interesting results, but it is not wholly reliable. The excised gland rapidly becomes oedematous during the course of the perfusion experiments and in this condition it is possible that the cells of the gland may become permeable to substances which would not normally gain access to the secretory tissue.

2. The analysis of the blood and milk of animals on varying rations, attempting to correlate any changes which may take place in the composition of these two fluids.

The work done by this method is scanty and uncoordinated. It requires an adequate knowledge of the correlation of blood chemistry with mammary secretion and dietary history such as does not exist at the present time. The work in this field is also retarded by the lack of suitable methods of blood analysis but when these initial difficulties are surmounted and the relation of the blood stream to the mammary secretion founded on a secure basis it may yield results of practical importance. It has been employed to some extent by Cary (1921, 1922).

3. Analysis of blood before entering and after leaving the tissues of the mammary gland in the expectation that absorption of the precursors of milk constituents from the blood can be demonstrated as apparent losses in the efferent blood.

This method was adopted with modifications by Kaufmann and Magne (1906) and employed by Meigs, Blatherwick and Cary (1919) with results summarised in the section on the precursors of milk constituents. It seemed probable that this method of experimentation would be the only one capable of affording conclusive evidence on the nature of the precursors of milk constituents which could be used as a basis for future work on the study of the synthetic activities of the mammary

gland and on the correlation of many factors in the animal economy.

Accordingly, I shall discuss the method in detail. It is first necessary to consider the blood supply of the mammary glands.

The mammary gland receives its blood supply from the external pudic arteries. Arterial blood, leaving the heart by way of the aorta, divides into the internal and external iliacs under the fifth or sixth lumbar vertebra. The external iliac descending becomes the femoral artery at the internal border of the pubis. Here the prepubic artery arises which later divides into the posterior abdominal and external pudic arteries. The latter continues downward and divides into the abdominal subcutaneous and mammary arteries. These mammary arteries enter the mammary gland. They are deeply embedded in the tissues and are inaccessible without serious disturbance to the animal.

Emerging from the mammary gland in a large number of vessels the venous blood is united in the mammary or milk veins at the base of the gland. Five veins carry the blood away from the udder - two subcutaneous abdominal, two external pudic and the perineal veins. The subcutaneous abdominal veins are usually termed the milk veins: they start from the fore part of the udder, pass

along the outside of the lower abdominal wall and through the foramens (or milk wells) to the internal thoracic vein, which empties into the anterior vena cava. The blood of the other three veins, which are smaller than the abdominal subcutaneous veins, unites in the external iliac and finally joins the posterior vena cava. The abdominal subcutaneous veins are readily accessible without disturbance to the animal. The ideal method, therefore, of determining whether any given blood constituent takes part in the secretory process would be to compare the concentration of that constituent in the blood before entering and after leaving the gland. A diminished concentration of any constituent in the efferent blood would indicate utilisation of that substance by the mammary gland.

Since the mammary artery is inaccessible the problem of securing samples of arterial blood with which the comparison of analytical figures for mammary venous blood is justified, is therefore one of considerable practical difficulty and was first encountered by Kaufmann and Magne (1906) who wished to investigate the consumption of blood glucose in the gland under varying conditions of activity. Their results are considered under the section on the precursor of lactose. These workers chose the concentration of sugar in the blood of the jugular vein as a standard

with which to compare the concentration of sugar in the mammary venous blood and justified their procedure on the following grounds.

According to the findings of Chauveau (1856) the concentration of sugar in venous blood is always lower than that in arterial blood, the difference being accounted for by the uptake of sugar during the passage of the blood through the tissues. Kaufmann and Magne therefore considered that jugular blood might be taken to represent mammary arterial blood minus the sugar actually oxidised in the secreting cells of the mammary gland. Any difference in concentration between jugular and mammary venous bloods would then be accounted for by the secretory activities of the mammary gland.

Later work on the nature of the non-sugar reducing substances of the blood, and the fact that the energy consumption of the mammary gland is not necessarily equal to that of the tissues drained by the jugular veins introduce new considerations into the question of the applicability of this technique to the study of the secretion of milk sugar. These will be considered in detail in the experimental section on milk sugar and I shall meantime pass to the consideration of the application of this technique to other possible milk precursors.

The technique of Kaufmann and Magne was adopted by

Meigs, Blatherwick and Cary (1919) and Cary (1920) the former in a study of the phosphorus metabolism of the mammary gland, and the latter in support of a claim that blood amino acids are the precursors of milk protein. (See sections on precursors of milk protein and fat.) These workers justified their procedure by an argument which differed from that employed by Kaufmann and Magne. They held that since the tissues drained by the jugular vein were mainly muscular, changes brought about in the phosphorus or sugar content of blood passing through them would be negligible in comparison with those brought about by passage of the blood through the mammary gland. Finally they concluded that the blood of the jugular vein might, for all practical purposes, be assumed to be identical with the arterial blood going to the tissues drained by that vein and hence be identical with the blood supply of the mammary artery.

In the absence of experimental evidence in support of their contention it is difficult to accept the many conclusions arrived at by these workers from their analytical results: a final conclusion can, in fact, only be reached if analytical values are available for arterial blood samples - a procedure which I have adopted.

In addition to the intervention of tissue metabolism between arterial blood and jugular (venous) blood and the

consequent difficulty in the interpretation of analytical values obtained for jugular blood, there are other effects, unrecognised by previous workers, which may effect modifications in the venous blood draining from either mammary tissue or from the tissues of the head and neck. Before proceeding to the comparison of the concentrations of any given constituent in two bloods drawn from different sources, it must be ascertained that no water has been lost from the blood either to the tissues or without the body. The lactating animal on the average secretes over a period of 12 hours about 10 litres of water as milk. This may result in concentration of the blood and the question must be considered whether or not the haemo-concentration so produced is of sufficient magnitude to be reflected in analytical figures.

A secretion of 10 litres per 12 hours is equal to a secretion of about 14 cc. of water per minute and would therefore require a velocity of flow of blood through the mammary gland of only about 1.5 litres per minute to give a 1% concentration of the blood. As the velocity of flow of blood is probably very much higher than this it follows that the haemo-concentration due to secretion of water by the mammary gland is negligible so far as analytical figures obtained for mammary venous blood are concerned.

The loss of water from the blood in its passage through the tissues of the head and neck might, however, be a quantity of greater magnitude. Schalk and Amadon (1928) estimated the activity of the salivary glands of the ox as constant at some 60 or more litres of saliva per 24 hours. Such a secretion may make heavy demands on the water of the blood supply, and when one considers that part of the blood in the jugular vein is drained from the salivary glands, it is apparent that this constant flow of saliva may result in appreciable haemo-concentration in the jugular (venous) blood.

Although previous workers have failed to recognise that analytical values for jugular and mammary bloods may not be strictly comparable there are indications of such a possibility to be found in their work. For example, Meigs, Blatherwick and Cary have noted that samples of jugular and mammary bloods taken simultaneously from the same animal do not always have the same percentage corpuscular volumes. They do not discuss this fact fully in their paper nor do they seem to have realised the possible effect of such a difference on their analytical values for other blood constituents.

Loss of water from the blood will be reflected in the concentration of the indiffusible constituents. Accordingly the first question I have investigated experi-

mentally is the concentration of indiffusible constituents in samples of blood taken simultaneously from different regions of the bodies of lactating and non-lactating animals. The constituents chosen - percentage corpuscular volume and iron content - are those most likely to be accurate indices of the amounts of water contained in equal volumes of blood from different sources. The amount of iron in milk is so small that it may be taken as negligible for the quantitative aspects of this investigation.

The Modifying Influence of Salivary Secretion on Jugular Blood and its Relation to the Technique of Kaufmann and Magne.

The Modifying Influence of Salivary Secretion on Jugular Blood and its Relation to the Technique of Kaufmann and Magne.

If arterial blood on passage through the tissues of the head and neck loses water to the salivary glands the efferent blood will be more concentrated than the arterial blood with respect to those of its constituents which are not utilised by the tissues in their metabolic and secretory activities. This may be illustrated diagramatically as follows:

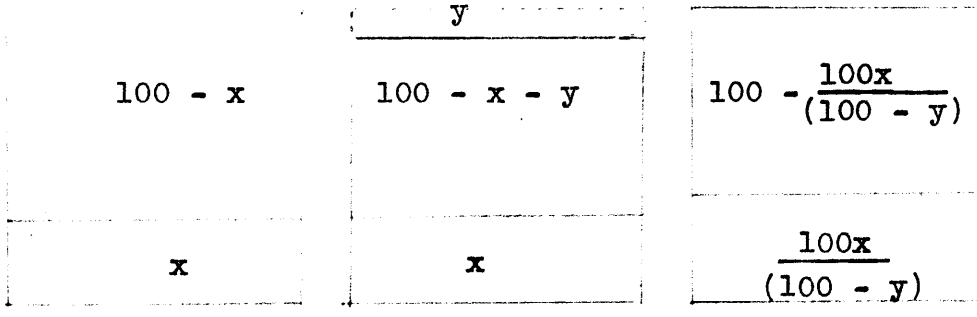

Fig. 1. Fig. 2. Fig. 3.

If Fig. 1 represents 100 cc. of arterial blood containing x cc. of corpuscles, then the volume of the plasma associated with x cc. of corpuscles is (100 - x) cc. Let this arterial blood be subjected to the influence of the sali-

vary glands where it will lose y cc. of water. 100 cc. of arterial blood has now become (100 - y) cc. of venous blood (Fig. 2.). Associated with (100 - y) cc. of venous blood there are still x cc. of corpuscles. In 100 cc. of venous blood there are then $\frac{100x}{(100 - y)}$ cc. of corpuscles, i.e. it is possible that jugular blood may show a concentration effected by the salivary glands in an increased corpuscular volume. (Fig. 3.)

Loss of water to the salivary glands will also affect the haemoglobin content of the blood. This point may be investigated by determining the iron content of the blood since the iron associated with haemoglobin is indiffusible and hence independent of interchanges between the blood and tissues other than that of water.

Experimental.

The arterial blood for the purposes of this investigation has been taken from the radial artery which is accessible in the intact animal, although not always without serious disturbance. Blood from the jugular and mammary veins may usually be readily obtained. Blood samples were taken simultaneously from the jugular and mammary veins and from the radial artery of lactating and non-lactating cows. The animals were not disturbed

until a few minutes before sampling, and usually no anti-kicking device was necessary other than a rope on the rear legs. A sterile needle was quickly inserted in one of the abdominal subcutaneous veins close to its point of emergence from the tissues of the udder. A steady flow of blood was readily obtained. Within two minutes (maximum interval) a similar needle was inserted in one of the jugular veins - from which a sample of blood was also quickly obtained.

Using a shorter needle of narrow bore a sample of blood was then collected from the radial artery. It was found that arterial puncture of non-aesthetised cows was a procedure attended by considerable disturbance with most animals. Whenever it became apparent that the disturbance was likely to be pronounced the sample was abandoned, since, unless a rapid flow of true arterial blood was obtained, the results were likely to be vitiated. Only in eight cases were full blood samples from all three sources obtained without disturbance.

The samples, each about 60 cc. volume, were received in sterile glass-stoppered bottles containing 0.1 g. of potassium oxalate as anti-coagulant.

It may be noted here that this experimental tech-

nique differs considerably from that of Meigs, Blatherwick and Cary (1919) and Cary (1920) who reported that the samples analysed by them were frequently obtained after considerable disturbance. Their samples were taken by means of a trochar and cannula inserted in the jugular and mammary veins, the samples collected simultaneously from each vein being as large as 400 - 500 cc. - a volume equal to almost $\frac{1}{20}$th of the total blood of the average cow. By the use of improved micro-methods I have found it possible to carry out a full series of analyses in duplicate on a volume of blood which can be much more readily obtained.

The samples were immediately analysed for:

1. Corpuscular Volume.

Blood from each source was centrifuged in duplicate hematocrit tubes at 3,500 revs. per minute until constant volume was attained. It has been shown (Osgood 1926) that the presence in blood of anti-coagulants such as potassium oxalate may affect corpuscular volumes, but since the amount of oxalate used was always minimal and the same for each blood, the slight effect of its addition was negatived.

2. Iron Content.

The concentration of iron in 2 cc. samples of each

blood was determined by a micro-method elaborated for the purpose of this investigation and reported in the appendix.

The results of analyses of samples of blood from seventeen different animals, ten of which were lactating and seven non-lactating at the time of sampling, are given in Table 1.

Table 1.

Cow No.	Lactating or Non-lactating	Corpuscular volume (per cent)			Iron content (mgms Fe per 100 cc. blood)		
		jugular	arterial	mammary	jugular	arterial	mammary
1	Lactating	33.5	30.5	30.5	37.04	35.26	35.06
2	"	36.5	34.0	33.5	42.32	39.34	38.94
3	"	41.0	39.0	39.0	38.75	37.71	37.33
4	"	39.0	38.0	37.0	36.69	35.14	34.26
5	"	37.0	36.0	36.0	40.86	39.29	39.50
6	"	36.0	-	34.5	39.65	-	38.39
7	"	33.0	-	31.0	40.25	-	38.66
8	"	36.0	-	36.0	37.96	-	37.07
9	"	43.0	-	42.0	38.75	-	36.94
10	"	42.5	-	40.5	41.83	-	39.77
11	Non-lactating	41.0	38.5	38.5	39.31	36.28	36.81
12	"	36.5	35.5	35.5	34.74	-	32.94
13	"	32.5	29.0	30.0	34.23	32.56	33.46
14	"	38.3	34.3	34.8	37.28	34.60	34.89
15	"	30.0	-	28.0	38.12	-	37.27
16	"	34.0	-	31.5	43.56	-	41.88
17	"	36.0	-	33.5	37.70	-	35.77

Table II has been constructed from the data of Table I and shows, in each case, the differences between jugular, arterial and mammary bloods in corpuscular volume and iron content expressed as percentages of jugular values.

Table II.

Cow No.	Lactating or Non-lactating	Differences in corpuscular volume			Differences in iron content.		
		jugular-mammary as % jugular	jugular-arterial as % jugular	mammary-arterial as % jugular	jugular-mammary as % jugular	jugular-arterial as % jugular	mammary-arterial as % jugular
1	Lactating	8.95	8.95	0	5.34	4.81	0.54
2	"	8.23	6.85	1.3	7.96	7.04	0.92
3	"	4.87	4.87	0	3.68	2.71	0.95
4	"	5.14	2.57	2.57	6.63	4.24	2.30
5	"	2.70	2.70	0	3.30	3.80	0.52
6	"	4.10	-	-	3.20	-	-
7	"	6.06	-	-	3.91	-	-
8	"	0	-	-	2.30	-	-
9	"	2.30	-	-	4.60	-	-
10	"	4.70	-	-	4.90	-	-
11	Non-lactating	6.09	6.09	0	6.37	7.70	-1.3
12	"	2.75	2.75	0	5.18	-	-
13	"	6.24	9.37	-3.1	2.26	4.87	-2.6
14	"	9.14	10.44	-1.5	4.50	6.42	-0.8
15	"	6.66	-	-	3.03	-	-
16	"	7.35	-	-	3.86	-	-
17	"	6.90	-	-	5.11	-	-

Discussion.

The differences in the contents of indiffusible constituents are found in lactating and non-lactating animals alike, and cannot therefore be the result of the state of activity of the mammary gland. This conclusion receives support from the observation that mammary venous and radial arterial bloods bear a close resemblance to one another and that jugular blood differs from each in approximately the same degree. Any differences between arterial blood and mammary venous blood analyses are not consistent and are not of a magnitude which may be regarded as significant when the various analytical procedures are considered. A scrutiny of the differences between jugular and arterial, and jugular and mammary bloods show that they are consistently in the same direction and of a magnitude outwith the range of any experimental error.

The conclusion may then be drawn that the circumstance responsible for the differences obtained is conditioned by interchange between blood and tissues in the course of the circulation of the blood through the head and neck. It has been shown that loss of water from the arterial blood might be expected to cause an increase in concentration of venous blood constituents. This appears to be the point which the data presented illustrate, and

it is thence apparent that the withdrawal of water from the arterial blood by the salivary glands is of sufficient magnitude to bring about an appreciable concentration of the blood resulting in a consistent increased value for corpuscular volume and iron content of jugular samples.

The concentration of diffusible blood solutes, as well as of those indiffusible, will be affected by loss of water to the salivary glands and erroneous interpretations of analytical figures are likely to arise unless this fact is realised.

The determination of the degree of haemoconcentration, however, enables the effect of this factor on the concentration of a diffusible blood constituent to be allowed for.

Let x = mg. Iron per 100 cc. jugular blood.

y = mg. Iron per 100 cc. mammary blood

then the degree of concentration of jugular blood is given by $\frac{x}{y}$

Let p = mg. sugar (or amino acid nitrogen etc.) per 100 cc. jugular blood,

then $p\frac{y}{x}$ = mg. sugar per 100 cc. jugular blood if no loss of water to the salivary glands had taken place.

The application of such a factor enables analytical values for jugular blood to be compared directly with values for arterial and mammary venous bloods since it

negatives the effect of loss of water to the salivary glands.

For the calculation of the correction factor I have chosen the values given by iron determinations in preference to those given by corpuscular volume measurements because the estimation of iron is not attended by those inaccuracies inherent in haematocrit technique.

In addition to these factors there is the possible influence of the lymph drainage on the concentration of venous blood solutes which might be considered. Sampling the lymphatic circulation would be a matter of great difficulty and could not be considered when drawing up this experimental plan. It is probable, however, that its effect would be in the same direction as that of the salivary glands but it is unlikely that its magnitude would be appreciable.

The Relation of Blood Sugar Absorption to Lactose Secretion.

The Relation of Blood Sugar Absorption to Lactose Secretion.

The amount of blood sugar absorbed by the mammary gland is a quantity the accurate determination of which is necessary, not only to decide the nature of the precursor of lactose, but also in problems concerning the relationship of mammary secretion to the general metabolism of the animal. There are certain considerations which suggest that the results of Kaufmann and Magne do not give a true measure of this quantity.

In the first place, the assumption that the energy requirements of the mammary gland are identical with those of the tissues of the head and neck remains as yet unjustified. This point can indeed be decided only when sugar values for arterial blood samples are available for comparison with those of jugular and mammary blood samples taken simultaneously. Further, since the investigation by Kaufmann and Magne was reported it has become evident, principally from the work of Somogyi (1926 - 29), that a considerable fraction of the material estimated as blood sugar by the older methods of analysis consists of non-sugar reducing substances. It is apparent that absorption of such substances by the mammary gland would result in a low "sugar" value for mammary venous blood and would

lead to erroneous estimates of the amount of sugar utilised by the gland.

In measuring the absorption of blood sugar by the mammary gland, there appear then to be three requirements.

1. The correction of jugular blood sugar values for the influence of salivary secretion.

2. The use of a blood sugar technique which would afford information on the values of both true sugar and non-sugar reducing substances.

3. The consideration of the relative energy requirements of the tissues of the head and neck and the active and inactive mammary gland.

Experimental.

Samples of blood were taken simultaneously from the jugular and mammary veins and radial artery of lactating and non-lactating animals as previously described, and iron and blood sugar analyses carried out immediately.

Iron Content.

This was determined as described in the appendix.

Sugar Values.

The method of blood sugar analysis employed was that of Hagedorn and Jensen as applied to Folin-Wu blood filtrates by Hiller, Linder and Van Slyke (1925).

Somogyi (1927) and Herbert, Bourne and Groen (1930) have shown that the Folin-Wu blood filtrate contains practically all of the non-sugar reducing substances so that by this method information on both "true sugar" and the so-called "rest reduction" can be obtained.

The total reducing power of the Folin-Wu filtrate from the freshly drawn blood was first determined. A sample of the blood was then incubated at 37°C for 24 hours and the reducing power of the Folin-Wu filtrate from the incubated sample determined. The second figure gives the amount of non-sugar reducing substances in terms of glucose, and the difference between the two figures gives the true sugar content of the blood as glucose. I have satisfied myself that incubation at 37°C for 24 hours removes added glucose quantitatively.

In Table III are given the iron contents and true sugar values for all the blood samples obtained - in each case the values for jugular blood sugar are corrected as determined in the first investigation of this series.

Table III.

No. of Cow.	Lactating or Non-lactating	Iron content (mgms Fe per 100 cc. blood)			True sugar (mgms glucose per 100 cc. blood)			
		jugular	arterial	mammary	jugular	jugular (corrected)	arterial	mammary
1	Lactating	37.04	35.26	35.06	53	50	52	47
2	"	42.32	39.34	38.95	26	24	30	25
3	"	38.75	37.71	37.33	37	36	49	33
4	"	40.86	39.29	39.50	46	44	48	40
5	"	36.69	35.14	34.26	51	49	56	41
6	"	41.83	-	39.77	36	34	-	29
7	"	37.96	-	37.07	43	42	-	40
8	"	38.75	-	36.94	43	41	-	27
9	Non-lactating	39.31	36.28	36.81	44	41	46	44.5
10	"	34.74	-	32.94	42	40	51	43
11	"	34.23	32.56	33.46	49	47	56	51
12	"	37.28	34.60	34.89	60	55	60.5	60.5
13	"	37.70	-	35.77	43	41	-	44

In Table IV are given the values for non-sugar reducing substances for each sample of blood - the jugular value being in each case corrected for loss of water to the salivary glands.

Table IV.

No. of Cow.	Lactating or Non-lactating	Non-sugar Reducing Substances (as mg. glucose per 100 cc. blood)			
		arterial	jugular	jugular (corrected)	mammary
1	Lactating	23	26	25	27
2	"	30	36	33	28
3	"	30	31	30	32
4	"	35	38	36.5	36
5	"	28	33	32	25
6	"	-	27	26	25
7	"	-	33	32	27
8	"	-	44	42	41
9	Non-lactating	28	28	26	29.5
10	"	32	32	30	31
11	"	27	20	19	22
12	"	31.5	31	29	31.5
13	"	-	40	38	37

In Table V the differences in blood sugar content shown in Table III are expressed for comparative purposes as percentages of the arterial blood sugar where arterial samples were obtained and of the jugular blood where only jugular and mammary samples were available for analysis.

Table V.

No. of Cow.	Lactating or Non-lactating.	Differences in sugar content of jugular, arterial and mammary bloods (as percentage arterial). arterial-jugular	arterial-mammary	jugular-mammary
1	Lactating	3.8	9.6	5.8
2	"	20	16.6	-3.3
3	"	26.5	32.6	6.1
4	"	8.3	16.6	8.3
5	"	12.5	26.7	14.2
6	"	-	-	14.7
7	"	-	-	4.7
8	"	-	-	34.1
9	Non-lactating	13.0	3.2	-9.8
10	"	21.5	15.6	-5.9
11	"	16.0	9.1	-6.9
12	"	7.4	0	-7.4
13	"	-	-	-7.3

Discussion.

Blood sugar values for lactating cattle have been recorded by previous workers. The values and methods used are as follows:

Hayden and Fish (1928) 46 mg. glucose per 100 cc. of blood (Benedict),

Widmark and Carlens (1925) 60 mg (Bang and Hagedorn-Jensen),

Hayden and Scholl (1924) 52 mg (Folin-Wu),

Little, Keith and Fawns 75 mg (1928) (Hagedorn-Jensen),

Allardyce, Fleming, Fowler and Clark (1930) 50 - 65 mg (Benedict 1928).

The results given in Table III would indicate that about 30 per cent of the material estimated by many former workers is to be accounted for by non-sugar reducing substances.

The values obtained for true sugar are very variable, ranging from 24 to 60 mg glucose per 100 cc. of blood. This is not surprising when it is remembered that there are four distinct phases in the life cycle of the dairy cow, (1) non-lactating and non-pregnant, (2) non-lactating and pregnant, (3) lactating and non-pregnant, and (4) lactating and pregnant. At the present time no data are available on the variation of blood sugar level through-

out pregnancy and lactation, but it is hardly to be expected that the value would remain constant during periods physiologically so widely different. The variation in blood sugar level obtained may therefore be due to the animals being in different stages of the life cycle.

Turning now to differences in the true sugar content of the bloods taken from different sources in the same animal it is seen that they are greater than can be accounted for by the modifying influence of salivary secretion and independent of differences in the amounts of non-sugar reducing substances present. In non-lactating animals the sugar content of the jugular blood is lower than that of the arterial blood, a circumstance which is to be ascribed to the withdrawal from the blood of the sugar which is utilised in the metabolic activities of the tissues of the head and neck. In such animals, however, the sugar content of the mammary venous blood, while usually less than that of the arterial blood, is always greater than that of the jugular blood. It is to be concluded that the sugar required by the inactive mammary gland for oxidative purposes is less than that required by the tissues of the head and neck.

In lactating animals, however, it will be observed that the sugar content of the mammary venous blood is lower than that of the jugular blood and very much lower than

that of arterial blood. The question therefore arises as to whether this great lowering of mammary venous blood sugar in lactating animals represents absorption of glucose by the mammary gland for the purpose of meeting increased energy requirements during secretion, or for the synthesis of lactose. Nothing is known of the energy requirements of the mammary gland, but interesting and relevant facts have been discovered for other secretory organs. Barcroft and Brodie (1905), for example, have shown that the respiratory quotient of the active kidney is unity, representing oxidation of carbohydrate only. Barcroft and Piper (1912) have shown that the oxygen consumption of the resting submaxillary gland of the cat is about 0.2 cc. of oxygen per gram per minute, while in the active state it is as much as 0.084 cc. of oxygen per gram per minute. Anrep and Cannan (1922) report that the resting submaxillary gland of the dog utilises about 2.1 mg. glucose per gram of tissue per hour. In the active state much more sugar is utilised, an additional quantity of about 1.5 mg. glucose being required for each cubic centimetre of saliva secreted.

If these figures of Anrep and Cannan for the submaxillary gland are applied to the case of a cow with 5,000 g. of mammary tissue, and a milk yield of 10 litres containing 500 g. of lactose per 12 hours, some indication

is got of the relative proportions of glucose absorbed by the gland for oxidative and synthetic purposes.

In the resting state the gland will utilise (5,000 x 2.1 x 12) or 126 g. of glucose per 12 hours.

In the active state (1.5 x 10,000) or 15 g. of glucose must be added to the foregoing quantity to meet the energy requirements of the secretion of 10 litres of milk.

The amount of glucose used then in the active gland for oxidative purposes in 12 hours will be (126 + 15) or 141 g.

The total amount of glucose used in the active mammary gland in 12 hours is therefore (500 + 141) or 641 g.

The sugar requirement of the active gland for oxidative purposes is then about $\frac{3}{10}$ of the sugar requirement for synthetic activities on the basis of these calculations. Since milk is a much more concentrated product than saliva it is probable that figures calculated from the data of Anrep and Cannan are not strictly applicable to the mammary gland. Indeed it is more than likely that the glucose utilisation of the mammary gland in oxidative processes must be put at a higher figure than 141 g. per 12 hours.

From these observations there is reason to believe that the energy requirements of the mammary gland will be greater in the active state than in the inactive state. Part of the increased sugar absorption must therefore be

ascribed to an increased demand for energy producing substanceswhile secretion is in progress. It is hardly likely that this would account for all the sugar absorbed if the foregoing calculation be even approximately correct, although this is a point which can only be settled by an investigation of the gaseous metabolism of the mammary gland resting and active. It seems justifiable therefore to conclude that part of the sugar absorbed by the active mammary gland is utilised in the synthesis of lactose.

It will be observed that the amounts of sugar absorbed by the mammary gland of the different animals are widely different. This is important in certain aspects of milk production, since it would clearly be of interest to determine if high sugar absorption could be correlated with high lactose secretion and high milk yield. For this purpose an accurate method of determining the amount of sugar actually absorbed for the purpose of lactose synthesis is desirable. The results given indicate that until knowledge of the energy requirements of the mammary gland is available, comparative blood sugar analyses alone are not an entirely reliable guide.

Table IV shows that there are variations in the content of non-sugar reducing substances between the three bloods examined. This fact illustrates the importance of using only "true sugar" values as a basis for conclusions.

THE RELATION OF AMINO-ACID ABSORPTION

TO PROTEIN SYNTHESIS.

------oOo------

THE RELATION OF AMINO-ACID ABSORPTION TO PROTEIN SYNTHESIS.

The work of Cary (1920) indicates the possibility of blood amino acids being absorbed by the mammary gland for the synthesis of caseinogen. This work may be criticised on the grounds of the assumptions it permits - these have already been outlined and investigated in the section on the Kaufmann-Magne technique.

In addition, the analytical method used by Cary for the estimation of the amino-acid nitrogen content of the blood is extremely laborious. It consists of incubating the blood with urease to remove urea, deproteinising with boiling dilute acetic acid, treatment with kaolin, concentrating and tri-chloracetic acid treatment. After three hours boiling to destroy tri-chloracetic acid, the deproteinised filtrate is further concentrated, neutralised and the amino-acid nitrogen determined in the volumetric apparatus of Van Slyke. Any differences in amino acid nitrogen found appear to be significant but it is almost impossible to assess the magnitude of the real error which such an analytical procedure may involve. It has been used by Bock (1917) and Okada (1918) whose results did not agree with those given by Cary. In 1929, Van Slyke published a report of the application of his manometric apparatus to the direct estimation of amino acid nitrogen in blood filtrates prepared by tungstic acid treatment.

I have tested this method at great length and found

it to be both rapid and accurate when all the precautions recommended by Van Slyke are scrupulously observed. By estimating the amino-acid nitrogen in blood filtrate without further treatment there is a great decrease in the probable error due to manipulation. The use of this manometric method permits estimations to be carried out in a smaller quantity of blood than was previously necessary.

Experimental.

Blood samples were taken simultaneously from the jugular and mammary veins and radial artery of lactating and non-lactating animals as previously described. The samples were immediately analysed for:

Iron Content. This was estimated as described in the appendix.

Amino-Acid Nitrogen Content. The manometric method of Van Slyke (1929) was used.

Urea Nitrogen Content. The manometric method of Van Slyke (1927) was used.

Corpuscular Volume. Duplicate blood samples were centrifuged in haematocrit tubes until constant volume was attained.

In Table VI are given the results of a preliminary series of observations carried out to ascertain if the differences between jugular and mammary bloods in amino-acid nitrogen content reported by Cary could be detected by these methods and established as normal findings.

Table VI.

No. of Cow.	Lactating or Non-lactating	Date of Analysis	Urea Nitrogen mg. N per 100 cc. blood		Amino-Acid Nitrogen mg. N per 100 cc. blood		Difference J - M % J
			Jugular	Mammary	Jugular	Mammary	
1	Lactating	27/10/30	12.12	12.93	7.036	6.739	4.2
2	"	4/11/30	10.02	10.04	5.884	4.825	18.0
3	"	10/11/30	7.192	6.969	6.420	5.768	10.1
4	"	13/11/30	9.619	9.529	7.375	6.324	14.2
5	"	17/11/30	9.72	9.04	7.257	6.276	13.5
6	"	20/11/30	10.76	10.58	6.087	5.059	16.9
6	"	26/10/31	6.09	6.44	8.314	7.863	5.4
7	"	24/11/30	11.49	11.31	7.593	6.145	19.0
7	"	1/3/32	10.92	11.34	8.129	6.900	15.2
8	"	27/11/30	13.57	13.475	7.551	6.792	10.0
9	"	3/12/30	11.472	11.290	6.985	6.945	0.6
10	"	8/12/30	9.53	9.709	7.290	6.210	14.8
10	"	2/11/31	10.85	10.10	8.357	7.489	13.9
11	"	15/12/30	11.51	11.69	7.695	6.857	10.9
12	"	19/ 3/31	11.00	11.00	5.479	4.785	12.6
12	"	14/ 9/31	10.08	9.24	7.554	6.754	15.9
2	Non-lactating	19/ 1/31	8.01	7.83	5.771	5.790	0.3
12	"	14/ 1/31	9.95	9.77	5.922	5.934	0.2
12	"	22/ 1/31	10.20	9.77	5.949	5.934	0.2
13	"	9/11/31	10.85	11.06	6.925	7.187	3.6
14	"	23/11/31	5.46	6.34	6.556	6.643	1.3
15	"	26/3/31	8.62	8.56	5.676	5.718	0.8

Table VII.

No. of Cow.	Lactating or Non-lactating	Iron (mg. Fe per 100 cc. blood)			Urea (mg. N per 100 cc. blood)			Amino-Acids (mg. N 100 cc. blood			
		Jugular	Arterial	Mammary	Jugular	Arterial	Mammary	Jugular	Jugular (corrected)	Arterial	Mammary
1	Lactating	37.04	35.26	35.06	10.08	8.68	9.24	7.554	7.148	7.382	6.754
2	"	38.75	37.71	37.33	9.59	9.87	10.99	8.549	8.235	9.438	8.170
3	"	40.86	39.29	39.50	12.18	12.25	12.60	7.766	7.508	7.126	6.062
4	"	36.69	35.14	34.26	10.36	10.57	10.85	6.241	5.977	5.475	4.813
5	Non-lactating	39.31	36.28	36.81	6.58	6.86	7.21	6.921	6.480	6.591	6.993
6	"	34.74	-	32.94	11.41	11.03	11.34	7.653	7.257	7.848	7.653
7	"	34.23	32.56	33.46	8.61	9.31	8.96	7.147	6.985	7.204	7.384
8	"	37.28	34.60	34.89	11.74	11.20	11.74	6.413	6.003	6.101	6.388

Table VII gives the results of a second series in which complete analytical results (jugular, arterial and mammary) for eight cows were available. The figures for jugular blood have been corrected as previously described for the influence of salivary secretion.

In Table VIII the differences in amino-acid nitrogen contents of the blood samples are expressed as percentages of the arterial value for each animal.

Table VIII.

No. of Cow.	Lactating or Non-lactating	Differences in Amino-acid N content) (as percentages arterial amino-acid N) arterial-jugular	arterial-mammary	jugular-mammary
1	Lactating	3.16	8.49	5.33
2	"	12.75	13.44	0.69
3	"	-5.36	14.93	20.29
4	"	-9.17	12.09	21.25
5	Non-lactating	1.68	-6.1	-7.78
6	"	7.51	2.49	-5.05
7	"	3.04	-2.5	-5.54
8	"	1.61	-4.70	-6.31

Table IX gives the results of a number of cases in which corpuscular volumes were available for the calculation of the amino-acid nitrogen content of the corpuscles.

Table IX.

No. of Cow	Lactating or Non-lactating	Corpuscular Volume		Amino-acid N (Blood) mg N per 100 cc. blood		Amino-acid N (Plasma) mg. N per 100 cc. plasma		Amino-acid N (Corpuscles) mg. N per 100 cc. corpuscles	
		jugular	mammary	jugular	mammary	jugular	mammary	jugular	mammary
1	Lactating	33.5	30.5	7.554	6.754	4.248	3.380	14.11	14.44
2	"	37.0	36.0	7.766	6.062	4.808	2.242	12.80	12.85
3	"	41.0	39.0	8.549	8.170	4.687	4.206	14.10	14.37
4	"	39.0	37.0	6.241	4.813	3.224	1.419	10.96	10.59
5	"	40.0	39.0	5.479	4.785	2.357	1.857	10.16	9.37
6	"	43.0	42.5	8.314	7.863	3.714	2.601	14.41	14.71
7	Non-lactating	41.0	38.5	6.921	6.993	3.949	4.094	11.19	11.62
8	"	36.5	35.5	7.653	7.731	4.584	4.658	12.99	13.31
9	"	32.5	30.0	7.147	7.384	4.790	4.874	12.04	13.24
10	"	38.3	34.8	6.413	6.388	4.056	3.978	10.21	11.00
11	"	41.6	37.5	5.922	5.934	3.917	3.948	8.736	9.145
12	"	40.0	39.0	5.949	5.934	3.819	3.857	9.154	9.202

Discussion.

Cary (1920) gives values for the amino-acid nitrogen content of the jugular blood of cows which range from 3.99 to 5.34 mg. N per 100 cc. These are lower than the values given by Bock (1917) and Okada (1918) who give 7 mg. N per 100 cc. as an average. Allardyce et al. (1930) give 5 - 8 mg. N per 100 cc. of blood, a range which is in close agreement with the values given here (5.8 - 8.5 mg.).

Hayden and Fish (1928) give 5.30 - 18 mg. N per 100 cc. of blood as the range of urea nitrogen encountered by them in the analysis of the blood of dairy cows, while Allardyce et al. (1930) give 12 - 20 mg. N. The figures obtained here range from 5.4 - 12.6 mg. N per 100 cc. of blood and were used for the correction of amino-acid nitrogen values as determined by Van Slyke.

The results presented in Table VI show that the differences between the amino-acid nitrogen content of jugular and mammary blood are generally very considerable. This is in agreement with the results of Cary.

From Tables VII and VIII it is seen that the values for jugular blood are slightly lower than those of arterial blood in the non-lactating animals, a result which might be expected to arise from utilisation of amino-acids by the tissues of the head and neck. The mammary

venous blood of the four non-lactating animals examined is however slightly richer in amino-acid nitrogen than arterial blood, a fact which can only be explained by the uptake of amino-acids by the blood in its passage through the inactive gland. There is evidence of another kind to support this finding. There is usually a period of three months between the end of one lactation and the beginning of the next in dairy cows. At the end of the lactation period the udder tissue which, in high-milking breeds, may be very extensive is resorbed. After a period of rest the animal again begins to proliferate mammary tissue in preparation for the next lactation. As the observations on non-milking animals have been carried out soon after the end of a lactation period, it may be that the difference between arterial and mammary venous bloods in amino-acid nitrogen content can be explained by the breakdown of tissue protein in the udder and the gradual re-entry of amino-acids so formed into the circulation.

With lactating animals however such a consideration is absent and the content of radial arterial blood may be taken as the true level of amino-acid nitrogen supplied to the mammary gland. It will be seen from Table VIII that in each of the four lactating animals examined, the mammary amino-acid nitrogen content is markedly and con-

sistently lower than that of arterial blood. Only a small proportion of this absorbed nitrogen can be accounted for by the renewal of glandular tissue so that the theory that milk protein is synthesised from the free amino-acids of the blood appears to be firmly established.

It is interesting to note however that the work of Crowther and Raistrick (1916) indicates that the globulin of milk is identical with the globulin of blood. Lactalbumin and caseinogen, however, appear to be specific products of mammary glandular activity. As recently as 1927 the suggestion that milk proteins may arise by modification of blood proteins is to be found in the literature. In this connection the observation of Rimington (1927) may be quoted. "In considering the problem of mammary secretion the possibility has always to be kept in mind that one or other of the blood proteins might conceivably furnish the starting point for the synthesis of caseinogen". The work of Foà (1911 - 12) already detailed gave no evidence of such a possibility.

The assumption that the amino-acid nitrogen content of jugular blood is the same as that of arterial blood is not supported by the data given. In all of the non-lactating animals and in two of the lactating

ones it is lower, while in two of the lactating subjects it is higher. Whether this latter observation can be regarded as normal or not cannot be decided, but it is certain that the amino-acid nitrogen content of jugular blood cannot be taken as an accurate representation of arterial blood. From the qualitative standpoint this is not important as the results show, but where data are interpreted quantitatively the position is different.

Meigs (1922), for example, has attempted to establish a figure for the rate of flow of blood through the mammary gland by correlating the amount of one milk constituent secreted in a given time with the difference in the amounts of its precursor found in equal quantities of jugular and mammary blood. Cary (1920) has found that differences in the amino-acid nitrogen contents of the jugular and mammary blood of different cows bear no relation to the amount of protein secreted in a given time and concludes that the most important factor governing milk yield is the rate of flow of blood through the udder. Meigs has also stated that the mammary gland absorbs milk precursors from the blood plasma in the proportions in which the respective constituents occur in milk, citing as evidence in support of this contention a comparison of the figures obtained by Kaufmann and Magne (1906) for the utilisation of sugar by the mammary

gland with those for the absorption of blood phosphatide obtained by Meigs et al (1919) using the jugular-mammary technique. While these conclusions seem a priori reasonable, it cannot be said that they are established on the evidence alone of data which have been obtained by the application of a technique which does not give a true indication of the absorption of any precursor from the blood stream.

To illustrate the difficulties which attend any attempt to compare results of blood chemistry investigations with analytical figures for milk yield, figures calculated from the data given may be considered. There are available the differences in sugar and amino-acid nitrogen contents of the arterial and mammary venous blood of four lactating cows. From these it is possible to calculate the ratio of sugar absorbed by the mammary gland to the amino-acid nitrogen absorbed.

Table X.

No. of Cow.	Sugar absorbed from 100 cc. blood		Amino-acid N absorbed from 100 cc. blood		Sugar Nitrogen
	Arterial	Mammary	Arterial	Mammary	
	mg. Glucose		mg. N		
1		5		0.628	7.9
2		16		1.268	12.6
3		8		1.064	7.5
4		10		0.662	15.0

The differences in the sugar-nitrogen ratio indicate the great influence of individual variations on analytical data. These individual variations are, as previously indicated, probably conditioned by the stage in the life cycle attained by each animal.

It has been noted above that although the amino-acid nitrogen content of jugular blood cannot be taken to represent that of arterial blood in a strictly quantitative fashion, significant differences between jugular and mammary venous bloods may be accepted as indications of absorption. The procedure adopted in Table IX, the comparison of corpuscular amino-acid nitrogen figures for jugular and mammary bloods, is therefore justified in so far as information of a qualitative nature is desired. The fact that salivary secretion does not appear to affect the corpuscles in any way allows figures for jugular and mammary corpuscles to be compared directly without correction. It will be seen that the ratio of amino-acid nitrogen in the corpuscles to amino-acid nitrogen in the plasma is approximately 3 to 1. Data on this point have not been published before although Martens (1928) gives the figure of 3 or 4 to 1 for the human subject. In general, although rather large differences are shown by the whole bloods and by the plasmas of lactating animals, only very small differences

exist between the corpuscles. In most cases indeed the amino-acid nitrogen content of mammary corpuscles is even higher than that of jugular corpuscles. When one considers the ratio of amino-acid nitrogen in the corpuscles to that in the plasma one is inclined to explain the difference in content of corpuscles and plasma by indiffusibility of the amino-acids across the corpuscular cell membrane. If such be the case, it is hardly to be expected that corpuscular amino-acids would take part in exchanges between the blood and the mammary gland.

THE PHOSPHORUS METABOLISM OF THE MAMMARY GLAND.

THE PHOSPHORUS METABOLISM OF THE MAMMARY GLAND.

The mammary gland appears to have the power of effecting molecular transformations involving phosphorus. Thus phosphoric acid must be linked up with the amino acids, hydroxyglutamic acid. amino-hydroxybutyric acid and serine in the phosphopeptone which Rimington (1927) has shown to be the phosphorus containing group of caseinogen. That phosphorus is concerned in the secretion of fat is suggested by Meigs, Blatherwick and Cary (1919) who claim that the mammary gland produces milk fat from lecithin and allied substances. Kay (1925) has shown that from 15 - 25% of the total phosphorus of milk is in the form of acid soluble compounds hydrolysed by phosphatases. In view of the number of different phosphorus compounds occurring in milk it seemed desirable to investigate the nature of the phosphorus containing substances absorbed by the mammary gland from the blood stream.

The only attempt to solve this particular problem - that of Meigs et al (1919) - was instigated before the recognition of the important part played by organic acid soluble phosphorus compounds in biochemistry. This work has been so frequently quoted as a demonstration of the synthesis of milk fat from lipoids that some discussion of the data on which the findings are based is desirable.

Estimating the amounts of organic and lipoid phosphorus in samples of blood and plasma from the jugular and mammary veins of five lactating and three non-lactating cows, Meigs et al reported their analytical figures for blood in the case of 2 lactating and 2 non-lactating cows, and for plasma in the case of 3 lactating and 2 non-lactating cows.

The lipoid phosphorus of mammary blood was lower than that of jugular blood in the case of two lactating and one non-lactating animals by 4.6, 6.8 and 1.8 per cent respectively. In the case of the second non-lactating cow mammary lipoid phosphorus was higher than jugular by 2.7 per cent. Mammary plasma lipoid phosphorus was lower than that of jugular plasma in the case of two lactating animals by 8.0 and 10.2 per cent, while in a third there was no difference. For the two non-lactating animals there was no difference. For inorganic phosphorus only plasma figures are given, mammary values being higher than jugular values in lactating and non-lactating animals alike, by 9.5, 10.0, 26.0, and 8.0 and 12.0 per cent respectively. Meigs and his co-workers claim that these results indicate that the active mammary gland absorbs lipoids from the blood stream, that fat is formed from the lecithin so obtained and that the inorganic phosphorus liberated in excess of that required for the other milk

constituents is returned to the blood stream where it serves to raise the inorganic phosphorus content of the mammary blood. The fact that the inorganic phosphorus content of the mammary plasma is higher than that of the jugular plasma in lactating and non-lactating animals alike was explained by Meigs by assuming that the gland in the latter cases had not become inactive.

While the conclusions put forward by Meigs et al. (1919) seem a priori reasonable, the paucity of the experimental work reported and the equivocal nature of the data available should be borne in mind. It cannot be said that a priori reasonable theories are supported by such data as are given.

In addition, this work is open to the criticisms already levelled against the jugular-mammary technique. The assumption is made that the phosphorus content of jugular blood is identical in amount and in partition between the various phosphorus containing compounds with that of mammary arterial blood. The foregoing experimental sections have shown that jugular blood is not a true representation of arterial blood, and that the utilisation of sugar by the tissues drained by the jugular vein is considerable. Irving and Bastedo (1928) analysed blood from arteries and veins of exercising and asphyxiated muscles. Even when the inorganic phosphate of the muscle had risen to an extreme degree, as the result of ester hydrolysis, no significant difference between the inorganic phosphorus of the arterial and venous

bloods was observed. This has not yet been demonstrated for arterial and jugular bloods nor has the relation of organic phosphorus in the two bloods been established. Meigs and his co-workers have further assumed that the only organic phosphorus compound in plasma, and therefore the only phosphorus compound which can be abstracted by the tissues from the blood, is in the form of lipoid phosphorus. The significance attached at the present time to readily hydrolysable phosphoric esters in cellular activities, and the presence of these in the blood is not in harmony with the assumption made by Meigs and his collaborators. In the light of this recent work on the significance of the phosphorus containing compounds it seemed advisable to concentrate attention on the rôle of the acid soluble phosphorus compounds of the blood in relation to milk secretion. In this investigation, as in the others reported, arterial samples have been taken when possible and the modifying influence of salivary secretion on jugular blood determined.

Experimental.

Samples of blood were taken simultaneously from the radial artery and the jugular and mammary veins of lactating and non-lactating animals as already described.

The samples were analysed for.

Iron Content.

This was determined as described in the appendix.

Corpuscular Volume.

The hæmatocrit technique was used.

Total acid soluble phosphorus and inorganic phosphorus.

The colorimetric method of Fiske and Subbarow (1925) was used; organic acid soluble phosphorus being estimated by difference.

Table XI summarises the results obtained for four lactating and four non-lactating cows. In Table XII the differences implicit in Table XI are expressed as percentages of the arterial values. Table XIII shows the partition of acid soluble phosphorus compounds between plasma and corpuscles in the jugular blood of lactating and non-lactating cows. Table XIV gives similar data for the mammary blood.

Table XI.

No. of Cow.	Lactating or Non-lactating	Iron content mg. Fe 100 cc. blood			Total acid sol. phosphorus mg. P 100 cc. blood				Inorganic phosphorus mg. P 100 cc. blood				Organic acid sol. phosphorus mg P 100 cc. blood			
		Jugular	Arterial	Mammary	Jugular	Jugular (corrected)	Arterial	Mammary	Jugular	Jugular (corrected)	Arterial	Mammary	Jugular	Jugular (corrected)	Arterial	Mammary
1.	Lactating	42.32	39.34	38.95	9.60	8.93	10.60	9.70	5.16	4.80	5.20	4.92	4.44	4.13	5.40	4.78
2.	"	38.75	37.71	37.33	9.50	9.24	10.30	9.00	5.26	5.12	5.48	5.34	4.24	4.13	4.82	3.66
3.	"	40.86	39.29	39.50	11.60	11.15	12.10	11.44	5.72	5.50	5.86	5.76	5.88	5.65	6.24	5.68
4.	"	36.69	35.14	34.26	9.25	8.86	10.05	9.50	4.44	4.25	4.72	4.70	4.81	4.64	5.33	4.80
5.	Non-lactating	39.31	36.28	36.81	9.10	8.40	9.70	9.60	5.76	5.32	6.42	5.58	3.34	3.08	3.28	4.02
6.	"	34.74	-	32.94	9.00	8.53	8.80	9.20	4.70	4.46	4.76	4.90	4.30	4.09	4.04	4.30
7.	"	34.23	32.56	33.46	9.90	9.41	11.00	10.60	5.62	5.35	5.48	5.64	4.28	4.07	5.52	4.94
8.	"	37.28	34.60	34.89	10.00	9.28	9.80	10.40	5.82	5.40	5.86	5.62	4.18	3.88	3.94	4.78

Table XII.

No. of Cow.	Lactating or Non-Lactating	Total Acid Soluble Phosphorus			Inorganic Phosphorus			Organic Acid Soluble Phosphorus		
		Jugular-Mammary	Arterial-Jugular	Arterial-Mammary	Jugular-Mammary	Arterial-Jugular	Arterial-Mammary	Jugular-Mammary	Arterial-Jugular	Arterial-Mammary
1.	Lactating	-7.30	15.80	8.5	-2.20	7.60	5.4	-12.03	23.52	11.48
2.	"	2.32	10.29	12.62	-4.02	6.57	2.56	9.75	14.32	24.07
3.	"	-2.40	7.85	5.45	-4.44	6.14	1.71	-0.48	9.45	8.96
4.	"	-6.40	11.9	5.5	-9.54	9.96	0.42	-3.01	12.94	9.94
5.	Non-lactating	-12.37	13.40	1.03	-4.05	17.14	13.08	-28.7	6.1	-22.56
6.	"	-7.61	3.07	-4.54	-9.25	6.30	-2.94	-5.14	-1.22	-6.36
7.	"	-10.8	14.4	3.6	-5.29	2.37	-2.92	-15.76	26.27	10.51
8.	"	-11.43	5.31	-6.12	-3.75	7.85	4.10	-22.84	1.52	-21.32

TABLE XIII.

Partition of Acid Soluble Phosphorus Compounds between Plasma and Corpuscles in Jugular Blood.

	Corpuscular Volume.	Blood. Total Acid Sol. P.	Blood. Inorganic P.	Blood. Acid Sol. Organic P.	Plasma. Total Acid Sol. P.	Plasma. Inorganic P.	Plasma. Acid Sol. Org. P.	Corpuscles. Total Acid Sol. P.	Corpuscles. Inorganic P.	Corpuscles. Acid Sol. Org. P.	Organic Acid Sol. P. Corpuscles. / Organic Acid Sol. P. Plasma.
Lactating	29.5	10.00	6.48	3.52	8.80	7.46	1.42	12.88	4.14	8.74	6.15
	38.0	11.20	6.60	4.60	9.60	7.58	2.01	13.81	5.00	8.81	4.38
	36.5	9.60	5.16	4.44	8.70	6.10	2.60	11.10	3.53	7.64	2.94
	41.0	9.50	5.26	4.24	8.90	5.78	3.12	10.36	4.51	5.85	1.87
	37.0	11.60	5.72	5.88	9.90	7.10	2.80	14.49	3.38	11.14	3.97
	39.0	9.25	4.44	4.81	6.80	4.42	2.38	13.08	4.46	8.61	3.61
	Mean	10.19	5.61	4.58	8.75	6.40	2.39	12.62	4.17	8.46	3.82
Non-lactating	34.0	11.60	6.34	5.26	8.65	7.30	1.35	17.30	4.47	12.83	9.5
	38.5	11.35	6.60	4.75	10.05	7.86	2.19	13.90	4.60	9.30	4.24
	41.0	9.10	5.76	3.34	7.20	6.80	0.40	11.82	4.27	7.56	18.9
	36.5	9.00	4.70	4.30	6.00	5.60	0.40	14.22	3.12	11.09	27.7
	32.5	9.90	5.62	4.28	7.50	6.66	0.84	14.89	2.49	13.20	15.7
	38.5	10.00	5.82	4.18	8.00	6.90	1.10	13.21	4.05	9.14	8.30
	Mean	10.16	5.81	4.35	7.90	6.85		14.22	3.83	10.52	14.06

TABLE XIV.

Partition of Acid Soluble Phosphorus Compounds between Plasma and Corpuscles in Mammary Blood.

	Corpuscular Volume.	Blood: Total Acid Sol. P.	Blood: Inorganic P.	Blood: Acid Sol. Organic P.	Plasma: Total Acid Sol. P.	Plasma: Inorganic. P.	Corpuscles: Organic Acid Sol. P	Corpuscles: Total Acid Sol. P	Corpuscles: Inorganic P	Corpuscles: Organic Acid Sol. P	Organic Acid Sol. P Corpuscles / Organic Acid Sol. Plasma.
Lactating	28.5	10.10	6.56	3.54	9.0	6.56	1.44	12.88	4.13	8.75	6.07
	36.0	10.8	6.3	4.5	9.0	6.8	2.2	14.00	5.30	8.70	3.95
	33.5	9.70	4.92	4.78	8.20	5.68	2.52	12.69	3.40	9.26	3.67
	39.0	9.00	5.34	3.66	9.00	6.38	2.62	9.00	3.72	5.28	2.01
	36.0	11.44	5.76	5.68	8.96	6.40	2.56	15.83	4.61	11.22	4.38
	37.0	9.50	4.70	4.80	7.00	5.34	1.66	13.76	3.54	10.22	6.15
	Mean	10.09	5.59	4.49	8.52	6.19	2.17	13.03	4.11	8.90	4.37
Non-lactating	33.0	11.60	6.40	5.20	8.70	7.30	1.35	17.50	4.45	13.05	9.7
	38.5	10.95	6.66	4.29	10.00	8.06	1.94	12.47	4.42	8.05	4.15
	38.5	9.60	5.58	4.02	8.70	6.96	1.74	11.04	3.38	7.80	4.48
	35.5	9.20	4.90	4.30	7.20	6.18	1.02	13.04	2.82	10.28	10.07
	30.0	10.60	5.64	4.94	8.70	6.82	1.88	15.03	2.90	12.00	6.38
	34.8	10.40	5.62	4.78	8.66	7.08	1.58	13.73	2.87	10.79	6.90
	Mean	10.39	5.80	4.59	8.66	7.07	1.58	13.80	3.47	10.33	6.95

Discussion.

Figures for the total acid soluble phosphorus and inorganic phosphorus content of cow's blood have been published by Hayden and Fish (1928) who give 5.70 mg. P per 100 cc. of blood and 9.29 mg.P per 100 cc. of blood as inorganic and total acid soluble phosphorus respectively. The mean values obtained by me for samples of jugular blood from twelve different cows are 5.71 mg. P per 100 cc. of blood as inorganic phosphorus and 10.18 mg. P per 100 cc. of blood as total acid soluble phosphorus.

Discussing first the hypothesis of Meigs and his co-workers (1919) that the phosphorus contents of arterial and jugular bloods are identical, reference to Tables I and II shows that this assumption is unjustified so far as acid soluble phosphorus is concerned. In every case which I have examined I have found the acid soluble phosphorus content of jugular blood to be lower than that of arterial blood. Further, this relationship appears to hold for inorganic and organic acid soluble phosphorus contents also. It is then to be assumed that, at the time when these blood samples were taken, both of these classes of phosphorus containing compounds were being absorbed from the blood stream by the tissues drained by the jugular vein. The qualification is necessary because there cannot be continual absorption of phosphorus, no matter how small the amount, by any tissue. The observations reported in

this investigation were carried out on samples of blood taken 4 hours after milking and immediately after a meal. It is feasible that at such a period, the arterial blood is losing material to the tissues, a process which might be reversed at a later period in the day. In this connection Palmer et al. (1930) have reported large diurnal variations in the inorganic phosphorus content of the venous blood of dairy cattle. Such an observation is in accordance with a post-prandial period of absorption of phosphorus by the tissues followed by a period during which phosphorus is returned to the blood. Whether this interpretation be accepted or not, it is evident that the phosphorus content of jugular blood cannot be taken to represent that of arterial blood. If then any information on the phosphorus metabolism of the mammary gland is to be obtained from blood analysis it is apparent that analytical values for mammary blood must be compared with those for arterial blood.

It will be seen that the four lactating cows of Table I show smaller amounts of total acid soluble phosphorus in mammary blood than in arterial blood. The difference appears to be due entirely to absorption of organic acid soluble phosphorus compounds since the amounts of inorganic phosphorus in the two bloods appear to differ but slightly. For the four lactating animals both bloods have practically the same amounts of total acid soluble phosphorus. With the exception of animal No.5 for which an anomalous result was obtained, the inorganic phosphorus contents of both

(presumably non-lactating cows under discussion)

bloods are equal. With the exception of one animal I have found that the organic acid soluble phosphorus content of mammary blood is higher than that of arterial blood - an observation which was at first surprising. The work of Kay and Robison (1924) Martland and Robison (1926) and Lawaczek (1924) has indicated however that there is probably a continual interchange of phosphorus between the inorganic and organic phosphorus compounds in the blood. It may be that as the blood proceeds further from the heart the ratio, organic acid soluble phosphorus to inorganic phosphorus, is increased without change in the total amount of acid soluble phosphorus present. Such a process would explain my finding that the organic acid soluble phosphorus content of mammary blood is higher than that of arterial blood in non-lactating cows. If such be the case the figures shown for the amounts of organic acid soluble phosphorus absorbed by the mammary gland are too low. Meigs et al.(1919) have stated that the active mammary gland excretes excess inorganic phosphorus back into the blood stream, basing their conclusion on the finding that the inorganic phosphorus content of mammary plasma is higher than that of jugular plasma. Figures for whole blood are likely to give more accurate information on this point. In every case examined - lactating and non-lactating animals alike - I have found that mammary blood contains more inorganic phosphorus than jugular blood. This fact cannot be related to the state of activity of the mammary gland in any way.

Moreover, if the mammary gland absorbs more phosphorus from the blood stream than is required for the secretion of milk - a point which has not yet been demonstrated - we should expect to find more inorganic phosphorus in mammary blood than in arterial blood. In no lactating animal was this the case and I believe that the difference between jugular and mammary bloods in this respect results from the fact that the mammary gland does not absorb the inorganic phosphorus compounds of the blood stream as does the tissues drained by the jugular veins.

My results support the view that the mammary gland utilises the organic acid soluble phosphorus compounds of the blood stream and the question arises as to the function which they perform in the secreting cells. Kay (1925) has found that the active mammary gland of the goat contains an enzyme capable of hydrolysing such phosphorus containing substances. The secretion of milk must to a certain extent resemble bone formation for both processes involve the separation of insoluble calcium phosphate from soluble phosphorus compounds. Robison and his co-workers (1924-1926) consider that the calcium phosphate of bone arises by enzymic hydrolysis of substances of the nature of hexose phosphates and I suggest that there is strong presumptive evidence for a similar process taking place in the mammary gland. a) The gland can absorb acid soluble phosphorus compounds from the blood stream, b) it contains

an enzyme capable of splitting such compounds and c) it secretes insoluble calcium phosphate. The evidence is of the same nature as that adduced for the intervention of acid soluble organic phosphorus compounds in bone formation.

One of the difficulties of accepting Robison's theory of bone formation is that most of the acid soluble organic phosphorus of the blood is carried in the cells. It is of interest therefore to consider some data on the distribution of acid soluble phosphorus compounds between corpuscles and plasma of cow's blood. In the first place it is to be noted that the total acid soluble phosphorus content of cow's blood is only about one-third of that of human blood. This is accounted for almost entirely by the extremely low acid soluble organic phosphorus content of cow's blood. In human blood, the plasma is almost devoid of organic acid soluble phosphorus. From Tables III and IV it will be seen that the plasma of lactating cows contains appreciable quantities of organic acid soluble phosphorus - amounts which, indeed, constitute a considerable fraction of that in whole blood. I have found the organic acid soluble phosphorus contents the jugular blood of lactating and non-lactating animals to be almost equal. The partition of this phosphorus between cells and plasma appears to differ in the two states. In non-lactating animals there is about fourteen

times as much organic acid soluble phosphorus in the corpuscles as in the plasma. In lactating animals there is only about four times as much. This would appear to indicate that in the lactating animal there is a greater demand for organic acid soluble phosphorus than in the non-lactating animal. In the former state a large proportion of the organic phosphorus is carried in the plasma -a medium of transport which would facilitate exchanges with the mammary gland. The ratio is not so high in the mammary blood of lactating animals (Table IV) although still higher than that in non-lactating animals. This is as one would expect if the active mammary gland absorbs organic acid soluble phosphorus. It would appear therefore that the acid soluble phosphorus compounds of blood play an important part in the secretion of milk.

In conclusion it should be stated that Meigs and his co-workers found no organic acid soluble compounds in the plasma, but their method of analysis involved samples of plasma standing in contact with precipitating reagents for twenty-four hours. Under such conditions any easily hydrolysable phosphorus present in the plasma would appear in the analysis as inorganic phosphate.

C O N C L U S I O N.

CONCLUSION.

The investigations reported in this thesis may be considered to contribute to our knowledge of the physiology of milk secretion in two ways.

Firstly, by establishing the fact that the technique employed by Kaufmann and Magne and later workers is untrustworthy, and by substituting the comparison of analytical values for mammary blood with those for arterial blood, accurate information has been obtained on the amounts of blood constituents absorbed by the mammary gland. It has been shown that there is strong evidence for the view that the sugar of the blood is the precursor of lactose, but it has been indicated that, owing to the demand for energy producing substances by the mammary gland, there cannot be equality between lactose secretion and the sugar absorbed from the blood stream. The extent to which amino-acids are utilised by the mammary gland has been investigated and it seems that there is little doubt that such substances are the precursors of caseinogen and lactalbumin. Only the amino-acids of the plasma appear to take part in the exchanges with the mammary gland. With regard to the phosphorus metabolism of the mammary gland the position appears to be more complicated than previous workers have realised. The mammary gland does not, as Meigs and his co-workers suggested, simply absorb lipoid phosphorus and pass inorganic phosphorus

back into the blood stream. It is probable indeed that all the organic phosphorus constituents of the blood stream enter into the chemical transformations taking place in the secreting cells. It seems a priori reasonable to assume that phosphatides are concerned in the secretion of milk fat but the treatment of milk secretion to be found in the preceding pages indicates that this fact has not yet been established with any certainty. It has been shown that acid soluble organic phosphorus compounds are absorbed by the mammary gland and it is suggested that such compounds may enter into the chemical reactions resulting in the appearance of insoluble calcium phosphate in milk. When it is remembered that such reactions would liberate hexoses in addition to inorganic phosphorus one finds some encouragement for the view that organic phosphorus compounds play a part in lactose secretion. Such a suggestion might prove a fruitful source of future work although at the present time it is purely speculative. There is no evidence for the statement that the mammary gland returns inorganic phosphorus to the blood stream.

Secondly, the work here reported has a bearing also on the quantitative aspects of milk secretion. It is known for example that different cows vary enormously in the amounts of milk secreted in equal times. It is therefore desirable to discover upon what factors this individuality depends. In this investigation it has been shown that different animals abstract different amounts of milk pre-

cursors from the blood stream and it might be thought that this is the factor which determines the amount of milk secreted in a given time. On this basis the all important factor in the secretion of milk would be the permeability of the membranes separating the secreting cells from the blood stream. There must however be other factors concerned, namely the rate at which the blood flows through the mammary gland and the area of the surface exposed to the blood. In order to determine the extent to which each of these factors condition milk yield it would be necessary to know the amount of each precursor absorbed by the gland in a given time. It has been shown by this investigation that the only accurate measure of the first of these can be obtained by comparison of arterial and mammary bloods. Further, the evidence adduced indicates that one cannot always equate input and output so far as the mammary gland is concerned. This certainly is the case so far as sugar is concerned, and it may also hold for phosphorus. It is probable however that the only change undergone by amino-acids in the secreting cells is synthesis to the proteins caseinogen and lactalbumin. It may reasonably be expected therefore that data of a satisfactory nature on the factors determining the yield of milk could be obtained by consideration of the input of amino-acid nitrogen to the mammary gland and the output of protein nitrogen.

So far capacity factors alone have been considered - i.e. the amounts of milk precursors absorbed from the blood. It may now be possible to study the effect of intensity factors on the secretion of milk - namely the influence of the concentration of the precursors of milk constituents in the blood perfusing the mammary gland. By studies developed in logical fashion it would be possible in time to build up a structure of biochemical knowledge of milk secretion which could not fail to be of service in the practical problems of milk production.

APPENDIX.

An Iodometric Method for the Estimation of Iron in Blood.

APPENDIX.

An Iodometric Method for the Estimation of Iron in Blood.

Preparatory to the investigation of the Kaufmann-Magne technique it was necessary to employ a routine but accurate method for the estimation of iron in small quantities of blood.

The well-known reaction of ferric ions with thiocyanate is the basis of colorimetric procedures used by Wong (1923), Walker (1925) and others, but the disadvantage attending the colorimetric comparison lead me to reject these methods.

Jahn (1911) has described a procedure in which the blood is submitted to dry ashing and the iron residue titrated with titanium chloride in the presence of potassium thiocyanate. I have found dry ashing of the blood to be unsatisfactory on account of the difficulty of obtaining the iron residue in a form in which it is completely soluble in concentrated hydrochloric acid. Moreover it is necessary to carry out titanium chloride titrations in an atmosphere of carbon dioxide.

Murray (1924) has employed dry ashing of the blood,

followed by reduction of the iron which is then titrated with standard permanganate in an atmosphere of hydrogen. Using dilutions commensurate with the small quantities of iron present in blood the permanganate end-point is difficult to judge and is therefore unreliable.

Accordingly I have developed an iodometric method, which, employing the sensitive starch-iodide indicator, gives results accurate to about 2% on 2 cc. portions of blood.

The reaction between ferric and iodide ions proceeds according to the reversible equation

$$Fe^{+++} + 3I^- \rightleftharpoons Fe^{++} + 2I^- + \tfrac{1}{2}I_2$$

I have been unable to find any information on the state of equilibrium of this system at the dilutions which must be employed in the analysis of small quantities of blood. My first efforts were accordingly directed to finding conditions suitable forthe employment of the above reaction in the estimation of the amounts of iron in 2 cc. of blood (about .7 mgm.).

It was found that in neutral solution the equilibrium of the iron-iodide system lies only a short distance from the left and is therefore useless for quantitative purposes. With increasing H-ion concentration, however, the equilibrium moves towards the right, until in sufficiently acid solution quantitative reduction takes place. The liberated iodine may then be removed by titration with N/200

thiosulphate solution.

For the present purpose it will be sufficient to refer to Table I which summarises the results of a series of titrations carried out by me. The figures were obtained by titrating with N/200 thiosulphate the iodine liberated by the interaction of a known quantity of ferric iron with potassium iodide.

Table I.

Initial concentration of iodide = 50 millimols per litre.

Concentration of hydrochloric acid = 5 mols per litre.

Initial concentration of ferric salt (millimols per litre)		1	2	3	4	5	6	7	8	9	10
Thiosulphate titration of 10 cc. of solution	Found at equilibrium	1.97	3.97	2.96	3.94	2.505	2.96	3.48	3.99	4.45	4.95
	Calculated for complete reduction	2.00	4.00	3.00	4.00	2.50	3.00	3.50	4.00	4.50	5.00
Strength of thiosulphate		.005N	.005N	.01N	.01N	.02N	.02N	.02N	.02N	.02N	.02
Ferrous salt at equilibrium (per cent)		98.5	99.2	98.6	98.5	100.2	98.6	99.4	99.7	98.8	99.0

It will be seen that in solutions which are 5 molar with respect to HCL and 50 millimolar with respect to potassium iodide it is possible to estimate quantitatively amounts of iron representing concentrations of from 1 - 5 millimols per litre. Under these conditions the end-point is sharp and there is no return of colour within 10 minutes.

It remains therefore to develop a preliminary treatment of 2 cc. of blood which will allow of the above conditions in the final titration. In practice it was found that the following method gave satisfactory results.

METHOD.

The following reagents, which should be iron free, are required.

Sulphuric acid.

Nitric acid.

Hydrochloric acid.

Potassium iodide (4% solution - free from iodate: this is made up fresh each day).

Starch indicator (1% solution of starch in sat. sodium chloride).

N/200 Sodium thiosulphate.

Merck's perhydrol.

Potassium dichromate.

Preparation of Thiosulphate.

N/200 sodium thiosulphate is best prepared according to the instructions given in Pregl's Quantitative Organic Micro-Analysis (2nd English Edition, 1930). N/10 sodium thiosulphate which has been ripened to constant strength, is diluted exactly to N/200 strength by means of boiled out distilled water. The diluted solution contains 5% by volume of amyl alcohol as a preservative and I have found that, when kept in amber coloured bottles attached to automatic burettes protected by soda-lime tubes, the reagent retains its strength over long periods.

Standardisation of Thiosulphate.

5 cc. of conc. hydrochloric acid are placed in a 8 x 1¼ inch Pyrex test-tube, and boiled gently for one minute, a glass bead being added to prevent bumping.

The tube is then cooled under the tap and 3 cc. of standard potassium dichromate solution (25 mg. $K_2Cr_2O_7$ in 100 cc.) and 2 cc. of 4% potassium iodide solution are added. A tightly fitting rubber stopper is the inserted and the tube is allowed to stand for exactly 10 minutes. The thiosulphate solution is now added drop by drop with shaking until the yellow colour of the solution almost disappears. At this point 10 cc. of distilled water are added followed by 5 drops of the starch indicator when the

characteristic blue colour of the starch iodide complex develops. After shaking, thiosulphate is added until the blue colour disappears. The last few drops should be added very slowly, drop by drop, and with constant shaking.

A blank titration is carried out exactly as above except that 3 cc. of distilled water take the place of the dichromate solution. The value of the blank is substracted from that of the first thiosulphate titration.

Normality of Thiosulphate = $\frac{3x}{4.9y}$.

where x = gms. $K_2Cr_2O_7$ in 100 cc. of solution.

and y = cc. of thiosulphate required.

The Estimation of Iron in Blood.

2 cc. of blood are drained under the surface of 2 cc. of distilled water contained in a Pyrex test-tube, the pipette (which is graduated to contain and not to deliver) being rinsed twice with the supernatant water.

5 cc. of conc. HNO_3 are then added and the tube is placed in a bath of boiling water until only 2 cc. of pale yellow solution are left. In this way the blood proteins are digested smoothly and most of the organic matter is oxidised.

The latter part of the combustion is best carried

out by hand. 0.5 cc. of conc. H_2SO_4 is added to the tube along with a glass bead to prevent bumping and the liquid is boiled gently over the flame of a small burner until charring is complete. The tube is allowed to cool a little, 1 cc. of conc. HNO_3 is added and the liquid is again boiled. If any organic matter still remains a few more drops of HNO_3 are usually sufficient to complete the destruction. 1 cc. of distilled water and 5 drops of perhydrol are added to the cooled liquid which is again boiled until white fumes appear. Two successive quantities of 1 cc. of distilled water are now added and after each addition the liquid is evaporated down in order to remove any remaining HNO_3 or perhydrol.

5 cc. of conc. HCl are introduced into the cooled tube and the liquid is boiled gently until a clear yellow solution is obtained. The tube is cooled under the tap, 3 cc. of distilled water and 2 cc. of 4% K.I. solution are added immediately and the tube is stoppered tightly.

The titration is then carried out, as before, at the end of exactly ten minutes.

A blank estimation is carried out using 2 cc. of water instead of blood. The blank value is substracted from the titration figure. In practice I have found that the blank value remains constant and need only be redetermined when any change is made in the reagents.

Mgms. Iron per 100 cc. blood = 2792 xy

where x = cc. of thiosulphate required.

and y = Normality factor of thiosulphate.

Results: The following table gives the results of estimations in which a solution of ferrous iron of known strength was used instead of blood. The complete procedure outlined above was followed.

Table II.

mgm. Iron present	mgm. Iron found	Percentage error.
.6055	.6149	1.4
.8074	.8088	0.17
1.0093	.9954	-1.3
1.2111	1.1920	-1.6

The following experiment, using cow's blood, illustrates the recovery of iron added to the blood before ashing.

Table III.

Iron in 2 cc. of blood. mgm.	Iron added mgm.	Total iron mgm.	Iron found mgm.	Iron recovered %
0.6829	-	0.6829	0.6829	-
0.6829	0.2830	0.9659	0.9576	99.2
0.6829	0.4000	1.0829	1.067	98.5
0.6829	0.4800	1.1629	1.143	98.2
0.6829	0.5600	1.2429	1.214	97.8

I have carried out analyses on over 50 samples of

cow's blood and have obtained excellent agreement between duplicates.

BIBLIOGRAPHY.

Abderhalden (1913) Zeit. f. physiol. chem. 88. 478.

Abel. Rowntree and Turner (1914) J. Pharm. Exp. Therap. 5. 275.

Allardyce, Fleming, Fowler and Clark (1930) Canad. J. Res. 3. (2). 120.

Anrep and Cannan (1922) J. Physiol. 56. 248.
ibid.57. 1.

Barcroft and Brodie. (1905). J. Physiol. 33. 52.

Barcroft and Piper (1912). J. Physiol. 44. 359.

Bert. (1884) C. R. Acad. Sci. 8. 775.

Bloor (1918) J. Biol.Chem. 36. 49.

Bock. (1917) J. Biol. Chem. 28. 357.

Cary. (1920) J. Biol. Chem. 43. 477.

(1921) J. Biol. Chem. 46. Proc.Soc.Biol.Chemists 13.

(1922) ibid. 50 Proc.Soc.Biol.Chemists 35.

(1922) reported by Meigs. Phys.Revs. 2. 2. 214.

Cary and Meigs. (1928) J. Biol. Chem. 78. 399.

Chauveau (1856) C. R. Acad. Sci. 42. 1008.

Crowther and Raistrick. (1916) Biochem. J. 10. 434.

Cori and Cori. (1928) Proc. Soc. Exp. Biol. and Med. 25.402.

Corley. (1928) J. Biol. Chem. 76. 31.

Delaunay (1913) C. R. Soc. Biol. 74. 639,- 767.

Eckles and Palmer (1916) Res. Bull. 25. Univ. of Missouri Agr. Exp. Sta.

Feigl (1917) Biochem. Zeit. 81. 380.

ibid. 83. 81 and 218.

ibid. 84. 231.

ibid. 86. 395.

Fiske and Subbarow (1925) J. Biol. Chem. 66. 375.

Foà (1907 - '08) Arch. d. Fisiol. 5. 533.

(1911 - '12) ibid. 10. 402.

Folin and others (1912) J. Biol. Chem. 11. 87. 161. 527.

ibid. 12.141. 253. 259.

Folin and Berglund. (1922) J. Biol. Chem. 51. 395.

Folin and Denis (1912) J. Biol. Chem. 11. 87.

ibid. 12.141.

Hayden and Fish. (1928) Cornell Veterinarian April 1928

Hayden and Scholl. (1923-'24) Rep. N.Y. State Vet. Coll. 1923-24.

Herbert, Bourne and Groen (1930) Biochem. J. 24. 291

Hiller Linder and Van Slyke. (1925) J. Biol. Chem. 64. 625

Irving and Bastedo (1928) Am. J. Physiol. 86. 225.

Jahn. (1911) Zeit. f. physiol. chem. 75. 308.

Kaufmann and Magne (1906) C. R. Acad. Sci. 143. 779.

Kay (1925) Biochem. J. 19. 433.

Kay and Robison (1924) Biochem. J. 18. 755.

Lawaczek. (1924) Biochem. Zeit. 145. 351.

Little, Keith and Fawns. Vet. Journ. 84. 6. 284.

Marshall and Kirkness. (1907) Biochem. J. 2. 1.

Martens (1928) Bull. soc. chem. biol. 10. 1336.

Martland and Robison. (1926) Biochem. J. 20. 847.

Meigs. (1922) Phys. Revs. 2. 2. 204.

Meigs, Blatherwick and Cary. (1919) J. Biol. Chem. 37. 1.

Morgen, Beger und Fingerling. (1904 - '05) Die Landwirt-
-schaftlichen Versuchs Stationen. 61. 1.

Morgen, Beger und Fingerling. (1905) Die Landwirt-
-schaftlichen Versuchs Stationen. 62. 251.

(1906) ibid. 64. 93.

Morgen, Beger und Westhauser (1907) Die Landwirt-
schaftlichen Versuchs Stationen. 66. 63.

Moore and Parker (1900 - '01) Amer. J. Phys. 4. 239.

Murray (1924) Biochem. J. 18. 852.

Okada (1918) J. Biol. Chem. 33. 325.

Osgood (1926) Arch. Int. Med. 37. 685.

Palmer, Cunningham and Eckles. (1930) J. Dairy Sci.
13. 3. 174.

Porcher (1909) Arch. internat. d. physiol. 8. 356.

Pregl Quantitative Organic Microanalysis. 2nd Eng.
Ed^{n}. 1930. J. & A. Churchill, London.

Rimington (1927) Biochem. J. 21. 272.

Schalk and Amadon (1928) North Dakota Agric. Exp. Sta.
Bull. 216.

Somogyi (1927) J. Biol. Chem. 75. 33.

(1928) ibid. 80 753.

(1929) ibid. 83.157.

Van Slyke (1917) Arch. Int. Med. 19. 56.

(1927) J. Biol. Chem. 73. 695.

(1929) ibid. 83. 425.

Van Slyke, Cullen and Maclean (1915) Proc.Soc.Exp.Biol.Med.
12. 92.

Van Slyke and Meyer (1912) J. Biol.Chem. 12. 399.

(1913) ibid. 16. 197.

Walker (1925) Analyst. 50. 1279.

Widmark and Carlens. (1925) Biochem. Zeit. 158. 3.

Witts. (1929) Quart. J. Med. 22. 477.

Wong. (1923) J. Biol. Chem. 55. 421.