XLI. THE ABSORPTION OF MILK PRECURSORS BY THE MAMMARY GLAND.

I. THE KAUFMANN-MAGNE TECHNIQUE.

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THE object of the present series of papers is to effect a correlation of our knowledge of milk secretion and of the chemistry of the blood.

Since the process of milk secretion involves complex molecular transformation within the secreting cells of the mammary gland, it is a matter of physiological importance to determine which constituents of the blood are selected by the gland for conversion into milk protein, carbohydrate and fat. Such data are also of immediate practical importance since effective control of the milk production of animals requires a co-ordination of diet and milk yield and, therefore, an adequate knowledge of the relationship between active gland and blood-stream—the intermediary between gland and digestive tract.

The secretory process of the mammary gland is extremely sensitive and can best be studied in the intact animal. The ideal method, therefore, of determining whether any given blood constituent takes part in the secretory process is to compare the concentration of that constituent in the blood before entering and after leaving the gland. A diminished concentration in the efferent blood would indicate utilisation by the mammary gland.

The mammary glands of the cow are drained by the abdominal subcutaneous veins which are superficial and therefore readily accessible without disturbance to the animal. The arterial blood, however, is supplied by the mammary artery which is deeply embedded in the tissues and is inaccessible without serious disturbance to the animal.

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.

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. In this assumption that jugular blood may be taken

to represent mammary arterial blood they have been followed 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. The intervention of tissue metabolism between arterial blood and jugular (venous) blood renders analytical values for the latter difficult of interpretation, an aspect which is to be considered in later papers.

In addition, there are other factors, 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 outside the body. The lactating cow on the average secretes over a period of 12 hours about 10 litres of water as milk. This must result in concentration of the blood and we have to consider whether 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] estimate 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 *et al.* [1919] 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.

Accordingly the first question we have investigated in this experimental survey is that of the concentrations 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.

EXPERIMENTAL.

Samples of blood have been taken simultaneously from the radial artery and the jugular and abdominal subcutaneous (mammary) veins of lactating and non-lactating cows.

The samples, each of about 60 cc. volume, were drawn into dry sterile glass bottles containing 0.1 g. sodium oxalate as anticoagulant. They were immediately examined.

1. *Corpuscular volume* was estimated by centrifuging in haematocrit tubes until constant volume was attained. As each blood contained the same amount of anticoagulant the slight effect of the addition of sodium oxalate on corpuscular volume is eliminated.

2. *Iron content* was estimated by a micro-method elaborated for the purpose of this investigation (see previous paper).

(*Note.* We have found arterial puncture of non-aesthetised cows to be a procedure attended by considerable disturbance with most animals. Only in eight cases have we obtained full blood samples from all three sources without disturbance.)

The results of analyses of samples of bloods from 17 different animals are given in Table I.

Table I.

Cow No.	Lactating or non-lactating	Corpuscular volume (%)			Iron content (mg. 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 expressed as percentages of jugular.

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	0.0	5.34	4.81	0.54
2	"	8.23	6.85	1.3	7.96	7.04	0.92
3	"	4.87	4.87	0.0	3.68	2.71	0.95
4	"	5.14	2.57	2.57	6.63	4.24	2.30
5	"	2.70	2.70	0.0	3.30	3.80	0.52
6	"	4.10	—	—	3.20	—	—
7	"	6.06	—	—	3.91	—	—
8	"	0.0	—	—	2.30	—	—
9	"	2.30	—	—	4.60	—	—
10	"	4.70	—	—	4.90	—	—
11	Non-lactating	6.09	6.09	0.0	6.37	7.70	-1.3
12	"	2.75	2.75	0.0	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 shows that they are consistently in the same direction and of a magnitude outside the range of any experimental error.

We are therefore forced to the conclusion 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. Loss of water from the arterial blood would cause an increase in concentration of venous blood constituents. This appears to be the point which our figures illustrate and we believe that the withdrawal of water from the arterial blood by the salivary glands is of sufficient magnitude to bring about the appreciable concentration of the blood resulting in a consistently increased value for corpuscular volume and iron content of jugular (venous) blood.

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

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

Let x = mg. Fe per 100 cc. jugular blood;

y = mg. Fe per 100 cc. mammary blood;

then degree of concentration of jugular blood = $\frac{x}{y}$.

Let p = mg. sugar (or amino-N, etc.) per 100 cc. jugular blood;

then $p \frac{y}{x}$ = mg. sugar per 100 cc. mammary 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 eliminates the effect of loss of water to the salivary glands.

For the calculation of the correction factor we 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 the inaccuracies inherent in haematocrit technique.

In addition to the factors considered in this paper there is the influence of the lymph drainage on the concentration of venous blood-solutes. Sampling the lymphatic circulation would be a matter of great difficulty and could not be considered when drawing up this experimental plan. It is highly 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.

SUMMARY.

1. The technique of Kaufmann and Magne as applied to the problem of milk secretion is discussed.

2. Experimental figures are given which show that the influence of salivary secretion on the analytical data of jugular (venous) blood is considerable.

3. A method of compensating for the influence of salivary secretion on these analytical figures is given, which permits the technique of Kaufmann and Magne to be applied to all diffusible blood solutes.

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