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

II. THE RELATION OF BLOOD-SUGAR ABSORPTION TO LACTOSE SECRETION.

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THE theory that the sugar of the blood is the precursor of lactose rests on experimental evidence which has been subjected to much criticism.

Bert [1884] removed the mammary glands of young goats and allowed the animals to become pregnant. Immediately after parturition sugar appeared in the urine. This did not take place with animals from which the mammary glands had not been extirpated before pregnancy. Bert concluded that immediately after parturition the blood receives an additional supply of sugar from some organ—presumably the liver, that normally this sugar is absorbed by the mammary gland for the synthesis of lactose and that, in the absence of the normal glandular function, the excess sugar is excreted in the urine.

This work was confirmed by Porcher [1909] who showed that the *post partum* glycosuria in animals from which the mammary glands had been removed was accompanied by hyperglycaemia and that the urinary sugar was glucose and not lactose. Neither Bert nor Porcher, however, was able to confirm these findings when guinea-pigs were used as experimental subjects. Evidence of a negative character was also obtained by Marshall and Kirkness [1907] while Moore and Parker [1900–01], who repeated Bert's original work, could not obtain unequivocal results.

Foa [1907, 1912] carried out perfusion experiments on the excised mammary glands of sheep. The glands were maintained at body temperature in Ringer's solution. Perfusion with Ringer's solution alone resulted in the secretion of a fluid free from lactose while on the addition of blood to the perfusate lactose was secreted. On replacement of the blood by glucose solution a watery fluid containing lactose was secreted by the glands, a result not obtained when galactose was used. These experiments have been criticised on the grounds that, in Ringer's solution, the excised glands rapidly became oedematous, a

condition in which the cells might be permeable to substances which would not normally penetrate the cell membrane.

In view of the unsatisfactory nature of the work reviewed above, the demonstration of the absorption of blood-sugar by the active mammary gland of intact animals becomes of considerable value.

The only previously recorded work of this description is that of Kaufmann and Magne [1906] who published data intended to illustrate the utilisation of blood-sugar by the mammary gland in different states of activity. From four cows (one of which was not lactating) these workers took samples of blood simultaneously from the jugular and abdominal subcutaneous (mammary) veins. They found that in the lactating animals the mammary venous blood contained less sugar than the blood from the jugular vein, while in their one non-lactating animal the two bloods had the same sugar content. On the basis of the finding of Chauveau [1856] that venous blood normally contains less sugar than arterial blood, Kaufmann and Magne suggested that jugular blood-sugar might be taken to represent the difference between arterial blood-sugar and the glucose oxidised in the cells of the mammary gland to provide energy. The difference between jugular and mammary blood-sugar levels would therefore represent sugar absorbed by the mammary gland for non-oxidative purposes. Such reasoning involves the assumption that the energy requirements of the mammary gland are identical with those of the tissues of the head and neck—the tissues drained by the jugular vein. Later workers have interpreted the results of this investigation as confirmation of the view that blood-sugar is the precursor of lactose, although this point is not explicitly made by Kaufmann and Magne.

The accurate estimation of the amount of blood-sugar absorbed by the mammary gland should be of value not only in deciding 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 the amount of sugar absorbed from the blood.

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 only be decided 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 [1927, 1928, 1929], 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 the present investigation we have studied the problem of the sugar

uptake of the mammary gland by the analysis of samples of blood taken simultaneously from the radial artery and the jugular and mammary veins. Analytical figures for arterial samples should afford some information on the comparative energy requirements of the tissues of the head and neck and of the mammary gland, while the application of the factor established in the first paper of this series [1932, 2] eliminates the effect of salivary secretion. The method of sugar estimation employed ensures that the data shall be independent of the possible absorption of non-sugar reducing substances by the mammary gland.

EXPERIMENTAL.

Samples of blood were taken simultaneously from the jugular and mammary veins and radial artery of lactating and non-lactating cows. The samples were taken at 9 a.m., that is, four hours after milking in the case of the lactating animals. The samples, each of about 60 cc. volume, were drawn into clean glass bottles containing 0.1 g. sodium oxalate as anticoagulant. The blood-sugar analyses were carried out immediately.

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]. We have chosen this method advisedly, because the Folin-Wu filtrate has been shown by Somogyi [1927] to give minimal precipitation of non-sugar reducing substances and we wished to have information of the amounts of such substances present in our samples. 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° 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. We have satisfied ourselves that incubation at 37° for 24 hours removes added glucose quantitatively.

The iron content of the blood samples was determined by the iodimetric method previously described by us [Blackwood and Stirling, 1932, 1].

In all we have taken samples of blood from thirteen animals—eight lactating and five non-lactating. In four cases, three lactating and one non-lactating, arterial samples were not obtained. In Table I are given the iron contents and true sugar values for all the blood samples obtained—in each case the values for jugular sugar are corrected, as indicated in the introductory part of this paper. In Table II 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. In Table III the differences in blood-sugar content shown in Table I 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 I.

No. of cow	Lactating or non-lactating	Iron content (mg. Fe per 100 cc. blood)			True sugar (mg. glucose per 100 cc. blood)			
		Jugular	Arterial	Mammary	Jugular (corrected)			
					Jugular	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

Table II.

No. of cow	Lactating or non-lactating	Non-sugar reducing substances (as mg. glucose per 100 cc. blood)			
		Arterial	Jugular	Jugular (corrected)	
				Arterial	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

Table III.

No. of cow	Lactating or non-lactating	Difference 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	"	20.0	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	0.0	-7.4
13	"	—	—	-7.3*

* As percentage jugular.

DISCUSSION.

Blood-sugar values for lactating cattle have been recorded by previous workers. The values and methods used are as follows: Hayden and Fish [1923] 46-52 mg. glucose per 100 cc. blood (Benedict); Widmark and Carlens [1925] 60 mg. (Bang and Hagedorn-Jensen); Hayden and Scholl [1928] 52 mg. (Folin-Wu); Little, Keith and Fawns [1928] 75 mg. (Hagedorn-Jensen). Our results would indicate that about 30 % of the material estimated by former workers is to be accounted for by non-sugar reducing substances.

The amounts of non-sugar reducing substances present in samples of blood taken simultaneously from different parts of the animal body do not appear to be equal. Such inequalities, we have found, are of sufficient magnitude to give erroneous estimates of the sugar absorbed by the mammary gland when only total reducing power of the blood is measured. This source of error is avoided when estimates are based on the true sugar content of blood as is done in Table I.

The values we have obtained for true sugar are very variable, ranging from 24 to 60 mg. glucose per 100 cc. 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 there are no data available as to the variation of blood-sugar level throughout pregnancy and lactation, but it is hardly to be expected that the value would remain constant during periods which physiologically are so widely different. The variation in blood-sugar level obtained by us may therefore be due to the animals being in different stages of the life cycle, a point which is receiving further attention.

Turning now to differences in the true sugar contents of the bloods taken from different sources in the same animal, we see at once that they are greater than can be accounted for by the modifying influence of salivary secretion and are 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 pur-

pose 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 0.02 cc. of oxygen per g. per minute, while in the active state it is as much as 0.084 cc. of oxygen per g. per minute. Anrep and Cannan [1922] report that the resting submaxillary gland of the dog utilises about 2.1 mg. glucose per g. 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.

From these observations there is reason to believe that the energy requirements of the mammary gland will be greater in the active than in the inactive state. Part of the increased sugar absorption must therefore be ascribed to an increased demand for energy-producing substances while secretion is in progress. It is hardly conceivable that this would account for all the sugar absorbed, although this is a point which can only be settled by an investigation of the gaseous metabolism of the mammary gland in a resting and an active state. 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 glands of 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. Our results indicate that until knowledge of the energy requirements of the mammary gland is available, comparative blood-sugar analyses alone are not a reliable guide.

SUMMARY.

In non-lactating animals the sugar content of the jugular blood is lower than that of the mammary venous blood, which is itself lower than that of arterial blood.

In lactating animals the sugar content of jugular blood is higher than that of mammary venous blood but lower than that of arterial blood.

The great lowering of mammary venous blood-sugar in lactating animals is to be ascribed partly to the increased energy requirements of the active gland and partly to lactose secretion.

On account of the lack of knowledge of the energy metabolism of the mammary gland comparative blood-sugar analyses alone have been shown to be unreliable guides to lactose secretion.

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