I. THE NATURE OF SUGAR IN THE MILK AND THE CARBOHYDRATE METABOLISM OF LACTATING DIABETIC WOMEN

By KAMEL MIKHAIL DAOUD

From the Biochemical Department, Faculty of Medicine, Cairo, Egypt

(Received 27 October 1939)

EXPERIMENTS performed by Bert [1884], Kaufmann & Hagne [1906], Porcher [1909, 1], Paton & Cathcart [1911] and Röhmann [1919] indicate that the glucose of the blood stream is the substrate for lactose synthesis by the mammary gland. As it is known that in diabetes mellitus the hyperglycaemia causes the appearance of glucose in various secretions such as saliva, sweat, bile, pancreatic secretion etc., it became of interest to investigate whether, in this condition, glucose is found in milk together with lactose or whether the mammary gland is still capable of secreting all the sugar of milk in the form of lactose. That the mammary glands, under certain conditions, can secrete unchanged glucose has been shown by Grant [1936] who injected prolactin for 2 weeks into animals which had gone completely dry.

Two cases of diabetic nursing women were available. Their milk sugar was examined for different periods during lactation. During pregnancy the two patients were receiving insulin injections whenever it was found necessary and care was taken to prevent glycosuria. It was noticed, however, that for a certain period after parturition and during lactation glucose ceased to appear in urine. The patients themselves could record the improvement in their state by the disappearance of symptoms such as thirst and polyuria despite the cessation of insulin and of the restrictions of the diet which then contained liberal amounts of carbohydrate. The weights of the patients were constant during this period of lactation. The sugar tolerance curves before pregnancy indicated moderate diabetes in case I and severe diabetes in case II (Table 1). During lactation the normal state continued for 8 months in case I and for 7 months in case II after which glucose started to reappear in the urine and the symptoms recurred.

The disappearance of glycosuria for a certain period during lactation suggested changes in carbohydrate metabolism in that condition. Some investigations were carried out on the milk, blood and urine of the two diabetic subjects in an attempt to throw some light on the nature of these metabolic changes. Further work on the latter problem is still proceeding on animals.

EXPERIMENTAL

The nature of sugar in the milk of diabetics

The two subjects of this investigation were suffering from typical diabetes mellitus as could be seen from the nature of their sugar tolerance (Table 1) which was worked out before pregnancy.

Sugar was present in the fasting urine in both cases and increased in amount after the ingestion of glucose.

Samples of milk were taken in both cases at different periods after parturition. The milk was always collected aseptically. The proteins and fat were precipitated

Table 1

Time	Blood sugar	mg. per 100 ml.
	Case I	Case II
Fasting	191	247
$\frac{1}{2}$ hr. after ingestion of 50 g. glucose	244	341
1	254	367
$1\frac{1}{2}$	233	376
2	215	361

by dialysed iron and the nature of the sugar was determined in the clear filtrate. The phenylosazone of the sugar present in the filtrates obtained from the different samples was always completely soluble in hot water, and crystallized from cold water giving the yellow clusters of fine needles with the microscopic appearance characteristic of lactose phenylosazone and showing no trace of phenylglucosazone crystals. The mean M.P. of these osazones after recrystallization was 199.5°. The M.P. of pure lactosazone under the same experimental conditions was 199.9° and that of pure glucosazone 207°. The characteristics of the phenylosazone derivative of the sugar were entirely those of phenyl lactosazone. This could not be taken as conclusive evidence of the entire absence of glucose but undoubtedly indicated that if glucose was present at all it would be in a low percentage since a large excess of lactosazone might increase the solubility of a small amount of glucosazone and the characteristics of the former would not, then, be appreciably masked by the latter. For further investigation advantage was taken of the fact that yeast (*Saccharomyces cerevisiae*) can ferment glucose but not lactose. 10 ml. of every sample of milk examined were diluted with 5 ml. of water, the mixture was deproteinized and defatted by treatment with 4 ml. of dialysed iron and filtered. The precipitate was repeatedly washed with small amounts of water. The filtrate and washings, adjusted to neutrality, were concentrated in a vacuum and the volume was adjusted to 10 ml. in a measuring flask. The liquid was acidified with a little tartaric acid to inhibit the growth of bacteria which might attack lactose and cause its fermentation by the yeast. 0.5 ml. of this liquid was treated with 3 drops of a suspension of yeast in water in Lohnstein's apparatus. A control experiment was made by dissolving 20 mg. of glucose in 8 ml. of the same acidified milk filtrate to bring about a 0.25 % concentration of glucose and 0.5 ml. of the latter liquid was treated with 3 drops of the same suspension of yeast. After about 5 hr. at 37° a quantity of CO₂ was evolved in the control experiment which, within the limits of experimental error, accounted for the added glucose while no evolution of gas took place in the milk filtrate alone. From this result it could be inferred that the samples of yeast used were active, that the milk filtrates did not contain anything which might inhibit the fermentative power of yeast and that glucose was absent from the samples of milk. The difference in solubility of glucose and lactose in cold absolute methyl alcohol was utilised for the confirmation of the absence of the former from the milk of lactating diabetics. Lactose is practically insoluble in methyl alcohol, while according to Trey [1901] 100 ml. of absolute methyl alcohol at 17.5° dissolve 1.25 g. of anhydrous glucose. The fractionation is better effected when the two sugars are in a crystalline form. The deproteinized and defatted milk filtrates were therefore neutralized, concentrated *in vacuo* and brought to dryness in a vacuum desiccator. The almost crystalline residues were extracted with cold methyl alcohol. No glucose could be identified by means of its osazone in these extracts after evaporation and resolution in small volumes of water.

It has already been mentioned that the diabetic symptoms disappeared for a certain period during lactation and then reappeared, but the results of the above experiments which were performed both in the presence and absence of these symptoms were always the same, thus indicating the ability of the mammary glands to prevent the leakage of blood glucose into the milk of diabetics even in the presence of a high concentration of blood glucose. The synthetic power of these glands appears to afford an efficient mechanism for transforming the whole of the sugar secreted in milk into lactose regardless of the level of the blood sugar. The absence of such a mechanism from other glands may explain the appearance of glucose in their secretions in diabetes.

Carbohydrate metabolism during lactation

Lactose synthesis by the mammary glands from blood sugar might be thought of, in the first instance, as one of the possible explanations of the disappearance of diabetic symptoms for a certain period during lactation. In the latter condition the blood sugar of both normal and diabetic persons finds a new route for its consumption and a normal person will be in a state approaching that of a diabetic in that there is a waste of sugar from the body with resulting polyphagia; in case of the diabetic the removal of sugar in the milk, which would otherwise be lost in the urine, may temporarily remove the diabetic symptoms. On this assumption the only difference between lactating normal and diabetic persons will be that in the former either the glucose stores are mobilized to replace the blood sugar lost in the synthesis of lactose and the glucose loss will be equivalent to the lactose formed or there may be equilibrium between a hyperglycaemia developed during lactation and lactose formation [Bert, 1884; Porcher, 1909, 2]; while in the case of a diabetic person the excess of blood sugar above the normal level is utilized for the lactose synthesis. On this basis the disappearance of diabetic symptoms necessitates the assumption that, during lactation, the whole of the glucose which would otherwise be lost in the urine must be transformed into lactose. In a case of severe diabetes, like case II of the present investigation, the urinary glucose excretion may amount to as much as 500 g. per 24 hr. [Beaumont & Dodds, 1931] on an ordinary mixed diet as was ingested during lactation while, if the milk composition and yield are the same as in a normal person, the lactose produced in 24 hr. would not exceed 51–85 g. Since glucose was not found as such in the milk of diabetics it is clear that the lactose yield should appreciably increase if it was the only factor responsible for the disappearance of the diabetic symptoms during lactation. Lactose occasionally appears in the urine of pregnant and lactating normal individuals, but the urine in the two cases described during the period of absence of diabetic symptoms showed no reduction of Fehling's solution on several occasions and the osazones prepared from these urines resembled the osazone mixture normally present in urine. Winter [1932] proved the absence of lactose from the blood during lactation and of a threshold for this sugar. It follows that there was no replacement of glycosuria by lactosuria in such cases and that any increment in the lactose yield above normal would appear almost entirely in the milk as there could be no retention of this sugar in the blood.

The estimation and comparison of the total lactose yield in the milk of diabetics with that in the milk of normal individuals involve several difficulties. The lactose in mature milk of normal individuals varies from 6 to 8%. It can be seen (Table 2) that the percentage in the milk of the two cases examined during the period of disappearance of diabetic symptoms is within the range for normal individuals. It cannot be said what percentage would be present if these

persons were normal, but the difference between the usual lower and upper limits is only 2% which does not appear to account for the disappearance of glycosuria, especially in a severe case. Still, there is the possibility of an abnormally excessive yield of a milk with a normal percentage of lactose but estimates of milk yields in women are subject to error from many sources.

In view of these difficulties in obtaining an accurate estimate of normal milk yield it was thought that at least some idea of milk yield might be gained from analytical data. On the assumption that in diabetes there is no reason for an increase in the yield of milk constituents other than lactose it is conceivable that if there is an increase of milk yield, in such cases, although the percentage of lactose may remain within the normal range yet the milk will be dilute as regards its other constituents. The fact that glycosuria reappeared after a certain period of lactation allowed a comparison between the percentages of lactose and the constitution of milks obtained during the periods when there was no loss and when there was a loss of sugar in the urine in one and the same person. Further, it would be expected that if the percentages of all the constituents of milk were within the normal range, during either of the two periods, an increase in milk yield would aggravate the condition of the lactating patient, which is contradictory to the actual observation.

For the collection of milk for analysis the instructions of Talbot [1919] were followed. The milk was collected at about 10 a.m.; 20 ml. were drawn both before and after nursing and the two samples were thoroughly mixed. The specific gravity, total solids, lactose, solids other than lactose and ash content were estimated in each case. Table 2 shows the constitution of mature milk during the periods of disappearance and of reappearance of glycosuria, a comparison between the two kinds of milk and between these and the constitution of mature milk of normal individuals.

Table 2

Conditions	Specific gravity average at 15°	Total solids average %	Lactose average %	Solids other than lactose average %	Ash average %
Case I: no glycosuria	1030.7	13.27	6.09	7.18	0.19
Case I: glycosuria	1031.0	13.28	6.10	7.18	0.20
Case II: no glycosuria	1032.6	14.24	7.04	7.20	0.21
Case II: glycosuria	1032.6	14.22	7.04	7.18	0.21
Normal range	1031.0*	11-15.25	6-8	5-7.25	0.18-0.25

* Average at 60° F.

It is clear that the constitution of the milk of lactating diabetics is the same during the disappearance and the reappearance of glycosuria and in both cases it does not differ from the constitution of the milk of normal individuals. The fat content was also estimated in some samples and was always within the normal range. It may be mentioned that the percentages of the constituents in all the individual samples examined did not differ appreciably from the average percentages given in Table 2.

After the reappearance of glycosuria it was arranged to draw the samples of milk at the close of 24 hr. during which the urine was collected. This technique was followed in case II; the samples of urine always showed a strong reduction and gave abundant typical glucosazone crystals. The amount of sugar in the urine varied from 26 to 33 g. per 24 hr. which suggested that the hyperglycaemia which recurred while lactation was still proceeding was of a milder nature than before pregnancy. This is further indicated by the estimations shown in Table 3

which also show that the hyperglycaemia, after the cessation of lactation, returns almost to the high level obtaining before pregnancy.

It might be thought that the disappearance of glycosuria for a certain period during lactation was due to a temporary rise in the renal threshold for glucose, but determination and comparison of the fasting blood sugar levels in case II in the different conditions indicated in Table 3 excluded this idea.

Table 3

Case II in different conditions	Fasting blood sugar level mg. per 100 ml.
Before pregnancy	247
During lactation: no glycosuria	99
During lactation: glycosuria	175
40 days after cessation of lactation	241

It is clear that in this case of severe diabetes the blood sugar level in absence of glycosuria was within the normal limits (90–110 mg. per 100 ml. blood) and that the appearance or absence of glycosuria was quite dependent on the level of the blood sugar.

Basal metabolic rate during lactation

The possibility of the prevalence of factors which raise the metabolic rate during lactation may explain the disappearance of glycosuria in this condition. Fluctuations in oxygen consumption in women are stated to occur; thus the oxygen consumption may fall to 170 ml. per min. during menstruation and rise to 195 ml. 10 days later. Such fluctuations appear to be correlated with variations in the production of sexual hormones which are at a minimum during menstruation and which, according to Verzár & Árvay [1931], increase the basal metabolism in females. Lee [1928] also observed an increase in basal metabolism in rats in dioestrus and pro-oestrus but no change in any other period of the cycle. The disappearance of glycosuria for a certain period during lactation and its reappearance cannot be ascribed to fluctuations in the production of the ovarian hormones since the glycosuria disappeared entirely, in the two cases examined, during the inhibition period of menstruation which follows parturition when it is supposed that there is a deficiency in the production of these hormones, while once the glycosuria starts to reappear during lactation it continues during every period of the menstrual cycle. It follows that if there is any increase in basal metabolism during lactation it will, in all probability, be caused by different factors which operate in this condition. For the comparison of the basal metabolism during lactation with that in the non-lactating condition it was thought advisable to make the comparison under conditions which cover all the cyclic fluctuations in the non-lactating state and, for this purpose, the comparison of the basal metabolism in short-cycle animals such as the rat in which the cycle occurs every 4 or 5 days is more instructive especially when the study is extended for 7 or 8 days.

A number of female rats of the same litter and weight were chosen. Some were allowed to become pregnant and some were left as controls. One week after parturition the oxygen consumption of both the lactating rats and the controls was measured in a closed system. Four pairs of rats of the same weight 160 g. were chosen of each batch. Two pairs of the four of each batch were alternately deprived of food at 4 p.m. on the day preceding the experiments which were performed on consecutive days. On the days of the experiments the oxygen consumption of each pair of the two of each batch was separately

estimated. The consumption in the first 30 min. was ignored in each case in order to avoid the effect of handling and fear of the apparatus. It was found that the oxygen consumption of the lactating rats was within the range for the non-lactating animals which varied from 160 to 175 ml. O₂ per pair per 30 min. during 8 days. It appears, therefore, that there is no recognizable increase in the basal metabolic rate during lactation and that this factor is unlikely to explain the disappearance of glycosuria in lactating diabetics.

SUMMARY

1. In lactating diabetics no glucose is secreted in the milk regardless of the existence or absence of hyperglycaemia. The amounts of lactose and other constituents of the milk remain within the range for normal individuals.

In diabetics, the active mammary glands, contrary to other glands, prevent glucose leakage, possibly by virtue of their synthetic power for lactose, and are capable of maintaining normal milk composition.

2. The diabetic symptoms in the two cases examined entirely disappeared for a certain period during lactation and reappeared while lactation was still proceeding. The hyperglycaemia recurring during lactation was of a milder nature than both before pregnancy and after cessation of lactation.

3. Lactation has no effect on the basal metabolic rate of rats.

4. The disappearance of hyperglycaemia and glycosuria during lactation could not be explained by either (1) secretion of glucose in milk, (2) excessive lactose secretion, (3) replacement of glycosuria by lactosuria or (4) increased sugar combustion.

REFERENCES

- Beaumont & Dodds (1931). Recent advances in medicine. London.
 Bert (1884). *C.R. Acad. Sci., Paris*, **98**, 775.
 Grant (1936). *Biochem. J.* **30**, 2033.
 Kaufmann & Hagne (1906). *C.R. Acad. Sci., Paris*, **143**, 779.
 Lee (1928). *Amer. J. Physiol.* **86**, 694.
 Paton & Cathcart (1911). *J. Physiol.* **42**, 179.
 Porcher (1909, 1). *Biochem. Z.* **23**, 370.
 — (1909, 2). *Arch. int. Physiol.* **8**, 356.
 Röhmman (1919). *Biochem. Z.* **93**, 237.
 Talbot (1919). *J. Amer. med. Ass.* **73**, 662.
 Trey (1901). *Z. phys. Chem.* **18**, 193.
 Verzář & Árvay (1931). *Biochem. Z.* **240**, 28.
 Winter (1932). *J. Physiol.* **77**, 100.