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Aerobic Lactate Production by Mammary Tissue

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Glucose uptake and L-lactate production were measured in cell, slice and intact tissue preparations of mammary glands from late-pregnant and lactating rats. The tissues showed extensive conversion of glucose into lactate *in vitro*, but not *in vivo*. Therefore aerobic lactate formation is not a normal feature of mammary tissue, but occurs *in vitro* as the result of some metabolic derangement.

While evaluating the metabolism of mammary epithelial cells, prepared by the collagenase technique, we observed a relatively high rate of aerobic lactate production. Similarly high rates of aerobic acid production were reported by Folley & French (1949) for mammary slices from pregnant rats, and by Rees & Eversole (1964) for slices from both pregnant and lactating rats. Moretti & Abraham (1966) also noted aerobic lactate production by mammary explants from pregnant mice.

Few mammalian tissues normally show aerobic lactate production, but in those cases it appears to serve a definite function (for a discussion see Krebs, 1972). We therefore investigated whether the transition of mammary tissue from the quiescent to the lactating state might be associated with a change from fermentative to oxidative metabolism of glucose. Glucose uptake and lactate formation were measured in cell preparations, tissue slices and intact mammary tissue from late-pregnant and lactating rats.

Experimental

Mammary cells were prepared by the method of Pitelka *et al.* (1969) with certain modifications. Thus vigorous reciprocal shaking (200 strokes/min) was necessary to disrupt the tissue in the presence of collagenase, and the hyperosmotic sucrose used by the above authors was replaced by 1% Ficoll. When examined under the microscope the final preparations contained mixtures of free and clumped cells; adipocytes and erythrocytes were scarce. The original tissue DNA was recovered in 25–50% yield (Burton, 1956). All preparations were quantified by their DNA content, which was converted into wet weight equivalent by assuming that 2.2mg of DNA is equivalent to 1g wet weight of tissue (Kuhn & Lowenstein, 1967).

Mammary slices were cut by hand with a razor blade. Single arterial (femoral) and mammary venous (pudic–epigastric) blood samples were taken into heparinized syringes from intact rats anaesthetized with 3% Fluothane in O_2 or, occasionally, with Nembutal. They were deproteinized with 3% $HClO_4$, neutralized with $KHCO_3$, and assayed for glucose (Slein, 1963) and L-lactate (Hohorst, 1963). Extraction of acid-soluble material, monitored in several cases with [^{14}C]lactose or [^{3}H]inulin, was in excess of 88%. Wistar-derived rats were used on day 19–20 of their first pregnancy or on day 15 of their first lactation.

Tissue slices (0.6g) were incubated with shaking for up to 90min at 37°C in 15ml of Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964) equilibrated with O_2+CO_2 (95:5). Initial glucose concentrations were 0.6 and 5.3mM for tissue from pregnant and lactating rats respectively. It was necessary to use a low glucose concentration in the former case in order to detect a sufficiently large fall in concentration during the incubation. Mammary cells were incubated under similar conditions in siliconized flasks with the addition of bovine serum albumin (1mg/ml).

Results

Table 1 shows the glucose uptake and lactate production of mammary cells and slices prepared from pregnant and lactating rats. Rates were approximately constant up to 90min, which was the longest time studied. Lactating tissue used glucose more rapidly than did tissue from pregnant rats, confirming the findings of Folley & French (1949), although the different initial glucose concentrations may have contributed to this effect. Table 1 also shows the relative lactate production, where 100% represents the formation of 2mol of lactate/mol of glucose consumed. All cell preparations showed a substantial relative lactate formation, especially when prepared from pregnant rats. Slices showed a large relative lactate formation when prepared from pregnant rats but rather little when prepared from lactating rats. The observations with slices confirm the findings of acid production by Folley & French (1949) but only partially confirm the findings of Rees & Eversole (1964).

Table 1. *Glucose uptake and lactate production in vitro by mammary tissue from pregnant and lactating rats, and arterial and venous plasma concentration of glucose and lactate across intact mammary tissue in pregnant and lactating rats*

Net uptakes of glucose and lactate by mammary tissue *in vivo* were calculated by using mean blood-flow rates of 13 and 37 ml/h per g wet wt. on day 19–20 of pregnancy and day 15 of lactation respectively (Linzell, 1969; Chatwin *et al.*, 1969). The values are the means of the given numbers of preparations ± S.E.M.

	Cells		Slices		Intact gland	
	Pregnancy	Lactation	Pregnancy	Lactation	Pregnancy	Lactation
No. of preparations ...	5	5	6	6	10	17
Glucose consumption (μmol/h per g wet wt.)	3.63 ± 0.51	8.38 ± 0.46	1.71 ± 0.32	33.3 ± 1.0	16.9	135
Lactate production (μmol/h per g wet wt.)	3.52 ± 0.64	4.07 ± 0.95	2.58 ± 0.58	6.96 ± 0.59	−3.2	−20.3
Arterial glucose concentration (μmol/ml of blood)	—	—	—	—	5.19 ± 0.24	5.80 ± 0.04
Venous glucose concentration (μmol/ml of blood)	—	—	—	—	3.86 ± 0.17	2.16 ± 0.13
Arteriovenous concentration difference (μmol/ml of blood)	—	—	—	—	1.33	3.64
Arterial lactate concentration (μmol/ml of blood)	—	—	—	—	2.06 ± 0.16	1.05 ± 0.02
Venous lactate concentration (μmol/ml of blood)	—	—	—	—	1.81 ± 0.04	0.50 ± 0.01
Arteriovenous concentration difference (μmol/ml of blood)	—	—	—	—	0.25	0.55
Relative lactate production (%)	51	21	75	11	0	0

By contrast with these observations *in vitro*, arteriovenous measurements *in vivo* revealed no net conversion of glucose into lactate (Table 1), except to a small extent in occasional individuals. In fact there was a detectable net uptake of lactate, especially during lactation. Hawkins & Williamson (1972) also noted a small lactate consumption by lactating tissue, but found a markedly lower arteriovenous glucose difference.

Discussion

These results show that aerobic lactate production is a feature of mammary preparations *in vitro*, especially those from pregnant rats. However, it is not shown by intact mammary tissue either before or during lactation, except perhaps to a small extent during starvation (Hawkins & Williamson, 1972). Preparations *in vitro* therefore show a definite metabolic derangement, and we suggest caution in their use for the study of mammary metabolism.

The lactate that is formed *in vitro* probably does not result from an excessive rate of glucose uptake, since a much greater rate appears to occur *in vivo* when this is calculated from the blood-flow data of Linzell (1969) and Chatwin *et al.* (1969). Nevertheless, these calculated values may somewhat overestimate the true values, since they appear to exceed the potential capacity of pyruvate dehydrogenase to metabolize the pyruvate that would be generated (Coore & Field, 1974; Katz & Wals, 1972).

Lactate production might reflect a decreased oxidation of pyruvate due to some inactivation of pyruvate dehydrogenase *in vitro*. However, dichloracetate (10 mM), which appears to reactivate this enzyme in some tissues (McAllister *et al.*, 1973), did not change the rate of lactate formation by mammary slices prepared from pregnant rats (results not given in detail). It remains possible, however, that an excessively reduced state of the NAD^+/NADH couple characterizes preparations of mammary tissue *in vitro*; this could both promote the conversion of pyruvate into lactate and block its oxidation through an inhibition of pyruvate dehydrogenase.

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