Biochem. J. (1986) 239, 269–274 (Printed in Great Britain)

Arteriovenous glucose differences across the mammary gland of the fed, starved, and re-fed lactating rat

Tony PAGE and Nicholas J. KUHN
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

1. Arteriovenous glucose difference across the mammary gland of the lactating rat was used as an 'instantaneous' monitor of mammary glucose uptake. Plasma [glucose] and arteriovenous glucose difference varied according to whether Halothane, diethyl ether or sodium pentobarbitone anaesthesia was used. 2. In pentobarbitone-treated rats a 60% glucose extraction in the fed state decreased to 5% after 18 h starvation, and recovered to 40% and 59% after 15 min and 60 min re-feeding respectively. The increase and decrease in plasma [fatty acids] and the depletion and restoration of hepatic glycogen mostly followed similar time courses. Re-feeding was accompanied by a brief surge of plasma [insulin]. 3. Starved lactating rats showed a markedly greater capacity than age-matched virgin rats in the oral and intraperitoneal glucose tolerance tests. 4. Mammary glucose uptake in the starved rat was significantly restored by oral or intraperitoneal glucose or by insulin, but not by acetoacetate or by heparin-induced elevation of plasma [fatty acids]. 5. The role of insulin and of possible changes in mammary sensitivity to insulin in the return of mammary glucose uptake on re-feeding is discussed.

INTRODUCTION

Although the timing of lactation is linked to the maternal reproductive cycle, its magnitude is geared to the nutritional status of the mother and the demands of the young. Dairy performance has been the subject of many nutritional studies, but the ruminant does not readily lend itself to the study of short-term control and may in any case not be representative of other mammals.

General lactational performance in the rat can be measured from the milk accumulation over several hours during the absence of suckling (Hanwell & Linzell, 1972), but rather more useful are the more recent radioisotopic methods that measure rates of synthesis specifically of fatty acid, cholesterol, lactose and protein in the mammary gland (Robinson *et al.*, 1978; Gibbons *et al.*, 1983; Carrick & Kuhn, 1978; Bussmann *et al.*, 1984; Sampson *et al.*, 1984). These rates appear to be 'physiological', as opposed to the frequently unphysiological rates given by measurements of excised tissues *in vitro*. Thus synthesis of fatty acid and lactose has been shown to vary diurnally, to decline within 6 h of food withdrawal, and to be nearly abolished by 16–24 h of starvation (Robinson *et al.*, 1978; Carrick & Kuhn, 1978; Wilde & Kuhn, 1979). When starved lactating rats are re-fed, these processes are rapidly restored to normal. Lactose synthesis reaches 70% of normal by 5 h, whereas fatty acid synthesis reaches normal rates by 2 h and exceeds them substantially by 5 h (Robinson *et al.*, 1978; Bussmann *et al.*, 1984). Uptake of 2-deoxyglucose by the mammary gland *in vivo* is inhibited by 90% by 16 h starvation and restored to normal by 1 h re-feeding (Threadgold & Kuhn, 1984). These findings have thus elicited a remarkably close relationship between lactation and maternal nutrition.

Such observations contrast sharply with those made on preparations of mammary acini incubated *in vitro*, where glucose uptake and lactose synthesis are affected little or not at all by starvation of the donor rat (Robinson & Williamson, 1977; Wilde & Kuhn, 1979; Williamson, 1983). Impairment of fatty acid synthesis has been recorded, and is of interest; yet it, too, is strikingly less than the change *in vivo* (Robinson & Williamson, 1977; Williamson, 1983).

The speed of response to re-feeding is such that these radioisotopic methods, which require 30–60 min for sufficient radioisotope incorporation, are now unsuitable for examining very early response times *in vivo*. In seeking a relatively 'instantaneous' measurement, we have employed arteriovenous [glucose] differences across the briefly exposed mammary glands of lactating rats. Hawkins & Williamson (1972), and later Elkin & Kuhn (1975), reported large arteriovenous differences, reflecting an uptake of 25–63% of glucose traversing the gland. Similar uptakes have been reported across the mammary gland of goats, cows and pigs (see Linzell, 1974). This remarkable extraction of nutrient is not peculiar to glucose, since Viña *et al.* (1983) have found the same for amino acid uptake in the fed rat, although not in the starved rat. Arteriovenous glucose differences alone do not give absolute rates of glucose uptake, for which data on blood flow are also required. However, the present paper shows that they offer a useful index of rapid changes in mammary glucose uptake.

MATERIALS AND METHODS

Animal treatment

Wistar rats were used at 4–4½ months of age, having been maintained on a 06:00 h–20:00 h light/20:00 h–06:00 h dark cycle with food available *ad lib.*, except where stated. Experiments were performed between 09:00 and 12:00 h, except when using 6 h-starved rats, where measurements were made between 14:00 and 15:00 h. Lactating females of weight 250±6 g were used on days 14–16 of their first lactation, being left with their pups until immediately before sampling. Litters averaged 10.4±0.3 pups. Virgin females, used for the glucose tolerance test, were age-matched and weighed 226±5 g (values are means±S.E.M.).

Acetoacetate was administered intravenously as 0.5 ml of 1 M-lithium acetoacetate in 0.9% (w/v) NaCl 5 min before blood sampling. Heparin was infused via the vena cava at 100 units/min for 20 min before blood sampling. Streptozotocin (50 mg/kg body wt.) was administered subcutaneously in 0.9% NaCl 3 h previously. Glucose (2 g/kg body wt.) was administered intraperitoneally or by stomach tube in 0.9% NaCl 30 min before blood sampling. Fat (2 ml of Intralipid) was given 45 min before sampling.

Blood and tissue sampling

Rats were anaesthetized with sodium pentobarbitone (Sagatal; 60 mg/kg body wt.), diethyl ether or Halothane (8%, followed by 2.5% in O_2). The carotid artery and main abdominal–inguinal mammary vein were exposed and simultaneous blood samples (about 0.1 ml) were collected into chilled heparinized microfuge tubes. The plasma was separated by centrifugation and was stored at −20°C until analysed. Samples (about 50 mg) of liver were transferred to weighed tubes containing 30% (w/v) KOH (1 ml). Blood samples for the glucose tolerance test were collected from the tips of the tails of conscious animals before, and at 15 min intervals after, the administration of glucose.

Analyses

Liver glycogen was extracted and hydrolysed to glucose (Bergmeyer, 1963), which was then assayed with a glucose oxidase kit (Boehringer). Plasma glucose was determined with a Beckman mk. II glucose analyser. Fatty acids were determined by the method of Duncombe (1961), and insulin was measured by radioimmunoassay, with a rat insulin standard (Novo) and antiserum purchased from Wellcome.

Presentation of results

The arteriovenous difference of [glucose] is expressed in this paper as a percentage of the arterial value, in order to minimize variations owing to variations in arterial [glucose] itself. The use of absolute [glucose] differences gives mean values with usually larger variance, but would not substantially alter the conclusions drawn. In Fig. 1, the data from all animals except those treated with streptozotocin are plotted to show the correlation between the two methods of expression. The calculated least-squares regression line is shown, and the regression coefficient is 0.79.

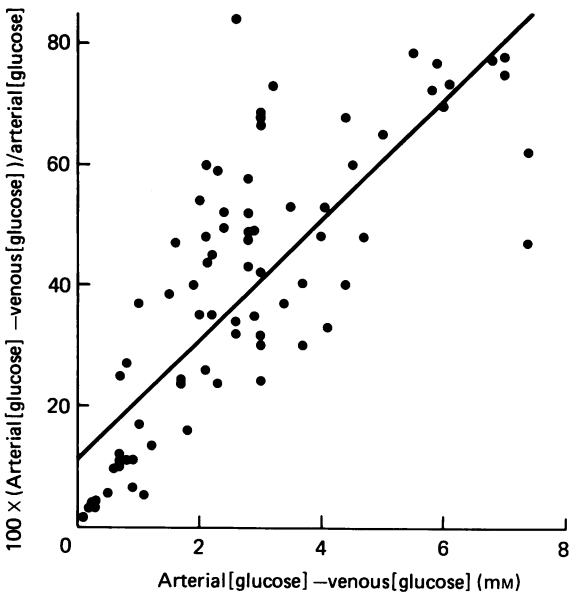

Fig. 1. Correlation between actual arteriovenous [glucose] differences and percentage arteriovenous [glucose] differences across the mammary gland

The calculated linear regression line is shown.

RESULTS

Comparison of different anaesthetics

Because earlier experiments in our laboratory (Elkin & Kuhn, 1975) found an arteriovenous glucose difference of about 63% across the gland of fed lactating rats anaesthetized with Halothane, whereas experiments by Williamson and co-workers gave values of only 25–30% with rats under pentobarbitone anaesthesia (Hawkins & Williamson, 1972; Robinson & Williamson, 1977), we first compared the uses of Halothane, pentobarbitone and diethyl ether in such measurements. Table 1 shows that Halothane and ether both gave high values of arterial glucose (11.5 mM and 11.1 mM respectively), compared with pentobarbitone (6.68 mM) or with mixed tail blood collected without anaesthesia (7.56 mM) in fed rats. In starved rats similar values were found for arterial blood collected under Halothane anaesthesia and for tail blood, but a slightly lower value was seen with pentobarbitone. However, both Halothane- and pento-

Table 1. Effect of various anaesthetics on the arteriovenous glucose difference across the mammary glands of fed and starved lactating rats

Results are shown as means ± S.E.M., with numbers of rats in parentheses.

Anaesthetic	Nutritional state	Plasma [glucose] (mM)			Arteriovenous glucose difference		
		Arterial	Venous	Mixed	Actual (mM)	(% of arterial)	
Halothane	Fed	11.53 ± 0.47	4.75 ± 0.62	—	6.75 ± 0.52	58.8 ± 4.7	(6)
Diethyl ether	Fed	11.06 ± 0.77	7.07 ± 0.69	—	3.99 ± 1.02	33.1 ± 8.3	(6)
Sodium pentobarbitone	Fed	6.68 ± 0.23	2.27 ± 0.40	—	4.42 ± 0.28	66.6 ± 5.2	(6)
Halothane	18 h starved	6.74 ± 0.20	6.51 ± 0.14	—	0.23 ± 0.17	7.3 ± 1.6	(8)
Sodium pentobarbitone	18 h starved	6.19 ± 0.29	5.64 ± 0.31	—	0.55 ± 0.18	10.8 ± 2.2	(7)
None	Fed	—	—	7.56 ± 0.20	—	—	(6)
None	18 h starved	—	—	6.66 ± 0.16	—	—	(6)

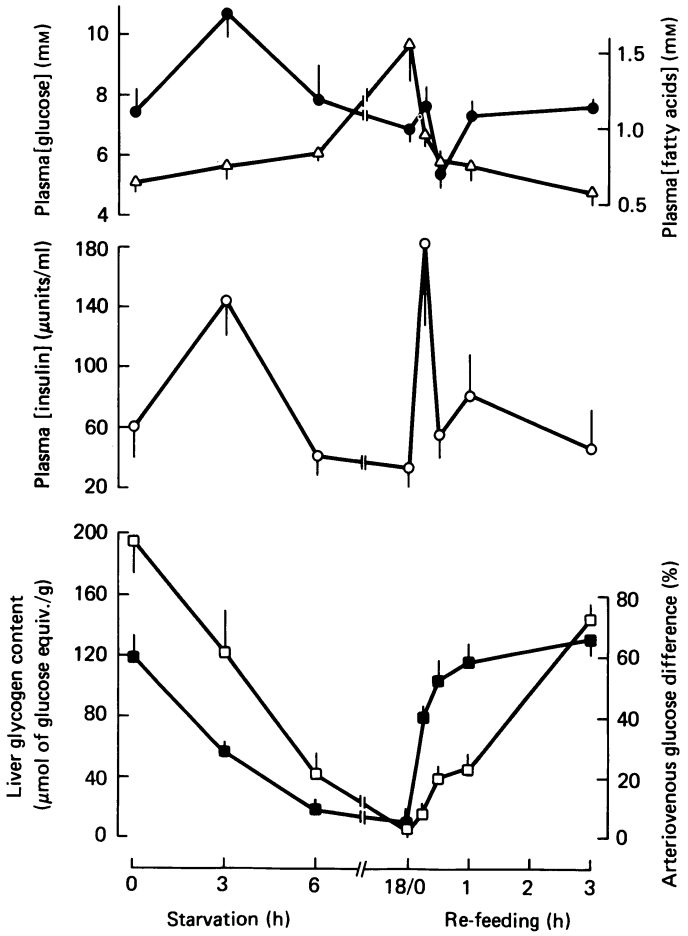

Fig. 2. Effect of feeding, starvation and re-feeding on mammary arteriovenous glucose (■), hepatic glycogen (□), and arterial plasma [glucose] (●), [fatty acids] (△) and [insulin] (○)

Each point is the mean for six rats; bars show the S.E.M. All parameters were measured in the same rats.

barbitone-anaesthetized rats gave arteriovenous glucose differences of about 60%, whereas ether-anaesthetized rats gave only 36%. These findings again do not correspond with those of Williamson and co-workers, but the difference has not been explained. In both Halothane- and pentobarbitone-anaesthetized rats, however, only very small arteriovenous glucose differences were observed in starved rats. Pentobarbitone anaesthesia was adopted for subsequent experiments, since it appeared to yield both satisfactory arterial glucose concentrations and large differences of mammary glucose extraction between the fed and starved states.

Changes in plasma glucose, fatty acids and insulin, and in liver glycogen, associated with starvation and re-feeding

In Fig. 2, the mean arteriovenous glucose difference of fed rats was $59 \pm 7\%$, in good agreement with the value reported in Table 1 for a separate group of rats. Food withdrawal caused a progressive decline in this value, that was significant ($P < 0.001$) already by 3 h and reached a nadir of only $4.9 \pm 2.1\%$ by 18 h. Liver glycogen content declined similarly, although significance was evident only by 6 h starvation ($P < 0.001$). Re-feeding *ad lib.* caused a remarkable recovery in arteriovenous glucose difference that was significant already by 15 min ($P < 0.001$) and that reached a value characteristic of the fed state by 1 h. The restoration of hepatic glycogen appeared to start equally soon, tissue glycogen contents being significantly elevated at 15 min ($P < 0.05$) and 30 min ($P < 0.01$), but requiring over 3 h to reach normal values.

These changes in mammary glucose consumption were not mirrored by similar changes in mean arterial [glucose], which was actually elevated ($P < 0.02$) to 10.7 ± 0.77 mM by 3 h before declining ($P < 0.05$) to 7.85 ± 0.51 mM at 6 h starvation. Similarly, the increased arteriovenous glucose difference seen during re-feeding was not mirrored by any elevation in arterial [glucose], which rather appeared to decline ($P < 0.05$) temporarily as mammary glucose uptake approached its maximum restored value.

Plasma fatty acids, which are often a useful index of nutritional status, showed a mean value of 0.62 ± 0.023 mM in the fed state and significantly increased in concentration at 6 h ($P < 0.01$) to reach a maximum of 1.55 ± 0.18 mM by 18 h starvation. These also declined sharply during the first 15 min ($P < 0.01$) and 30 min of re-feeding, by which time they resembled values typical of the fed state. Hawkins & Williamson (1972) reported similar values for [fatty acid] in fed lactating rats, but observed no increase during 16 h starvation.

Changes in plasma [insulin] are less easily described because of the large S.E.M. values. The apparent increase at 3 h starvation, although matching the elevation of plasma [glucose], was of low significance ($0.1 > P > 0.05$), but then significantly declined at 6 h ($P < 0.02$) and 8 h ($P < 0.01$) starvation. However, the values at 6 h and 18 h starvation were not significantly smaller than those in the fed state. Re-feeding was accompanied by an 8-fold increase by 15 min ($P < 0.05$), followed by a rapid decline to values at 30 min and 180 min that did not differ from those at 6–18 h of starvation.

Attempts to restore mammary glucose uptake in the starved rat

Rats starved for 18 h were variously treated in order to study possible factors in the recovery of mammary glucose uptake seen on re-feeding. Table 2 shows that the arteriovenous glucose difference was significantly elevated by glucose administered intravenously or orally, but not by orally administered fat. It is interesting that orally given glucose caused an apparent decrease in plasma glucose, possibly secondary to the extra secretion of insulin that oral glucose, as opposed to intravenous glucose, is known to evoke. The administration of insulin, causing the expected hypoglycaemia, also increased the arteriovenous glucose difference. Acetoacetate, administered to fed rats at a dose reported to give a marked elevation in plasma concentration (Viña *et al.*, 1983), did not significantly impair the glucose uptake. Heparin, infused intravenously at a rate sufficient to raise plasma [fatty acids] to 1.63 mM ($P < 0.001$), elevated arterial [glucose] and did not impair the arteriovenous glucose difference.

Glucose tolerance in lactating and non-lactating rats

Oral glucose feeding of starved lactating rats paradoxically resulted in a depression of plasma [glucose] 30 min later (Table 2). This prompted a comparison of the

Table 2. Effect of glucose, insulin and other treatments on arteriovenous glucose differences across the mammary gland

Results are means ± S.E.M., with numbers of rats in parentheses.

Treatment	Plasma [glucose] (mM)		Arteriovenous glucose difference (%)
	Arterial	Venous	
Fed	7.42±0.87	2.87±0.54	59.5±6.6 (6)
18 h starved	6.92±0.44	6.77±0.27	4.9±2.1 (6)
18 h starved + glucose (intraperitoneal)	6.90±0.51	4.86±0.59	33.4±4.8 (6)
18 h starved ± glucose (oral)	4.03±0.48	1.94±0.20	50.6±4.3 (6)
18 h starved + fat (oral)	7.40±0.31	6.95±0.33	8.4±1.6 (6)
18 h starved + insulin (0.5 unit/kg) (intraperitoneal)	3.68±0.31	1.65±0.34	55.0±8.6 (6)
Fed + streptozotocin	23.7	21.9	5.7 (2)
Fed + acetoacetate	9.4±0.4	4.9±1.0	48.0±8.7 (4)
Fed + heparin	11.2±1.0	6.6±1.0	41.1±5.4 (5)

glucose tolerance test in lactating and non-lactating rats, as another approach to eliciting the mammary response to re-feeding. Fig. 3 shows the results of administering glucose orally or intravenously to 18 h-starved lactating or virgin rats. Plasma [glucose] reached a peak at 15 min in all cases, as far as the sampling frequency allowed one to judge. Compared with the virgin rats, however, the return of plasma [glucose] to the normal value of the fed state was markedly hastened in both groups of lactating rats. Strikingly, it continued to fall rapidly in the orally fed group, even to the point of undershooting the starved value by 40 min. A similar effect of lactation in enhancing the tolerance of intravenously administered glucose has been shown by Burnol *et al.* (1983) in the post-absorptive rat.

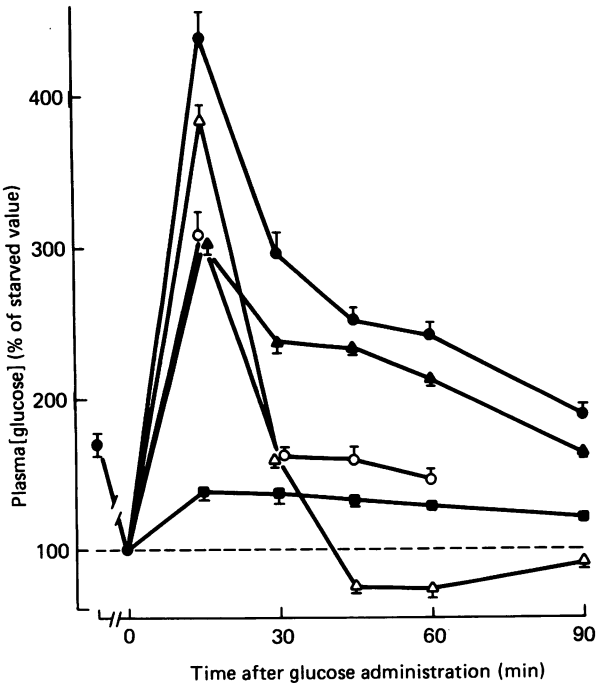

Fig. 3. Glucose tolerance test in virgin (●,▲) and lactating (○,△) rats given glucose orally (▲,△) or intraperitoneally (●,○)

Values are means for six rats (bars show the S.E.M.), expressed as a percentage of the arterial plasma [glucose] in the starved state. ■, Means of all (24) saline-treated controls.

DISCUSSION

Validity of the technique

The arteriovenous glucose difference across the mammary glands of our rats was about 60% when either Halothane or pentobarbitone was used, in agreement with Elkin & Kuhn (1975), who used these anaesthetics also. Use of ether led to apparently lower values, approaching the 25–30% reported by Hawkins & Williamson (1972) and Robinson & Williamson (1977), who used pentobarbitone. These discrepancies seem likely to reflect differences in rates of blood flow, which have been variously reported as 0.62 ml/min per g (Chatwin *et al.*, 1969), 0.75 ml/min per g (Jones & Williamson, 1984) and 0.43 ml/min per g (Viña *et al.*, 1985) for the fed lactating rat. An extraction of 60% of plasma glucose seems high in comparison with the 25%, 30% and 33% observed respectively in cows, pigs and goats (Linzell, 1974). However, combining such a value with an arterial plasma [glucose] of 7.5 mM, haematocrit of 0.60 and a blood flow of 0.43–0.75 ml/min per g yields an estimated glucose uptake of 1.16–2.02 μmol/min per g. This value agrees remarkably well with the range of 1.1–1.5 μmol/min per g estimated, without the need to know blood flow, from the transport $V_{max.}$ measured with 2-deoxyglucose uptake *in vivo* and from transport K_m measured *in vitro* (Threadgold & Kuhn, 1984; Threadgold *et al.*, 1982). Jones & Williamson (1984), who measured both arteriovenous glucose difference and blood flow, derived a glucose uptake of 1.47 μmol/min per g. Stress is difficult to avoid during experiments *in vivo* with small animals, and consistency of arteriovenous glucose difference is possibly more useful than normality.

Effect of starvation and re-feeding on mammary glucose uptake

The present values (Fig. 2) indicate a glucose uptake at 18 h starvation of only about 8% of that in the fed rat.

This would be even lower if allowance were made for the 53% decreased blood flow observed in starved rats (Viña *et al.*, 1985). Glucose transport monitored in the 15 h-starved rat by uptake of 2-deoxyglucose and of 3-*O*-methylglucose by the mammary gland was reported to be respectively 9% and 5% of that in control fed rats (Threadgold & Kuhn, 1984). Conversion of glucose into lactose (accounting for about 20% of glucose used) and into fatty acids (accounting for about 50% of glucose used) was decreased to about 3% and 1% respectively of fed values in intact 15–18 h-starved lactating rats (Bussmann *et al.*, 1984). These comparisons suggest that the present arteriovenous glucose difference measurements give a reasonably valid measure of changes in mammary activity during starvation. Earlier measurements in which the arteriovenous glucose difference decreased from only 25% to 14% (Hawkins & Williamson, 1972), or from 30% to 12% (Robinson & Williamson, 1977), on starvation seem likely to have underestimated the actual changes, unless accompanied by unusually large attenuation of blood flow. It appears, therefore, that 15–18 h of starvation almost totally shuts down the synthesis of milk solids *in vivo*, in marked contrast with their synthesis *in vitro*.

Of particular interest was the rapid recovery of mammary glucose uptake after re-feeding. The present measurements clearly establish that glucose extraction by the mammary gland has been substantially restored already at 15 min. This confirms the apparent increase in sugar transport at 15 min (Threadgold & Kuhn, 1984), although, as pointed out above, an elapse of 30 min was required for that measurement. Jones & Williamson (1984) found little restoration of mammary blood flow in 6 h-starved rats re-fed for 2 h.

Control of mammary glucose uptake during re-feeding

The resumption of mammary activity on re-feeding poses two problems. First, how is glucose homoeostasis maintained? Second, to what signal(s) does the mammary gland respond?

Re-feeding confronts the maternal homoeostatic system with the formidable challenge of rapidly re-activating a major glucose-consuming organ at a time when little hepatic glycogen exists to buffer changes in plasma glucose. The impact of the mammary gland that has to be accommodated is seen in the glucose tolerance test, where plasma glucose was cleared faster in lactating than in non-lactating animals (Fig. 3). It is probably also seen in the rise in plasma glucose that temporarily accompanies the cessation of mammary activity during the onset of starvation (Fig. 2; see also Jones *et al.*, 1984). From previous work (Carrick & Kuhn, 1978), this transient rise can probably not be ascribed to any diurnal variation in plasma [insulin]. Yet it is noteworthy that the rebuilding of hepatic glycogen is apparently given equal priority with the restoration of milk production. We estimate that at 15 min of re-feeding the rates of glucose uptake by the whole liver and mammary tissue are approx. 13 and 15 μmol/min respectively. The tight control that plasma glucose must exert on its own uptake by these tissues is indicated by the relatively small fluctuations in concentration that we observed. How far this is mediated solely by insulin is unclear. Jones *et al.* (1984) have commented that glucose cannot be the over-riding influence on insulin secretion during lactation.

At first sight the great elevation of plasma insulin is an obvious signal to re-activate mammary function. Burnol *et al.* (1983) have employed euglycaemic-insulin-clamp experiments to show the increase in rat mammary glucose utilization in the face of elevated plasma [insulin]. Jones *et al.* (1984) have reported a doubling of lipogenesis during infusion of insulin. Yet in our experiments, this elevation appeared largely to subside just as glucose uptake approached its maximum, as though a high concentration of insulin were required to throw a metabolic switch, but a low one sufficed to keep it there. This implies that, very soon after re-feeding has commenced, the mammary gland acquires some independence of circulating insulin, either through greater sensitivity towards it or through the by-passing of insulin-dependent controls (for discussion, see Zammit, 1985). Either mechanism would be consistent with the need to direct glucose and fatty acids away from other extrahepatic tissues towards the mammary gland itself (Williamson, 1980). There may be a parallel with lactogenesis, where a decline in plasma insulin also accompanies increased mammary function (Sutter-Dub *et al.*, 1974; Wilde & Kuhn, 1979). The importance of insulin sensitivity has been emphasized also by Jones *et al.* (1984), who, however, regard it as a possible cause of the low plasma [insulin] itself.

Our experiments with infused heparin, to increase plasma [non-esterified fatty acids], lend no support to the withdrawal of non-esterified fatty acids as being the basis of such sensitization. Changes in mammary sensitivity towards insulin might be mediated by adrenaline, which acts through β_2 receptors to stimulate the formation of cyclic AMP in mammary acini (Clegg & Mullaney, 1985). However, the coupling of this effect to control points on pathways of glucose utilization remains difficult to demonstrate *in vitro*, whereas the only effect of adrenaline seen *in vivo* appears to be a partial inhibition of glucose utilization for fatty acid synthesis relative to lactose synthesis (Bussmann *et al.*, 1984).

We thank the Agricultural and Food Research Council for generous financial support, and Dr. C. J. Bailey for providing facilities for the determination of insulin and automated determination of glucose.

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Received 10 April 1986/22 May 1986; accepted 12 June 1986