Biochem. J. (1977) **164**, 153–159
Printed in Great Britain

Comparison of Glucose Metabolism in the Lactating Mammary Gland of the Rat *in vivo* and *in vitro*

EFFECTS OF STARVATION, PROLACTIN OR INSULIN DEFICIENCY

By ALISON M. ROBINSON and DERMOT H. WILLIAMSON
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

(*Received* 5 *October* 1976)

1. Measurements of arteriovenous differences across mammary glands of normal and starved lactating rats, and lactating rats made short-term insulin-deficient with streptozotocin or prolactin-deficient with bromocryptine, showed that only in the starved animals was there a significant decrease in glucose uptake. This decrease was accompanied by release of lactate and pyruvate from the gland, in contrast with the uptake of these metabolites by glands of normal lactating rats. 2. There were no marked differences in metabolite concentrations in freeze-clamped glands in the four conditions studied, apart from a decrease in [lactate] and [pyruvate] and an increase in [glucose] in the glands of the streptozotocin-treated group. 3. Acini isolated from the glands of starved, insulin or prolactin-deficient rats had a higher production of lactate and pyruvate from glucose than did glands from normal rats; this is in agreement with the reported decrease in the proportion of active pyruvate dehydrogenase in these situations [Field & Coore (1976) *Biochem. J.* **156**, 333–337; Kankel & Reinauer (1976) *Diabetologia* **12**, 149–154]. 4. Addition of insulin did not increase the uptake of glucose by acini from normal glands, but it caused a significant increase in the utilization of glucose by acini from glands of starved rats. Insulin did not decrease the accumulation of lactate and pyruvate in any of the experiments. 5. It is concluded that isolated acini represent a suitable model for the study of mammary-gland carbohydrate metabolism in that they reflect metabolism of the gland *in vivo*.

The use of isolated cell preparations has considerable advantages in the study of tissue metabolism. The description of an isolated acinar preparation from lactating mammary gland of the rat (Katz *et al.*, 1974), which is capable of high rates of lipogenesis from glucose or lactate, seems to provide a suitable system *in vitro* for the investigation of the regulation of glucose metabolism in this tissue. However, Elkin & Kuhn (1975) have advised caution in the use *in vitro* of preparations of tissue slices and dispersed cells for the study of mammary-gland metabolism because of their high rate of aerobic lactate production. It therefore seemed important to compare the metabolic properties of the acinar preparation *in vitro* with the metabolism of the gland *in vivo* in various situations.

Alteration of the nutritional or hormonal status of lactating rats can have profound effects on the metabolism of the mammary gland. Starvation of lactating rats results in a decreased arteriovenous difference for glucose across the mammary gland and output of lactate and pyruvate (Hawkins & Williamson, 1972), in contrast with the fed state, where the gland takes up significant amounts of lactate and pyruvate (Hawkins & Williamson, 1972; Elkin & Kuhn, 1975). Evidence has suggested that prolactin and/or insulin may play a role in regulating the activity of pyruvate dehydrogenase in mammary gland (Field & Coore, 1975), and that the active form of this enzyme in the gland decreases in starvation (Kankel & Reinauer, 1976). In addition, it has been proposed that insulin may increase the rate of glucose entry into the gland (Walters & McLean, 1968*a*; Gumaa *et al.*, 1971) and/or act at other site(s) within the pentose phosphate pathway or lipogenesis (Abraham *et al.*, 1957; Walters & McLean, 1968*b*; Martin & Baldwin, 1971; Baldwin & Yang, 1974; Yang & Baldwin, 1975; Williamson *et al.*, 1975).

In the present paper we have compared the arteriovenous differences for glucose, lactate and pyruvate across the mammary gland of lactating rats with the rates of glucose utilization and of lactate and pyruvate production by isolated acini *in vitro*. In addition, the concentrations of key metabolites have been measured in freeze-clamped (Wollenberger *et al.*, 1960)

mammary gland. The four situations examined were fed and starved (24h) lactating rats, and lactating rats made short-term (2h) insulin-deficient by injection of streptozotocin (Schein *et al.*, 1971) or prolactin-deficient by injection of bromocryptine (Seki *et al.*, 1974).

Experimental

Materials

Rats. Rats of the Wistar strain (250–300g) with between 6 and 14 pups were used after a lactation period of between 10 and 18 days. Starved rats were deprived of food for 24h. Prolactin deficiency was induced by a subcutaneous injection of bromocryptine [10mg/kg body wt.; solution in 10% (v/v) ethanol containing 1% (w/v) tartaric acid] 24h before the experiments (Seki *et al.*, 1974). Streptozotocin (50mg/kg body wt.; solution in 0.01M-sodium citrate, pH4.5) was injected into the tail vein 2h before experiments to induce short-term insulin deficiency (Schein *et al.*, 1971). Rats were anaesthetized with Nembutal [50mg/kg body wt.; solution in 0.9% (w/v) NaCl] and the pups were allowed to continue to suckle before the operative procedure so as to maintain prolactin secretion (Amenomori *et al.*, 1970).

Biochemicals. Crystalline insulin free of glucagon was a gift from Dr. N. Z. Lazarus (Wellcome Research Laboratories, Beckenham, Kent, U.K.) and bromocryptine (2-bromo-α-ergocryptine mesylate) was a gift from Professor E. Flückiger and Dr. H. Friedli (Sandoz Ltd., Basle, Switzerland). Streptozotocin was obtained from the Upjohn Co., Kalamazoo, MI, U.S.A. All enzymes, including collagenase (grade II), and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Radioactive compounds. [1-^{14}C]Glucose was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Studies in vivo. For the measurements of arteriovenous differences, venous blood was collected from the left pudic–epigastric vein into a heparinized syringe by using a 25-gauge needle. The right inguinal mammary gland was then freed from surrounding tissue and rapidly cut free and clamped between tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). Arterial blood was then collected from the aorta into a heparinized syringe. A sample of the blood (0.5ml) was added to 2ml of 6% (w/v) $HClO_4$ and the mixture centrifuged at 3000rev./min for 10min to remove protein. The supernatant was neutralized with 20% (w/v) KOH and the $KClO_4$ precipitate was removed by centrifugation (3000rev. min, 10min). This final supernatan was decanted off and used for the determination of metabolites. The freeze-clamped tissue was powdered in liquid N_2 with a pestle and mortar, and a portion of the frozen powder (about 1g) was extracted with 4vol. (v/w) of 6% (w/v) $HClO_4$ by homogenization with a motor-driven Teflon homogenizer. The extract was centrifuged at 30000*g* for 20min at 4°C to remove protein and the final supernatant was neutralized with 20% (w/v) KOH. The $KClO_4$ precipitate was removed by centrifugation at 3000rev./min for 10 min and the supernatant passed through a Millipore filter (pore size 14μm) to clarify it. This filtered extract was used for metabolite determinations.

Studies in vitro. Acini were prepared by a modification of the procedure of Katz *et al.* (1974). The inguinal mammary glands (5–8g) were removed and finely chopped with a razor blade. The mince was washed three times by decantation in a 50ml measuring cylinder with warm saline (Krebs & Henseleit, 1932) and transferred to a 250ml plastic flask, where it was suspended in a total volume of 30ml of Krebs–Henseleit saline containing collagenase (1mg/ml). The suspension was gassed with O_2/CO_2 (19:1) and shaken at 37°C in a Dubnoff-type shaker. After about 1h the suspension was filtered through nylon gauze (mesh size 0.44mm) and the filtrate was centrifuged at 400rev./min for 1–2min. The resulting pellet (1–2g wet wt.), which contained the acini, was washed four times with about 30ml of warm Krebs–Henseleit saline. The time taken from the death of the animal to the start of incubation of the acini was 80–90min.

The acini (about 100mg wet wt.) were incubated in a total volume of 4ml of Krebs–Henseleit saline containing [1-^{14}C]glucose (5mM), with or without insulin (50munits/ml), in a silicone-treated 50ml conical flask fitted with a centre well. The flasks were gassed with O_2/CO_2 (19:1) and shaken at 37°C in a Dubnoff-type shaker for 20, 40 or 60min. The reaction was stopped with 0.4ml of 20% $HClO_4$ and $^{14}CO_2$ collected as described by Williamson *et al.* (1975). The acidified medium was transferred into weighed tubes and centrifuged for 10min at 3000rev./min to precipitate the acini. The supernatant was decanted and adjusted to pH7.0 with 20% (w/v) KOH. After centrifugation to remove the $KClO_4$ precipitate, the supernatant was decanted and used for metabolite determinations. Any residual solid material in the incubation flasks was removed with two 5ml portions of 50% (v/v) ethanol, which on each occasion were added to the precipitated acini and decanted off after centrifugation at 3000rev./min for 10min. These ethanol washings also served to remove electrolytes and soluble radioactive compounds from the acini (Katz *et al.*, 1974). The precipitated acini were finally extracted with 5ml of chloroform/

methanol (2:1, v/v). Radioactivity in this extract was taken to be a measure of the conversion of [1-^{14}C]-glucose into lipid (see below). The defatted residue was dried at 60°C and the tubes were weighed to obtain the defatted dry weight (usually 10–15mg).

Determination of metabolites. The following metabolites were determined by enzymic methods: glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962); glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963); AMP and ADP (Adam, 1963); glycerol 3-phosphate (Hohorst, 1963); lactose (Kurz & Wallenfels, 1974).

Measurements of radioactivity. These were carried out as described by Williamson *et al.* (1975).

Expression and interpretation of results. The metabolite content of blood and mammary gland is expressed as μmol/ml of blood or μmol/g of tissue respectively. Rates of glucose utilization and lactate and pyruvate production by acini were obtained from graphical plots of the 20, 40 and 60min values. These plots were usually linear; if not, the experiments were discarded. Rates of conversion of [1-^{14}C]glucose into $^{14}CO_2$ and incorporation into the lipid fraction were obtained in the same way.

It must be emphasized that the incorporation of [1-^{14}C]glucose into the lipid fraction does not give a measure of the absolute rate of lipogenesis, because (*a*) a considerable amount of radioactivity is lost as $^{14}CO_2$ in the pentose phosphate pathway, and (*b*) the incorporation of radioactivity into the glycerol and fatty acid moieties of the lipid was not measured separately. The latter is a comparatively minor source of error, as only about 10% of the glucose utilized is incorporated into the glycerol moiety (Katz *et al.*, 1974). Similarly, the $^{14}CO_2$ collected from [1-^{14}C]glucose is derived from two sources: glucose carbon oxidized to CO_2 in the hexose monophosphate pathway, and glucose carbon metabolized via the glycolytic pathway and subsequently oxidized to CO_2 in the tricarboxylic acid cycle. However, measurements of the contribution of the latter process with [6-^{14}C]glucose give values of less than 10% (Williamson *et al.*, 1975). Despite these reservations, the measurements of $^{14}CO_2$ and incorporation of radioactivity into the lipid fraction provide an index of alterations of the activities of the pentose phosphate pathway and the lipogenic pathway respectively.

Results

Arteriovenous differences

Although there was no difference in the arterial blood glucose concentration between fed and starved lactating rats (Table 1), glucose uptake by the mammary glands of starved rats was greatly decreased compared with the control rats (Table 2).

Table 1. *Concentrations of metabolites in arterial blood of lactating rats*

For details see the Experimental section. The results, expressed as μmol/ml of whole blood, are mean values±S.D. for the numbers of rats in each group shown in parentheses. Values that are significantly different by Student's *t* test from the normal group are shown: *$P<0.05$; **$P<0.01$.

	State of rats			
Metabolite	Normal (8)	Starved 24h (7)	Bromocryptine-treated (6)	Streptozotocin-treated (7)
Glucose	4.74±0.48	4.80±0.40	5.33±0.53*	10.6±3.36**
Lactate	1.75±0.43	0.98±0.37**	1.62±0.43	1.27±0.43*
Pyruvate	0.18±0.06	0.12±0.04*	0.21±0.07	0.14±0.06
Ketone bodies	0.14±0.03	0.54±0.22**	0.14±0.06	0.15±0.04

Table 2. *Arteriovenous differences across mammary glands of lactating rats*

For details see the Experimental section. The results, expressed as μmol/ml of whole blood, are mean values±S.D. for the numbers of rats in each group shown in parentheses. Values that are significantly different from those of the normal group are shown: *$P<0.05$; **$P<0.01$.

	State of rats			
Metabolite	Normal (8)	Starved 24h (7)	Bromocryptine-treated (6)	Streptozotocin-treated (7)
Glucose	−1.43±0.47	−0.58±0.59**	−1.56±0.51	−1.15±0.54
Lactate	−0.32±0.25	+0.42±0.22**	−0 33±0.17	−0.13±0.28
Pyruvate	−0.03±0.06	+0.06±0.06**	−0.06±0.05	+0.01±0.03
Ketone bodies	−0.005±0.09	−0.16±0.15*	+0.04±0.12	−0.03±0.02

The arterial blood concentration of lactate and pyruvate decreased on starvation (Table 1), and in contrast with the fed rats, lactate and pyruvate were released by the mammary gland rather than being taken up (Table 2). The arterial blood ketone-body concentration was increased 3-fold by starvation, and ketone bodies were taken up by mammary glands of starved lactating rats. These results are in general agreement with previous findings from our laboratory (Hawkins & Williamson, 1972) and with those of Elkin & Kuhn (1975) for fed lactating rats.

The arterial blood glucose concentrations were significantly increased in both bromocryptine-treated rats and streptozotocin-treated rats compared with normal rats (Table 1), but glucose uptake across the mammary glands of these two groups of rats did not differ significantly from normal rats (Table 2). Neither the arterial blood concentrations nor the arteriovenous differences for lactate and pyruvate were significantly different in bromocryptine-treated rats compared with normal rats. In the streptozotocin-treated rats there was a significantly lower arterial blood lactate concentration and a trend towards a decreased lactate uptake across the mammary gland. In contrast with starved rats, neither bromocryptine-treated nor streptozotocin-treated rats showed significantly different arterial blood concentrations or arteriovenous differences for ketone bodies compared with normal rats.

Metabolite concentrations in vivo

The marked changes in arteriovenous differences in starved rats were not accompanied by alterations in concentrations of metabolites in freeze-clamped mammary glands. Glucose, lactate and pyruvate concentrations were similar in freeze-clamped mammary glands from control, starved and bromocryptine-treated rats (Table 3). However, the [glucose] was elevated, and [lactate] and [pyruvate] were approximately halved, in the glands of streptozotocin-treated rats compared with the other three groups. The decreased [lactate] after streptozotocin treatment is in contrast with the increased [lactate] in the glands of lactating rats treated with anti-insulin serum (Martin & Baldwin, 1971). The [lactate]/[pyruvate] ratio was similar for all four groups of rats, whereas Martin & Baldwin (1971) reported a doubling of this ratio after anti-insulin treatment. The concentrations of glucose 6-phosphate and glycerol 3-phosphate were similar in all four groups of rats, despite changes in blood glucose concentration and assumed changes in hormonal balance.

[ATP] and [AMP] were similar in all four groups (Table 3), with the energy charge ([ATP]+½[ADP]/[ATP]+[ADP]+[AMP]) varying from 0.71 to 0.73, which agrees well with values of 0.77 and 0.73 reported for freeze-clamped mammary gland after halothane anaesthesia (Field & Coore, 1975) and ether anaesthesia (Martin & Baldwin, 1971) respectively. The high energy charge after streptozotocin treatment is in contrast with the value of 0.58 reported after anti-insulin treatment 2h before freeze-clamping (Martin & Baldwin, 1971). It is not clear why [ADP] is elevated in the bromocryptine-treated group of rats.

Metabolism of isolated acini

Acini isolated from the mammary glands showed changes in glucose metabolism after bromocryptine treatment, streptozotocin treatment or starvation for 24h (Table 4). Glucose uptake was significantly decreased in acini from starved rats compared with the other three groups. Lactate formation by the acini was significantly increased in all treated groups compared with the control group of rats, with acini from starved rats showing the highest lactate production (Table 4). Production of lactate plus pyruvate accounted for 16, 36, 53 and 65% of the glucose uptake by acini from normal, streptozo-

Table 3. *Metabolite concentrations in mammary glands of lactating rats*

For details see the Experimental section. The results, expressed as μmol/g fresh wt. of gland, are mean values ±S.D. for the numbers of rats in each group shown in parentheses. Values that are significantly different from the normal group are shown: $*P<0.05$; $**P<0.01$.

	State of rats			
Metabolite	Normal (8)	Starved 24h (7)	Bromocryptine-treated (6)	Streptozotocin-treated (5)
Glucose	1.55±0.48	1.68 ±0.51	1.90±0.38	3.72±1.02
Lactate	1.98±0.56	2.04 ±0.86	2.09±0.53	1.10±0.18**
Pyruvate	0.11±0.05	0.092±0.035	0.13±0.07	0.06±0.03*
Glycerol 3-phosphate	0.13±0.03	0.18 ±0.07	0.14±0.07	0.14±0.05
Glucose 6-phosphate	0.12±0.04	0.14 ±0.04	0.11±0.06	0.16±0.05
ATP	0.86±0.42	0.84 ±0.50	0.97±0.56	0.96±0.40
ADP	0.56±0.18	0.54 ±0.18	0.87±0.20**	0.47±0.06
AMP	0.15±0.06	0.14 ±0.05	0.11±0.09	0.10±0.06

Table 4. *Metabolism of acini prepared from mammary glands of lactating rats*

For details see the Experimental section. Where indicated insulin (50munits/ml) was added to the incubation medium. The results, expressed as μmol/min per 100mg defatted dry wt., are mean values ± S.D., for the numbers of rats in each group shown in parentheses. Values that are significantly different from the appropriate control for the normal group are shown: $*P<0.05$; $**P<0.01$. Values that are significantly different in the presence of insulin from the appropriate control without insulin are shown: $\dagger P<0.05$.

	State of rats							
	Normal		Starved 24h		Bromocryptine-treated		Streptozotocin-treated	
	(19)	+Insulin (6)	(9)	+Insulin (6)	(6)	+Insulin (6)	(7)	+Insulin (7)
Glucose removal	1.29 ± 0.21	1.24 ± 0.30	0.93 ± 0.15**	1.20±0.27†	1.05 ± 0.17*	1.20 ± 0.20	1.25 ± 0.26	1.45 ± 0.22
[1-^{14}C]Glucose → $^{14}CO_2$	0.56 ± 0.10	0.65 ± 0.12	0.24 ± 0.12**	0.32 ± 0.14	0.38 ± 0.06**	0.44 ± 0.12	0.38 ± 0.13**	0.51 ± 0.12
[1-^{14}C]Glucose → lipid	0.36 ± 0.07	0.45 ± 0.08†	0.14 ± 0.09**	0.21 ± 0.10	0.20 ± 0.06**	0.27 ± 0.14	0.24 ± 0.12*	0.37 ± 0.12
Lactate formation	0.34 ± 0.16	0.34 ± 0.14	1.06 ± 0.34**	1.31 ± 0.49	0.94 ± 0.45**	0.94 ± 0.43	0.76 ± 0.29**	0.85 ± 0.52
Pyruvate formation	0.08 ± 0.03	0.07 ± 0.02	0.14 ± 0.04**	0.14 ± 0.03	0.17 ± 0.06**	0.15 ± 0.06	0.13 ± 0.04**	0.11 ± 0.04

tocin-treated, bromocryptine-treated and starved rats respectively. The acini showed a low rate of lactose formation, which accounted for about 5% of the glucose uptake.

The oxidation of [1-^{14}C]glucose to $^{14}CO_2$ by mammary gland gives an indication of the activity of the pentose phosphate pathway, which provides a source of reducing equivalents for fatty acid synthesis. All three treatments decreased $^{14}CO_2$ production as well as the incorporation of [1-^{14}C]-glucose into lipid by the acini (Table 4), suggesting that fatty acid synthesis was decreased. Starvation had the greatest effect, causing a 60% decrease in both $^{14}CO_2$ production and ^{14}C incorporation into lipid compared with the 30–40% decrease found with bromocryptine and streptozotocin treatments.

The addition of insulin to the incubation medium did not affect the glucose uptake by acini isolated from the control group of rats, whereas it did significantly increase glucose uptake by acini from starved rats (Table 4). With [1-^{14}C]glucose as substrate, insulin tended to increase both $^{14}CO_2$ production and ^{14}C incorporation into lipid by the acini from all groups of rats, but only in the control acini was the increase significant and then only for lipid. Accumulation of lactate plus pyruvate was not decreased by insulin in any of the experimental groups.

Discussion

Comparison of results in vivo and in vitro

The use of preparations of mammary gland *in vitro* for metabolic studies has been questioned by Elkin & Kuhn (1975), who consider that the aerobic lactate production of cells and slices is indicative of a metabolic derangement. Acini used in the present work showed a twofold higher rate of glucose uptake (expressed as μmol/g wet wt.) compared with slices of rat mammary gland (Williamson *et al.*, 1975), and lactate production represented 13% of the glucose uptake in acini isolated from normal rats. In contrast, the mammary-gland cells used by Elkin & Kuhn (1975) had a glucose uptake of about 10% of that of the acini described in the present paper, and 21% of this glucose was converted into lactate. The fact that normal acini produce lactate at a low rate, whereas the intact gland takes it up, may merely reflect the initial absence of lactate from the medium. Support for this are the findings (Katz *et al.*, 1974; A. M. Robinson & D. H. Williamson, unpublished work) that acini take up lactate and convert it into lipid when it is present at a higher concentration (2mM) in the medium.

Increased production of lactate and pyruvate is the most marked change in the metabolism of the acini isolated from the mammary gland of rats after

starvation or treatment with bromocryptine or streptozotocin. This presumably reflects decreased activity of pyruvate dehydrogenase, because the increased lactate production was not accompanied by an increased glucose uptake compared with control rats.

Studies *in vivo* of the type reported here can only provide limited information on the behaviour of the intact gland. Comparison of the arteriovenous differences in the various experimental groups without comparable information on blood flow is open to criticism. Similarly, the rate of flux through a pathway may change without necessarily altering the concentrations of metabolites within the pathway. Thus the absence of any appreciable alteration in [lactate] or [pyruvate] within the gland itself (except in the streptozotocin-treated group) is presumably because the concentrations reflect the flux into the tissue pool of lactate plus pyruvate from glycolysis or the arterial blood and efflux via pyruvate dehydrogenase or the venous blood. The tissue [lactate] or [pyruvate] do not simply reflect the changes in arterial concentrations and consequent changes in the extracellular space (see Table 1 and 3). Nevertheless, except in the bromocryptine-treated group, there was a degree of parallelism between the results *in vivo* and *in vitro*.

The finding that *in vivo* mammary glands of starved lactating rats release lactate and pyruvate is in accord with the decrease in the proportion of pyruvate dehydrogenase in the active form (Kankel & Reinauer, 1976; A. Kerbey, personal communication) and with the high proportion of glucose taken up by isolated acini that is converted into lactate and pyruvate. Although the glands of streptozotocin-treated rats did not produce lactate, there was a decrease in the amount of lactate taken up by the gland (compared with the glucose uptake), which is consistent with the decrease in pyruvate dehydrogenase activity (Field & Coore, 1976) and the increased production of lactate by acini. The handling of glucose and lactate *in vivo* by glands of the bromocryptine-treated rats was not significantly different from that of glands of normal rats, whereas there is a marked decrease in pyruvate dehydrogenase activity (Field & Coore, 1975, 1976) and an increased lactate production by acini. The reason for this discrepancy is not apparent.

Effects of insulin

Acini have advantages over slices for studying the effects of insulin on mammary-gland metabolism, since contamination of the acinar parenchymal cells by adipose cells present in the gland is greatly decreased (Katz *et al.*, 1974). In adipose tissue, insulin promotes glucose uptake (Crofford & Renold, 1965), stimulates lipogenesis (Coore, *et al.* 1971; Sooranna & Saggerson, 1975) and causes an activation of pyruvate dehydrogenase (Coore *et al.*, 1971), but the site(s) of insulin action in mammary gland is by no means clear. Insulin *in vitro* (generally at concentrations of 50–200munits/ml) causes a marginal stimulation (20–50% increase) of glucose oxidation and glucose incorporation into lipid by mammary slices and dispersed mammary cells (Martin & Baldwin, 1971; Baldwin & Yang, 1974; Williamson *et al.*, 1975; Yang & Baldwin, 1975), and in mammary-gland slices from diabetic rats glucose oxidation and incorporation into lipid is decreased (Walters & McLean, 1968*a*).

In the present experiments, although the effects of insulin *in vitro* on any of the parameters measured were not striking, there was a consistent trend towards higher activity of the pentose phosphate pathway and greater incorporation of radioactivity into the lipid fraction when insulin was in the medium. Absence of marked effects of insulin on glucose metabolism in the acini is unlikely to be due to complete loss of insulin receptors, because insulin relieves the inhibition of glucose uptake brought about by acetoacetate in this preparation (A. M. Robinson & D. H. Williamson, unpublished work) as it does in mammary-gland slices (Williamson *et al.*, 1975).

The decreased rate of lipogenesis observed in the starved, bromocryptine- and streptozotocin-treated groups may be related to the inactivation of pyruvate dehydrogenase, and it appears that insulin is unable to influence the interconversion of this enzyme in the acini under the present experimental conditions, since accumulation of lactate and pyruvate was not decreased.

Starvation and the mammary gland

In starvation the metabolism of all tissues is controlled co-ordinately to maintain blood glucose, with most peripheral tissues using fatty acids or ketone bodies as alternative fuels to carbohydrate. Lactating mammary gland, with its high glucose uptake for the synthesis of the lactose and lipid secreted in milk, represents a potential drain on the body glucose supplies. The changes in mammary-gland metabolism on starvation seem ideally suited to the sparing of carbohydrate, since less glucose is removed from the circulation (assuming no change in blood flow) and more of the glucose taken up by the gland is released as lactate, which can return to the liver and be reconverted into glucose. In addition, the present studies provide evidence that less glucose is used for lipogenesis in starvation. Another adaptation to spare carbohydrate is the greatly decreased lactose synthesis found *in vivo* with starvation (N. J. Kuhn, personal communication).

It has been proposed by Hawkins & Williamson (1972) that the decreased glucose uptake and increased lactate output *in vivo* could result from

increased metabolism of ketone bodies, as has been found for other rat tissues (Williamson & Krebs, 1961; Randle *et al.*, 1964; Thompson & Williamson, 1975). A decreased insulin concentration has also been suggested as a possible cause for the decreased glucose uptake in starvation (Williamson *et al.*, 1975), but as yet the concentrations of insulin and prolactin have not been measured in starved lactating rats. Some support for a degree of insulin deficiency in the starved lactating rats is the finding that acini prepared from these rats show the greatest effect of insulin on glucose uptake. Acini from starved rats also convert a very high proportion of the glucose taken up into lactate and pyruvate, which suggests that the inhibition or inactivation of pyruvate dehydrogenase observed *in vivo* is retained *in vitro* and is therefore independent of the continued presence of ketone bodies.

The nature of 'signals' which bring about these complex metabolic changes in the gland when the lactating rat is starved remains to be identified, but the present study indicates that the use of isolated acini may be of considerable help in the elucidation of these and other outstanding questions of mammary-gland metabolism.

We thank Mr. P. Gregory for care of the lactating animals. This work was supported by the Medical Research Council and the U.S. Public Health Service (grant no. AM-11748). A. M. R. is a Commonwealth Scholar and an honorary New Zealand Ramsay Fellow. D. H. W. is a member of the External Staff of the Medical Research Council.

References

Abraham, S., Cady, P. & Chaikoff, I. L. (1957) *J. Biol. Chem.* **224**, 955–962

Adam, H. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 573–577, Academic Press, New York and London

Amenomori, Y., Chen, C. L. & Meites, J. (1970) *Endocrinology* **86**, 506–510

Baldwin, R. L. & Yang, Y. T. (1974) in *Lactation* (Larson, B. L. & Smith, V. R., eds.), vol. 1, pp. 364–366, Academic Press, New York and London

Coore, H. G., Denton, R. M., Martin, B. R. & Randle, P. J. (1971) *Biochem. J.* **125**, 115–127

Crofford, O. B. & Renold, A. E. (1965) *J. Biol. Chem.* **240**, 14–21

Elkin, A. R. & Kuhn, N. J. (1975) *Biochem. J.* **146**, 273–275

Field, B. & Coore, H. G. (1975) *Biochem. Soc. Trans.* **3**, 258–260

Field, B. & Coore, H. G. (1976) *Biochem. J.* **156**, 333–337

Gumaa, K. A., Greenbaum, A. L. & McLean, P. (1971) in *Lactation* (Falconer, I. R., ed.), pp. 197–238, Butterworths, London

Hawkins, R. A. & Williamson, D. H. (1972) *Biochem. J.* **129**, 1171–1173

Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 215–219, Academic Press, New York and London

Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18–46

Kankel, K.-F. & Reinauer, H. (1976) *Diabetologia* **12**, 149–154

Katz, J., Wals, P. A. & Van de Velde, R. L. (1974) *J. Biol. Chem.* **249**, 7348–7357

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210** 33–66

Kurz, G. & Wallenfels, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., vol. 3, pp. 1180–1184, Academic Press, New York and London

Lamprecht, W. & Trautschold, I. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 543–551, Academic Press, New York and London

Martin, R. J. & Baldwin, R. L. (1971) *Endocrinology* **88**, 868–871

Randle, P. J., Newsholme, E. A. & Garland, P. B. (1964) *Biochem. J.* **93**, 652–665

Schein, P. S., Alberti, K. G. M. M. & Williamson, D. H. (1971) *Endocrinology* **89**, 827–834

Seki, M., Seki, K., Yoshihara, T., Watanabe, N., Okumura, T., Tajima, C., Huang, S.-Y. & Kuo, C.-C. (1974) *Endocrinology* **94**, 911–914

Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London

Sooranna, S. R. & Saggerson, E. D. (1975) *Biochem. J.* **150**, 441–451

Thompson, M. P. & Williamson, D. H. (1975) *Biochem. J.* **146**, 635–644

Walters, E. & McLean, P. (1968*a*) *Biochem. J.* **109**, 407–417

Walters, E. & McLean, P. (1968*b*) *Biochem. J.* **109**, 737–741

Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96

Williamson, D. H., McKeown, S. R. & Ilic, V. (1975) *Biochem. J.* **150**, 145–152

Williamson, J. R. & Krebs, H. A. (1961) *Biochem. J.* **80**, 540–547

Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399–412

Yang, Y. T. & Baldwin, R. L. (1975) *J. Dairy Sci.* **58**, 337–343