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Regulation of the activity of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in mammary gland and liver of lactating rats

Effects of starvation, prolactin and insulin deficiency

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1. Starvation caused a marked decrease in the activity of ornithine decarboxylase in mammary gland, together with a lesser decrease in the activity of *S*-adenosylmethionine decarboxylase and a marked fall in milk production. Liver ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities were unaffected. 2. Refeeding for 2.5 h was without effect on ornithine decarboxylase in mammary gland, but it returned the *S*-adenosylmethionine decarboxylase activity in mammary gland to control values and elevated both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in liver. 3. Refeeding for 5 h returned the activity of ornithine decarboxylase in mammary gland to fed-state values and resulted in further increases in *S*-adenosylmethionine decarboxylase in mammary gland and liver and in ornithine decarboxylase in liver. 4. Prolactin deficiency in fed rats resulted in decreased milk production and decreased activity of ornithine decarboxylase in mammary gland. The increase in ornithine decarboxylase activity normally seen after refeeding starved rats for 5 h was completely blocked by prolactin deficiency. 5. In fed rats, injection of streptozotocin 2.5 h before death caused a decrease in the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase in mammary gland, which could be reversed by simultaneous injection of insulin. Insulin deficiency also prevented the increase in *S*-adenosylmethionine decarboxylase in liver and mammary gland normally observed after refeeding starved rats for 2.5 h.

Ornithine decarboxylase (EC 4.1.1.17) and *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), two enzymes required for spermidine synthesis from ornithine, are increased in activity in rat mammary gland during lactation (Russell & McVicker, 1972). In mammary-gland explants obtained from mice in mid-pregnancy, ornithine decarboxylase activity and spermidine synthesis increase as the gland differentiates in response to prolactin, insulin and glucocorticoid (Oka & Perry, 1974*b*). Spermidine synthesis is generally considered to be one component of the pleiotypic or growth response of any cell (Russell *et al.*, 1976), and in early stages of lactation the mammary gland does grow rapidly (Baldwin & Milligan, 1966). When lactation is firmly established, however, there is no further growth of mammary gland (Topper *et al.*, 1975), but this tissue is very active in synthesizing and secreting lactose, protein, lipid and other components of milk. At this stage, the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase are still elevated (Russell & McVicker, 1972). It is possible, therefore, that polyamine synthesis may play a role in the regulation of milk production or of some component thereof.

The metabolism of rat mammary gland, and thus milk production, is very sensitive to alterations in the dietary and hormonal state of the animal (Williamson, 1980). During short-term starvation, for example, the synthesis of lactose (Carrick & Kuhn, 1978) and lipid (Robinson *et al.*, 1978) are decreased.

The aim of the present study was to determine

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whether the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase alter in mammary gland and liver in situations in which milk production is known to be affected; in particular, in response to starvation and refeeding, and to short-term deficiency of insulin or of prolactin.

Materials and methods

Animals

Rats of the Wistar strain with between 8 and 11 pups were used after a lactation period of between 9 and 13 days. Rats were given food and water *ad libitum*, except where described, and were maintained on a constant lighting schedule (lights on 07:30 to 19:30h). Starved rats were deprived of food for 24h; refeeding commenced at 09:00h. Prolactin deficiency was induced by subcutaneous injection of bromocryptine [10mg/kg body wt.; solution in 10% ethanol (w/v) containing 1% tartaric acid] at 09:00h and 17:00h the day before the experiment. Rats refed for 5h received a third injection of bromocryptine at 09:00h on the day of the experiment. Where indicated, ovine prolactin (2mg in 0.2ml of 154mM-NaCl, pH 10) was injected subcutaneously immediately after the injection of bromocryptine. Streptozotocin (50mg/kg body wt.; solution in 0.01M-sodium citrate, pH4.5) was injected into the tail vein under ether anaesthesia 2.5h before death to induce short-term insulin deficiency. Where indicated, insulin (5 units; Isophane insulin; Nordisk Insulin-laboratorium, Copenhagen, Denmark) was injected subcutaneously. Pups were allowed to continue to suckle until the death of the mother so as to maintain the normal stimulus for milk production.

Radioactive compounds

L-[1-^{14}C]Ornithine and *S*-adenosyl-L-[*carboxy*-^{14}C]methionine were obtained from Amersham International Ltd., Amersham, Bucks., U.K.

Biochemicals

All enzymes and coenzymes were obtained from Boehringer Corp. (London) Ltd.

Treatment of tissues

Rats were dissected under pentobarbital anaesthesia (60mg/kg body wt.). One inguinal mammary gland was freed from surrounding tissue and rapidly cut free and clamped between tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). The other inguinal mammary gland was minced and rapidly cooled in homogenizing medium {0.25M-sucrose / 2mM-EDTA / 5mM-dithiothreitol / 2mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH7.4}. Arterial blood was then collected from the abdominal aorta into a heparinized syringe. A sample of the blood was immediately deproteinized as described by Robinson & Williamson (1977) and the neutralized extract used for assay of glucose (Slein, 1963). A sample of liver was also minced and rapidly cooled in homogenizing medium. The freeze–clamped tissue was extracted with 6% (v/v) $HClO_4$ as described by Robinson & Williamson (1977) and the neutralized extract used for assay of lactose (Kurz & Wallenfels, 1974).

A portion of fresh tissue (mammary gland or liver) was weighed and placed in 10ml of ice-cold homogenizing medium per g of tissue. Mammary gland was homogenized in a Silverson-model laboratory mixer/emulsifier for 20–25s.

Liver was homogenized in a smooth-glass Potter–Elvehjem homogenizer by five strokes of a motor-driven loose-fitting Teflon pestle. Homogenates were centrifuged at 27000*g* at 4°C for 35min and the supernatant used for assay of enzymes and protein.

Assay of enzymes

Ornithine decarboxylase activity in cytosol was measured by monitoring the release of $^{14}CO_2$ from L-[1-^{14}C]ornithine (Brosnan *et al.*, 1980). L-Ornithine was present in the incubation mixture at a final concentration of 0.2mM for liver or 0.4mM for mammary gland and at a specific radioactivity of 0.2μCi/μmol.

Putrescine-dependent *S*-adenosylmethionine decarboxylase was assayed by monitoring the release of $^{14}CO_2$ from *S*-adenosyl-L-[*carboxy*-^{14}C]methionine as described previously (Symonds & Brosnan, 1977), except that assays were carried out for 20min at 37°C.

Protein was determined as described by Lowry *et al.* (1951), after solubilization of membranous material with deoxycholate (Jacobs *et al.*, 1956). Bovine serum albumin was used as standard.

Results

Milk production

The weight gain of young rats is dependent on a continued supply of milk from their dam, and changes in pup weight have been used as an estimate of milk production (Cowie & Folley, 1947). Starvation for 24h in the presence of the pups to maintain the suckling stimulus and prolactin secretion resulted in a decline in milk production as indicated by the fall in weight of the pups over this period (Table 1). Milk release did not resume immediately on refeeding the mother, but one half of the litters did show a gain in pup weight by 5h after refeeding the dam. In fed rats, bromocryptine treatment caused a decrease in milk production (about 50%) that was not observed if rats were treated simultaneously with prolactin.

Table 1. *Effect of dietary and hormonal manipulations on weight of rat pups and on lactose content of mammary gland*
Experimental details are described in the text. Lactose is expressed as μmol per g fresh wt. of tissue. The results presented are the means ± S.D. of (*n*) determinations. Litters of pups were weighed before the start of the experimental period and again at the end. For refeeding experiments, pups were weighed before and after food was returned to the mother. Values for lactose content that are significantly different from those for fed rats are shown: $*P < 0.05$; $**P < 0.005$. Values that are significantly different from starved rats are shown: $\dagger P < 0.05$; $\dagger\dagger P < 0.005$.

Condition of dam	Change in weight of pups (%)	Lactose content of mammary gland
Fed (24h)	8.5 ± 2.1 (6)	24.3 ± 8.9 (22)
Fed, bromocryptine-treated (24h)	4.2 ± 2.5 (7)	13.2 ± 4.9 (6)**
Fed, bromocryptine + prolactin-treated (24h)	7.7 ± 1.6 (3)	13.7 ± 0.9 (3)*
Fed, streptozotocin-treated (2.5h)	—	25.8 ± 6.0 (12)
Fed, streptozotocin + insulin-treated (2.5h)	—	6.1 ± 4.3 (5)**
Starved (6h)	2.4 ± 2.5 (3)	8.6 ± 2.2 (3)**
Starved (24h)	−2.8 ± 2.7 (18)	2.9 ± 2.7 (14)**
Starved (24h) + refed (2.5h)	0 (2)	7.0 ± 1.2 (9)††
Starved (24h) + refed (5h)	0.5 ± 1.2 (9)	12.7 ± 3.6 (10)††
Starved (24h), bromocryptine-treated (29h) + refed (5h)	0.1 ± 0.4 (6)	10.5 ± 1.3 (10)††
Starved (24h), streptozotocin-treated (2.5h) + refed (2.5h)	—	6.1 ± 4.3 (5)†

Lactose content of mammary gland

The lactose content of mammary gland has been used as a measure of the amount of milk stored in the gland (Baldwin & Milligan, 1966). Table 1 gives the lactose concentration in mammary gland. On food removal, lactose content fell rapidly, presumably reflecting a rapid decline in its rate of synthesis (Carrik & Kuhn, 1978). When food was given to the mother, the rate of lactose synthesis increased rapidly, as can be seen by the increasing concentration of lactose in the mammary gland at a time when very little milk was being secreted. Less lactose was stored in the mammary gland of bromocryptine-treated rats, but prolactin deficiency did not interfere with the increased lactose accumulation on refeeding for 5h. Streptozotocin did not affect the lactose content of mammary gland of fed rats or of rats refed for 2.5h. This finding is in agreement with the observation of Kyriakou & Kuhn (1973) that short-term insulin deficiency does not affect accumulation of lactose in the gland at parturition. The decrease in lactose content on injection of insulin is presumably due to the hypoglycaemia.

Ornithine decarboxylase activity in mammary gland

Starvation caused a marked decrease in ornithine decarboxylase activity in mammary gland that was evident within 6h of food removal (Table 2). The enzyme activity was barely detectable after 24h of starvation. When rats were refed, no change in activity of ornithine decarboxylase occurred within the first 2.5h, but by 5h, activity had returned to levels found in the fed rat.

Induction of prolactin deficiency with bromocryptine caused a marked suppression of ornithine decarboxylase activity in fed rats and blocked the increase in activity normally evident after 5h refeeding. Treatment of rats simultaneously with prolactin and bromocryptine prevented the fall in ornithine decarboxylase activity.

Induction of short-term insulin deficiency by injection of streptozotocin also caused a suppression of ornithine decarboxylase activity in fed rats to 43% of control, which could be reversed by treating rats with insulin at the time of streptozotocin injection.

S-Adenosylmethionine decarboxylase activity in mammary gland

Table 2 shows that there was a decrease in *S*-adenosylmethionine decarboxylase activity after 24h starvation to about 60% of fed values. Refeeding rats for 2.5h resulted in a return of this enzyme to control levels, whereas a marked increase above control values was evident by 5h refeeding. Bromocryptine did not affect the increase observed on refeeding, but streptozotocin injected immediately before food was given completely blocked the increase usually observed after 2.5h refeeding. Streptozotocin also caused a small but significant decrease in *S*-adenosylmethionine decarboxylase activity in mammary gland of fed rats, which was reversed by insulin treatment.

Ornithine decarboxylase activity in liver

Ornithine decarboxylase activity in liver, shown in Table 3, was low compared with that in mammary gland from fed lactating rats, although it was higher than that in liver of fed virgin rats (39 ± 34 pmol/h per mg protein for five rats; S.D.). It did not alter significantly on starvation. Refeeding starved lac-

Table 2. *Ornithine decarboxylase and S-adenosylmethionine decarboxylase activities in mammary gland of lactating rats*
Experimental details are described in text. Ornithine decarboxylase activity is expressed as pmol of CO_2 formed/h per mg of soluble protein and *S*-adenosylmethionine decarboxylase as pmol of CO_2 formed/20min per mg of soluble protein. The results presented as means ± S.D. for (*n*) determinations. Values that are significantly different from those for fed rats are shown: $*P < 0.01$; $**P < 0.001$.

Condition of dam	Ornithine decarboxylase	*S*-Adenosylmethionine decarboxylase
Fed	376 ± 175 (23)	118 ± 23 (14)
Fed, bromocryptine-treated (24h)	17 ± 11 (6)**	180 ± 66 (6)*
Fed, bromocryptine + prolactin-treated (24h)	196 ± 117 (3)	234 ± 8 (3)
Fed, streptozotocin-treated (2.5h)	135 ± 110 (12)**	97 ± 16 (11)*
Fed, streptozotocin + insulin-treated (2.5h)	252 ± 175 (3)	111 ± 35 (3)
Starved (6h)	83 ± 23 (4)*	107 ± 25 (4)
Starved (24h)	17 ± 27 (15)**	69 ± 34 (7)*
Starved (24h) + refed (2.5h)	20 ± 15 (12)**	132 ± 39 (7)
Starved (24h) + refed (5h)	391 ± 201 (11)	272 ± 69 (11)*
Starved (24h), bromocryptine-treated (29h) + refed (5h)	46 ± 29 (6)**	276 ± 83 (7)**
Starved (24h), streptozotocin-treated (2.5h) + refed (2.5h)	6 ± 3 (5)**	88 ± 9 (5)*

Table 3. *Ornithine decarboxylase and S-adenosylmethionine decarboxylase activities in liver of lactating rats*
Experimental details are described in the text. Ornithine decarboxylase activity is expressed as pmol of CO_2 formed/h per mg of cytosol protein and *S*-adenosylmethionine decarboxylase as pmol of CO_2 formed/20min per mg of cytosol protein. The results presented are means ± S.D. for (*n*) determinations. Values that are significantly different from those for fed rats are shown: $*P < 0.01$; $**P < 0.001$.

Condition of dam	Ornithine decarboxylase	*S*-Adenosylmethionine decarboxylase
Fed	83 ± 52 (15)	188 ± 44 (6)
Starved (6h)	30 ± 17 (3)	171 ± 100 (3)
Starved (24h)	97 ± 86 (10)	210 ± 69 (4)
Starved (24h) + refed (2.5h)	636 ± 467 (8)*	297 ± 16 (3)*
Starved (24h) + refed (5h)	879 ± 170 (3)**	369 ± 34 (3)*
Starved (24h), bromocryptine-treated (29h) + refed (5h)	739 ± 56 (3)**	253 ± 19 (3)*
Starved (24h), streptozotocin-treated (2.5h) + refed (2.5h)	673 ± 427 (8)*	202 ± 47 (5)

tating rats with the normal chow diet for 2.5h resulted in a 7-fold stimulation of ornithine decarboxylase in liver. The increased activity was still evident 5h after refeeding began. Neither bromocryptine nor streptozotocin affected the response of hepatic ornithine decarboxylase to refeeding.

S-Adenosylmethionine decarboxylase activity in liver

S-Adenosylmethionine decarboxylase activity in liver of fed lactating rats was higher than that of mammary gland and was unaffected by starvation for 24h (Table 3). Refeeding caused an increase in enzyme activity that was still evident despite bromocryptine administration, but which was blocked by short-term insulin deficiency.

Discussion

Polyamine synthesis in mammary gland of lactating rats

Ornithine decarboxylase and *S*-adenosylmethionine decarboxylase are the rate-limiting enzmyes for the synthesis *de novo* of polyamines from ornithine and methionine. Elevated activities of these decarboxylases are normally present in rapidly growing cells or tissues, and polyamine synthesis appears to be required for cell proliferation. This is true of mammary gland during the phase of rapid growth and differentiation of the tissue around parturition *in vivo* (Russell & McVicker, 1972) and in mammary glands in culture that have been stimulated to differentiate (Oka & Perry, 1974*b*). Although mammary gland is no longer synthesizing deoxyribonucleic acid at mid-lactation (Russell & McVicker, 1972; Topper *et al.*, 1975), it still has a relatively high activity of ornithine decarboxylase. For example, mammary gland of fed rats had an activity of approx. 400pmol/h per mg of cytosol protein (Table 2), which compares with the activities reported by Raina *et al.* (1976) of less than 50pmol/30min per mg of cytosol protein for liver, lung, pancreas, brain, heart, skeletal muscle, spleen and uterus. It has been

shown by Russell & McVicker (1972) that the concentrations of spermidine and spermine in mammary gland are high (approx. 5 and 0.5 mM respectively) during peak lactation, and only begin to decrease at weaning. It has been suggested that spermidine synthesis might be an important regulatory step in the development of the mammary gland (Oka *et al.*, 1977).

Effects of starvation and refeeding

Starvation is known to halve the blood flow to the mammary gland of rats (Hanwell, 1972) and goats (Linzell, 1967), and to decrease plasma levels of insulin and glucagon, although the plasma concentrations of prolactin and glucose were unchanged (Robinson *et al.*, 1978). This decreased rate of delivery of hormones and nutrients to the mammary gland would be expected to result in decreased stimuli for milk production. This is borne out of the fact that the pups fail to gain weight (Hanwell, 1972; present Table 1), and the synthesis of lipid (Robinson *et al.*, 1978), lactose (Carrick & Kuhn, 1978) and protein (Viña & Williamson, 1981) is decreased. At the same time, the capacity of the mammary gland to synthesize polyamines *de novo* from ornithine, as indicated by the activities of ornithine and *S*-adenosylmethionine decarboxylases, is also decreased. In fact, both lactose synthesis and ornithine decarboxylase activity were significantly decreased by 6h food deprivation. Decreased substrate supply is one factor that has been suggested as a regulator of ornithine decarboxylase (Brosnan *et al.*, 1980), but there was no change in mammary-gland ornithine concentration in these rats (fed 108 ± 37 nmol/g of fresh tissue for six rats; starved 102 ± 28 nmol/per g of fresh tissue for four rats; S.D.).

Ornithine decarboxylase activity in liver of female rats has been reported to be increased during lactation (Lundgren & Oka, 1978). This increased activity is apparently not due to the increased dietary intake observed in these rats (Williamson, 1980), since, in the present study, ornithine decarboxylase activity was unaffected by starvation. This is in contrast with the marked decrease in ornithine decarboxylase activity reported for the liver of male rats which were starved for 48h (Conover *et al.*, 1980).

Milk release did not resume immediately on refeeding the mother, but five out of nine of the litters did show a gain in pup weight by 5h refeeding. Linzell (1967) showed that the flow of blood to the mammary gland and the yield of milk from starved goats did not increase for 2–3h after refeeding, and thereafter increased rapidly to reach control levels by 6 and 10h after refeeding respectively. In the present study, ornithine decarboxylase activity in mammary gland followed a similar pattern, with no detectable change at 2.5h refeeding, but returned to control levels by 5h. In contrast, the activity of *S*-adenosylmethionine decarboxylase in the present study, and the decreased rate of lipogenesis (Robinson *et al.*, 1978) in mammary gland observed in starved rats, were returned to normal by 2.5h refeeding. Table 1 shows that lactose synthesis as indicated by the lactose content, too, had increased by this time, although there was no detectable release of milk to the pups. Plasma insulin levels returned to normal by 2h refeeding, and glucagon concentration overshot the control level (Robinson *et al.*, 1978), but it is not known how quickly blood flow to the mammary gland or protein synthesis in the gland return to normal after a period of starvation. It appears unlikely that the delayed response of mammary-gland ornithine decarboxylase was due to an inability of the enzyme to respond so quickly to refeeding, since hepatic ornithine decarboxylase activity in the same rats was increased 7-fold by 2.5h refeeding, an increase similar to that observed in starved male rats (Conover *et al.*, 1980). In the mammary gland, the earlier rise in *S*-adenosylmethionine decarboxylase activity would allow more rapid removal of putrescine.

Effects of prolactin and insulin

In mammary-gland explants, it has been shown that the enzymes of spermidine synthesis can be influenced by hormones: arginase by prolactin (Oka & Perry, 1974*a*; Rillema *et al.*, 1977), ornithine decarboxylase by prolactin (Oka & Perry, 1976; Rillema *et al.*, 1977) and insulin (Aisbitt & Barry, 1973; Oka & Perry, 1976), *S*-adenosylmethionine decarboxylase by insulin and cortisol (Oka & Perry, 1974*b*), and spermidine synthase by insulin and cortisol (Oka *et al.*, 1977). These increases in enzyme activity ensure a marked increase in spermidine concentration from the low level initially observed in the explants (Oka & Perry, 1974*b*; Rillema *et al.*, 1977) at a time when epithelial-cell multiplication and/or initiation of milk-protein synthesis is occurring. It has been proposed that spermidine could be a regulatory agent for these developmental events in the mammary epithelium (Oka & Perry, 1976). There have been no studies to date, however, on the hormonal regulation of spermidine synthesis in the lactating mammary gland *in vivo*. Once lactation is fully established, epithelial-cell proliferation is no longer required (Topper *et al.*, 1975) and the cells are continuously engaged in synthesizing protein, lactose and lipid for the milk.

Prolactin deficiency can be induced *in vivo* by injection of bromocryptine, which prevents synthesis and secretion of prolactin in the adenohypophysis (Dannies & Rudnick, 1980). In the rat, it has been demonstrated that a continued supply of

prolactin is required for the maintenance of normal lactation (for review, see Williamson, 1980). In the present study, treatment of lactating rats for 24h with bromocryptine did cause a decrease in the lactose content of mammary gland and a decrease in milk production, as assessed by pup weight gain. Together with the decreased milk production, there was a marked fall in the activity of ornithine decarboxylase in the mammary gland, which could be reversed by prolactin administration. Both the return to normal of overall milk production and of ornithine decarboxylase activity after refeeding were also prevented by prolactin deficiency. These results would indicate that prolactin plays an important role in the regulation of ornithine decarboxylase activity in mammary gland of lactating rats *in vivo*, as it does during development of mammary-gland explants *in vitro*.

Insulin also appears to have some influence on ornithine decarboxylase activity in lactating mammary gland, although its major influence appeared to be on *S*-adenosylmethionine decarboxylase activity. The postulated increased delivery of these two hormones to the mammary gland on refeeding would thus increase the synthesis of spermidine from ornithine and methionine as the production of milk was re-established. Thus the present data would be in agreement with a continuing active role for spermidine in the synthesis of milk components throughout lactation.

In conclusion, these experiments suggest that the activity of ornithine decarboxylase in mammary gland correlates with the alterations in milk production, but not necessarily with the synthesis of all the major components of milk. Prolactin appears to play a major role in the maintenance of activity of this enzyme in mammary gland, but not in liver *S*-Adenosylmethionine decarboxylase activity in both liver and mammary gland appears to be responsive to insulin. The finding that the hepatic activity of ornithine decarboxylase responds more rapidly to refeeding than does that of the mammary gland, and that the response is unaffected by administration of either bromocryptine or streptozotocin, raises the interesting question as to the signals that regulate the activity and the role of ornithine decarboxylase in liver of lactating rats *in vivo*.

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References

Aisbitt, R. P. G. & Barry, J. M. (1973) *Biochim. Biophys. Acta* **320**, 610–616

Baldwin, R. L. & Milligan, L. P. (1966) *J. Biol. Chem.* **241**, 2058–2066

Brosnan, M. E., Roebothan, B. V. & Hall, D. E. (1980) *Biochem. J.* **190**, 395–408

Carrick, D. J. & Kuhn, N. J. (1978) *Biochem. J.* **174**, 319–325

Conover, C. A., Rozovski, S. J., Belur, E. R., Aoki, T. T. & Ruderman, N. B. (1980) *Biochem. J.* **192**, 725–732

Cowie, A. T. & Folley, S. J. (1947) *J. Endocrinol.* **5**, 9–13

Dannies, P. S. & Rudnick, M. S. (1980) *J. Biol. Chem.* **255**, 2776–2781

Hanwell, A. (1972) D.Phil. Thesis, University of Cambridge

Jacobs, E. E., Jacob, M., Sanadi, D. R. & Bradley, L. B. (1956) *J. Biol. Chem.* **223**, 147–156

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

Kyriakou, S. Y. & Kuhn, N. J. (1973) *J. Endocrinol.* **59**, 199–200

Linzell, J. L. (1967) *J. Physiol. (London)* **190**, 333–346

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Lundgren, D. W. & Oka, T. (1978) *Am. J. Physiol.* **234**, E451–E456

Oka, T. & Perry, J. W. (1974*a*) *Nature (London)* **250**, 660–661

Oka, T. & Perry, J. W. (1974*b*) *J. Biol. Chem.* **249**, 7647–7652

Oka, T. & Perry, J. W. (1976) *J. Biol. Chem.* **251**, 1738–1744

Oka, T., Perry, J. W. & Kano, K. (1977) *Biochem. Biophys. Res. Commun.* **79**, 979–986

Raina, A., Pajula, R.-L. & Eloranta, T. (1976) *FEBS Lett.* **67**, 252–255

Rillema, J. A., Linebaugh, B. E. & Mulder, J. A. (1977) *Endocrinology* **100**, 529–536

Robinson, A. M. & Williamson, D. H. (1977) *Biochem. J.* **164**, 153–159

Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346

Russell, D. H. & McVicker, T. A. (1972) *Biochem. J.* **130**, 71–76

Russell, D. H., Byus, C. V. & Manen, C. A. (1976) *Life Sci.* **19**, 1297–1305

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

Symonds, G. W. & Brosnan, M. E. (1977) *FEBS Lett.* **84**, 385–387

Topper, Y. J., Oka, T. & Vonderhaar, B. K. (1975) *Methods Enzymol.* **39**, 443–454

Viña, J. R. & Williamson, D. H. (1981) *Biochem. J.* **194**, 941–947

Williamson, D. H. (1980) *FEBS Lett.* **117**, Suppl. K93–K105

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