Biochem. J. (1981) **196**, 757–762
Printed in Great Britain

Utilization of L-alanine and L-glutamine by lactating mammary gland of the rat

A role for L-alanine as a lipogenic precursor

Juan R. VIÑA and Dermot H. WILLIAMSON
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

(*Received 9 December 1980/Accepted 9 February 1981*)

1. Lactation is associated with an increase in the arterial blood concentration of L-alanine and L-glutamate, but a decrease in that of L-glutamine compared with the corresponding values for virgin rats. 2. Virgin rats fed a 'cafeteria diet' that induces hyperphagia have increased arterial concentrations of L-alanine, L-glutamate and L-glutamine. During lactation L-alanine and L-glutamate concentrations are even higher. 3. The removal of L-alanine is decreased in hepatocytes from lactating rats fed either a chow or cafeteria diet. 4. Measurements of arteriovenous differences across lactating mammary glands indicate that appreciable amounts of L-glutamine and L-alanine are extracted by the gland. 5. A high proportion of the L-alanine metabolized by isolated acini from fed lactating rats is converted into lipid. 6. Metabolism of L-alanine in acini from starved lactating rats is limited by the activity of pyruvate dehydrogenase. 7. It is concluded that L-alanine and certain other amino acids taken up by the gland in excess of the requirements for protein synthesis can be converted into lipid.

It is now well established that L-alanine and L-glutamine have an important function in the transport of carbon and nitrogen between tissues. L-Alanine is released into the circulation by a number of tissues, but skeletal muscle is the most significant contributor (for review see Snell, 1980), and it is removed mainly by the liver for conversion into glucose (Mallette *et al.*, 1969; Felig *et al.*, 1970; MacDonald *et al.*, 1976). L-Glutamine is also released from muscle and is taken up by the small intestine, where it is both an important respiratory fuel (Windmueller & Spaeth, 1978; Watford *et al.*, 1979) and also contributes carbon and nitrogen for L-alanine formation and subsequent release (Windmueller & Spaeth, 1975, 1977; Hanson & Parsons, 1977; Rémésy *et al.*, 1978). In addition, L-glutamine is taken up by the kidney in acidosis to provide ammonia for excretion (for review see Lund, 1980).

During lactation the mammary gland of the rat becomes an additional site of net protein synthesis and it would therefore be expected to have increased requirements of amino acids for the formation of milk proteins. A recent study has shown that appreciable amounts of plasma amino acids are extracted by the gland during lactation (Viña *et al.*, 1980). Work from this laboratory has demonstrated that L-leucine is taken up by the gland *in vivo* and that part of the carbon is used for lipogenesis (Viña & Williamson, 1981).

The present paper is concerned with the effects of lactation on the blood concentrations of L-alanine, L-glutamate and L-glutamine, and on the relative uptake of these amino acids by the gland *in vivo*. In addition, a quantitative assessment of the metabolism of L-alanine in isolated acini from mammary glands of fed and starved lactating rats is reported. A novel finding is that a considerable proportion of the carbon of L-alanine utilized by the acini from fed lactating rats *in vitro* is converted into lipid; this observation extends qualitative measurements with lactating-mammary-gland slices (Abraham *et al.*, 1964).

Experimental

Materials

(*a*) *Rats.* Lactating rats of the Wistar strain (250–325 g) with between nine and ten pups were used at peak lactation (10–15 days); virgin rats weighed 190–210 g. Rats were anaesthetized with nembutal (50 mg/kg body wt.) and the pups were allowed to continue to suckle to maintain prolactin secretion (Amenomori *et al.*, 1970) before removal of the inguinal mammary glands. The rats were fed

0306-3283/81/060757-06$01.50/1

ad libitum on Oxoid breeding diet (Oxoid, London, U.K.). One group of lactating rats was fed from parturition with a 'cafeteria diet' in which the animals were offered a variety of palatable high-energy foods in addition to their normal chow diet (Rolls *et al.*, 1980). One group of virgin rats was fed with the cafeteria diet for a period of 12–14 days. This type of diet induces hyperphagia (Sclafani & Springer, 1976; Rolls *et al.*, 1980). Starved lactating rats (with pups) were deprived of food for 24h. Some lactating rats had their pups removed for 24h and are referred to in the text as 'weaned' rats.

(*b*) *Biochemicals.* All enzymes, including collagenase (grade II) and coenzymes were obtained from Boehringer Corp. (London), Lewes, East Sussex, U.K. Purified insulin was a gift from Dr. N. Lazarus, Wellcome Research Laboratories, Beckenham, Kent, U.K.

(*c*) *Radioactive compounds.* L-[1-^{14}C]Alanine and L-[U-^{14}C]alanine were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Studies in vivo. For the measurements of arteriovenous differences across the gland, venous blood was collected from both pudic-epigastric veins into a heparinized syringe. Other blood samples were collected from the portal vein and from the abdominal aorta. The blood (0.5ml) was added to 2ml of 6% (w/v) $HClO_4$ and the mixture was 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 for 10min). This final supernatant was decanted off and used for the determination of amino acids by enzymic methods. In the case of the venous blood the results for the two samples on each rat were combined to produce a mean value.

Studies in vitro. Preparation of acini. Acini were prepared by a modification of the procedure of Katz *et al.* (1974). For full details see Robinson & Williamson (1977) and Williamson & Robinson (1979).

Preparation of liver cells. Isolated hepatocytes were prepared from fed rats essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974).

Incubation procedure. The acini (about 100mg wet wt.) were incubated in a total volume of 4ml of Krebs–Henseleit (1932) saline containing glucose (5mM) and other additions where indicated. The following concentrations of other compounds were used where stated: L-alanine (1mM), dichloroacetate (0.5mM) and insulin (30m-units/ml). The isolated hepatocytes (about 80mg wet wt./4ml) were incubated in Krebs–Henseleit (1932) saline with L-alanine (5mM).

Determination of lipid. Lipid was extracted from the acini at the end of the experiment as described by Robinson & Williamson (1977*a*). In some experiments the lipid was saponified and the fatty acids were extracted as described by Stansbie *et al.* (1976).

Determination of metabolites. These were by enzymic methods: D-glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); L-alanine (Williamson *et al.*, 1967); L-glutamate and L-glutamine (Lund, 1974).

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

Expression of results. Rates of alanine utilization and glucose, lactate and pyruvate production by isolated hepatocytes were obtained from graphical plots of 20, 40 and 60min values. The rates of $^{14}CO_2$ formed from [1-^{14}C]alanine, ^{14}C-labelled lipid from [U-^{14}C]alanine, glucose removal and pyruvate and lactate production by the acini were obtained from the 30min and 60min values.

Table 1. *Arterial whole-blood concentrations of glutamate, glutamine and alanine in virgin and lactating rats*
For details see the Experimental section. The results are means ± S.D. with the numbers of observations in parentheses. Results that are significantly different from those for fed virgin rats are shown: $*P < 0.05$; $**P < 0.005$.

State of rats	[L-Glutamate] (mM)	[L-Glutamine] (mM)	[L-Alanine] (mM)
Virgin, fed	0.152 ± 0.019 (4)	0.522 ± 0.152 (4)	0.404 ± 0.054 (7)
Lactating, fed	0.202 ± 0.038* (11)	0.356 ± 0.097* (11)	0.599 ± 0.141** (16)
Lactating, starved (24h)	0.213 ± 0.052* (4)	0.285 ± 0.052* (4)	0.370 ± 0.114 (6)
Lactating, 'weaned'	0.187 ± 0.016* (3)	0.526 ± 0.064 (3)	0.574 ± 0.098** (4)
Virgin, cafeteria diet	0.237 ± 0.031** (5)	0.683 ± 0.048* (5)	0.592 ± 0.060** (5)
Lactating, cafeteria diet	0.516 ± 0.092** (15)	0.600 ± 0.160 (15)	0.996 ± 0.221** (15)

Results and discussion

Amino acid concentrations in arterial blood

At peak lactation the dietary intake of the rat is increased about 3-fold (Fell *et al.*, 1963). This hyperphagia was associated with an increase in the concentration of L-alanine and L-glutamate and a decrease in the concentration of L-glutamine in arterial blood of lactating rats compared with virgin rats (Table 1). Inhibition of lactation by removal of the pups (24h) did not affect the concentration of L-alanine and L-glutamate compared with normal lactating rats, but L-glutamine values returned to the concentration found in virgin rats fed a chow diet. Starvation (24h with pups) of lactating rats produced a significant decrease in the concentration of L-alanine and L-glutamine compared with corresponding values for fed lactating rats (Table 1). Virgin rats fed a cafeteria diet showed a significant increase in the concentration of L-alanine, L-glutamate and L-glutamine in arterial blood compared with virgin rats fed a chow diet (Table 1). In lactating rats on the cafeteria diet the concentration of L-alanine and L-glutamate was further increased, whereas the concentration of L-glutamine did not show a significant change compared with virgin rats fed the chow or cafeteria diets (Table 1).

Comparison of the concentrations of L-alanine, L-glutamate and L-glutamine in portal vein and in arterial blood of lactating rats fed a chow or a cafeteria diet (Table 2) showed that the arterial L-alanine concentration was significantly lower ($P < 0.05$) than that in the portal vein, whereas the arterial L-glutamine tended to be higher in lactating rats fed the chow diet and was significantly higher ($P < 0.05$) in cafeteria-fed lactating rats. There was no difference in the L-glutamate concentration between portal vein and arterial blood (Table 2).

Alanine removal by hepatocytes

The increase in alanine concentration in the arterial blood of virgin rats fed the cafeteria diet and of lactating rats (Table 1) might be due to the hyperphagia and/or decreased removal of alanine by the liver. To test this latter possibility we have measured the rate of alanine removal by hepatocytes from virgin and lactating rats fed the chow or cafeteria diet (Table 3). The hepatocytes from lactating rats fed with the chow or cafeteria diet removed significantly less ($P < 0.05$) L-alanine than those from virgin rats fed the chow diet (Table 3). This lower L-alanine uptake was accompanied by decreased output of glucose, L-lactate and pyruvate. Lactation is characterized by hypertrophy of liver (Chatwin *et al.*, 1969) and therefore the apparent decreased removal of alanine *in vitro* may not necessarily indicate any change in removal on a whole-liver basis. However, virgin rats fed the cafeteria diet have an increase in dietary intake but no change in liver weight (L. Agius & D. H. Williamson, unpublished work) and in this situation the hepatocytes removed less L-alanine compared with hepatocytes from virgin rats (Table 3). The glucose, L-lactate and pyruvate output was also lower, but only in the case of lactate was the decrease significant. In the three situations where L-alanine removal by isolated hepatocytes was lower compared with the values for virgin rats fed the chow diet the food intake is known to be increased. Taken together these two facts may explain the relative hyperalaninaemia that occurs in virgin rats fed the cafeteria diet and in lactating rats (Table 1). The decrease in alanine removal was associated in all situations studied with a lower activity of hepatic alanine aminotransferase (results not shown).

Uptake by the mammary gland

Arteriovenous differences for L-glutamate, L-glutamine and L-alanine were measured across the mammary gland of lactating rats fed on the chow or cafeteria diet (Table 2). On both diets the mammary gland extracted appreciable amounts of L-alanine and L-glutamine. However, the amounts removed by the glands of rats fed the cafeteria diet were significantly higher ($P < 0.05$) than those fed the chow diet. The amount of L-glutamate extracted by

Table 2. *Arteriovenous differences for glutamate, glutamine and alanine across inguinal mammary gland of lactating rats fed the chow or cafeteria diet and portal vein concentrations*

The results are means ± S.D. expressed as μmol/ml of whole blood. The numbers of rats are shown in parentheses. Venous blood samples were taken from the pudic-epigastric vein.

State of rat ...	Lactating, fed chow diet			Lactating, cafeteria diet		
	L-Glutamate (5)	L-Glutamine (5)	L-Alanine (5)	L-Glutamate (7)	L-Glutamine (7)	L-Alanine (7)
Portal vein	0.213 ± 0.057	0.313 ± 0.083	0.801 ± 0.185	0.522 ± 0.042	0.507 ± 0.160	0.988 ± 0.119
Arterial	0.204 ± 0.062	0.358 ± 0.091	0.620 ± 0.197	0.519 ± 0.036	0.662 ± 0.163	0.880 ± 0.137
Venous	0.186 ± 0.054	0.218 ± 0.094	0.516 ± 0.180	0.489 ± 0.051	0.493 ± 0.173	0.667 ± 0.153
Arteriovenous differences	0.019 ± 0.008	0.140 ± 0.014	0.103 ± 0.022	0.051 ± 0.038	0.169 ± 0.029	0.212 ± 0.118

Table 3. *Alanine uptake and glucose, lactate and pyruvate output by hepatocytes from fed lactating and virgin rats*
For details see the Experimental section. The results are means ± S.D. expressed as μmol/min per g wet wt. with the numbers of observations in parentheses. Results that are significantly different by Student's *t* test from those for fed (chow) virgin rats are shown: *$P < 0.05$; **$P < 0.005$.

State of rats		Alanine removal	Glucose production	Lactate production	Pyruvate production
Virgin, chow fed	(5)	1.07 ± 0.32	0.96 ± 0.16	0.67 ± 0.21	0.16 ± 0.04
Lactating, chow fed	(5)	0.74 ± 0.23*	0.44 ± 0.14**	0.44 ± 0.11*	0.10 ± 0.01*
Virgin, cafeteria-fed	(6)	0.77 ± 0.20*	0.82 ± 0.21	0.43 ± 0.13*	0.14 ± 0.03
Lactating, cafeteria-fed	(4)	0.63 ± 0.14*	0.43 ± 0.05**	0.43 ± 0.04*	0.09 ± 0.02**

Table 4. *Metabolism of* L-[*1*-^{14}C]*alanine and* L-[*U*-^{14}C]*alanine in isolated acini from mammary glands of fed and starved lactating rats*
For details see the Experimental section. The final concentrations in the incubation were: L-alanine, 1 mM; glucose, 5 mM; insulin, 30 m-units/ml; dichloroacetate, 0.5 mM. The results are means ± S.D. expressed as μmol/min per 100 mg defatted dry wt. with the numbers of observations in parentheses. Values that are significantly different from those for L-[1-^{14}C]alanine + glucose are shown: *$P < 0.05$; **$P < 0.005$.

	[1-^{14}C]- or [U-^{14}C]-Alanine incorporated into:		Glucose removed	Lactate formed	Pyruvate formed
	CO_2	Lipids			
Acini from fed rats					
L-[1-^{14}C]Alanine + glucose	0.11 ± 0.02 (6)	—	0.98 ± 0.14 (5)	0.23 ± 0.04 (6)	0.07 ± 0.01 (6)
L-[1-^{14}C]Alanine + glucose + insulin	0.12 ± 0.02 (5)	—	0.98 ± 0.13 (5)	0.25 ± 0.04 (5)	0.08 ± 0.02 (5)
L-[U-^{14}C]Alanine + glucose	0.04 ± 0.01 (4)	0.09 ± 0.01 (4)	1.07 ± 0.15 (4)	0.29 ± 0.03 (4)	0.08 ± 0.02 (4)
L-[U-^{14}C]Alanine + glucose + insulin	0.05 ± 0.01 (4)	0.10 ± 0.03 (4)	1.22 ± 0.18 (4)	0.28 ± 0.04 (4)	0.07 ± 0.02 (4)
Acini from starved rats					
L-[1-^{14}C]Alanine + glucose	0.024 ± 0.005 (5)	—	0.91 ± 0.17 (5)	0.82 ± 0.08 (5)	0.19 ± 0.03 (5)
L-[1-^{14}C]Alanine + glucose + diehloroacetate	0.070 ± 0.020 (5)**	—	0.71 ± 0.08 (4)*	0.30 ± 0.08 (5)**	0.045 ± 0.011 (5)**
L-[U-^{14}C]Alanine + glucose + dichloroacetate	0.022 ± 0.003 (5)	0.051 ± 0.009 (5)	0.69 ± 0.10 (4)*	0.40 ± 0.03 (4)**	0.061 ± 0.009 (4)**

the mammary gland is relatively small compared with the values for alanine and glutamine. The results of the measurements of arteriovenous differences reported here are in qualitative agreement with similar data on plasma of lactating (day 16) rats fed a chow diet (Viña *et al.*, 1980).

Metabolism of L-*alanine in isolated acini*

The first irreversible step in L-alanine catabolism is the reaction catalysed by pyruvate dehydrogenase (EC 1.2.4.1), which results in the formation of $^{14}CO_2$ from the $C_{(1)}$ position of alanine. Measurement of the rate of $^{14}CO_2$ produced from L-[1-^{14}C]alanine therefore indicates the flux of the amino acid into the major catabolic pathway (Table 4). In the absence of glucose in the medium the rate of $^{14}CO_2$ production from L-[1-^{14}C]alanine was 35% lower than when glucose is present (results not shown). This finding may be explained by a deficiency of oxo acids (mainly oxoglutarate) for transamination when exogenous precursors are omitted from the medium. The rate of production of $^{14}CO_2$ (in the presence of glucose) from L-[1-^{14}C]alanine was five times greater in isolated acini from glands of fed (chow diet) than starved (24 h with pups) lactating rats (Table 4). This finding is in agreement with the decrease in the proportion of pyruvate dehydrogenase in the active form in mammary glands of starved lactating rats (Kankel & Reinauer, 1976; Baxter & Coore, 1978). Further evidence that it is the activity of pyruvate dehydrogenase that limits the catabolism of L-alanine

by acini from starved lactating rats was the increase (about 300%) in the rate of $^{14}CO_2$ production when dichloroacetate (an activator of pyruvate dehydrogenase; Whitehouse & Randle, 1973; Whitehouse *et al.*, 1974) was present in the medium (Table 4). This inhibition of alanine utilization is in direct contrast with the finding that catabolism of L-leucine is not altered in acini from starved lactating rats (Viña & Williamson, 1981).

It has been reported that L- or DL-[2-^{14}C]alanine can be converted into lipid in mouse adipose tissue (Feller & Feist, 1962) and in slices of lactating rat mammary gland (Abraham *et al.*, 1964) that contain adipocytes. To study the fate of the alanine carbon in isolated acini we have measured the production of $^{14}CO_2$ from L-[U-^{14}C]alanine and the incorporation of radioactivity into lipid (Tables 4). In acini from fed lactating rats the rate of incorporation of L-[U-^{14}C]alanine into lipid was similar to the rate of production of $^{14}CO_2$ from L-[1-^{14}C]alanine; insulin had no significant effect on either measurement (Table 4). As expected, because of the low rate of L-[1-^{14}C]alanine oxidation, the rate of incorporation of L-[U-^{14}C]alanine into lipid was extremely low in acini from starved lactating rats (results not shown). Addition of dichloroacetate increased the rate of incorporation of L-[U-^{14}C]alanine into lipid to a value that was 50% lower than that of acini from fed lactating rats (Table 4).

The incorporation of L-[U-^{14}C]alanine into lipid need not necessarily involve net utilization of the amino acid because exchange of radioactivity with intracellular pyruvate can occur via the alanine aminotransferase reaction. Measurements of L-alanine removal with an enzymic assay (Williamson *et al.*, 1967) gave a value of 0.085 ± 0.03 (3) μmol/min per 100mg defatted dry wt. of acini or about 80% of the L-[1-^{14}C]alanine decarboxylated. This rate of net L-alanine utilization may be an underestimate because the amino acid is formed by the acini when glucose is the sole substrate (0.035; 0.042μmol/min per 100mg defatted dry wt.). At least 85% of the radioactivity from L-[U-^{14}C]alanine incorporated into lipid was found in the fatty acid moiety (mean for three experiments; results not shown).

Comparison with other lipid precursors

There is now a considerable amount of information on the rates of lipid synthesis from various precursors in isolated acini (Table 5). In terms of C_2 units glucose is the most active; the rate with L-leucine is about 25% of that with glucose, whereas that with L-alanine is only about 7%.

Physiological implications

These experiments indicate that at peak lactation the mammary gland of the rat removes considerable amounts of L-alanine and L-glutamine from the circulation. By using the available data on mammary gland blood flow and gland weight (Chatwin *et al.*, 1969), and the arteriovenous differences given in Table 3 it can be calculated that the lactating gland of the rat fed the chow diet utilizes 2.4mmol of L-alanine/24h and 3.0mmol of L-glutamine/24h. Presumably the increased demand for amino acids during lactation is mainly satisfied by the higher food intake. A major fate of the amino acids taken up by the gland is synthesis of milk proteins. However, the present experiments show that at least *in vitro* L-alanine can act as a precursor of lipid. This evidence, taken together with previous studies on mammary-gland slices (Abraham *et al.*, 1964) and the results of similar experiments on the conversion of L-leucine into lipid in the gland *in vivo* and in isolated acini (Viña & Williamson, 1981), indicate that certain amino acids can act as lipogenic precursors in lactating mammary gland. This means that amino acids taken up by the gland in excess of the requirements for protein synthesis could be channelled into lipid synthesis. This would be a physiological advantage, particularly if the lactating rat was fed a high protein diet; the necessity to first convert the gluconeogenic amino acids into glucose in the liver would be less if amino acids could directly form lipid within the gland. A key question

Table 5. *Comparison of the rates of lipogenesis from various substrates in isolated acini*

The acini were isolated from fed lactating rats of the Wistar strain. For other experimental details see various references. Glucose (5mM) was present in all experiments; the incubations with [3-^{14}C]acetoacetate also contained insulin (30 m-units/ml).

Substrate added	Concn. (mM)	C_2 units converted to lipid (μmol/min per 100mg defatted dry wt.)	References
D-[6-^{14}C]Glucose	5	1.48	Robinson & Williamson (1977*b*)
L-[U-^{14}C]Lactate	2	0.86	Katz *et al.* (1974)
[3-^{14}C]Acetoacetate	2	0.58	Robinson & Williamson (1977*b*)
L-[U-^{14}C]Leucine	1	0.36	Viña & Williamson (1981)
L-[U-^{14}C]Alanine	1	0.10	The present paper

is the fate of the amino groups released from the amino acids that form lipids, because as demonstrated here they are not released as alanine or glutamine.

We thank Dr. Patricia Lund for helpful discussions and Mrs. Vera Ilic for skilled assistance. This work was supported by the U.S. Public Health Service (Grant no. AM-11748) and by the Medical Research Council (U.K.). D. H. W. is a member of the External Staff of the Medical Research Council, U.K.

References

Abraham, S., Madsen, J. & Chaikoff, I. L. (1964) *J. Biol. Chem.* **239**, 855–864

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

Baxter, M. A. & Coore, H. G. (1978) *Biochem. J.* **174**, 553–561.

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Chatwin, A. L., Linzell, J. L. & Setchell, B. P. (1969) *J. Endocrinol.* **44**, 247–254

Felig, P., Pozefsky, T., Marliss, E. & Cahill, G. F. (1970) *Science* **167**, 1003–1004

Fell, B. F., Smith, K. A. & Campbell, R. M. (1963) *J. Pathol. Bacteriol.* **85**, 179–188

Feller, D. D. & Feist, E. (1962) *Metabolism* **11**, 448–455

Hanson, P. J. & Parsons, D. H. (1977) *Biochem. J.* **166**, 509–519

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

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

Katz, J., Walls, 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

Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) *Alfred Benzon Symp.* **6**, 718–743

Lund, P. (1974) in *Methoden der enzymatischen analyse* (Bergmeyer, H. U., ed.), 2nd edn., pp. 1670–1673, Verlag Chemie, Weinheim

Lund, P. (1980) *FEBS Lett.* **117**, K86–K92

MacDonald, M., Neufeldt, N., Park, B. N., Berger, M. & Ruderman, N. (1976) *Am. J. Physiol.* **231**, 619–625

Mallette, L. E., Exton, J. H. & Park, C. R. (1969) *J. Biol.. Chem.* **244**, 5713–5723

Rémésy, C., Demigné, C. & Aufrère, J. (1978) *Biochem. J.* **170**, 321–329

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

Robinson, A. M. & Williamson, D. H. (1977*b*) *Biochem. J.* **168**, 465–474

Rolls, B. J., Rowe, E. A. & Turner, R. C. (1980) *J. Physiol. (London)* **298**, 415–427

Sclafani, A. & Springer, D. (1976) *Physiol. Behav.* **17**, 461–471

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

Snell, K. (1980) *Biochem. Soc. Trans.* **8**, 205–213

Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416

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

Vinã, J., Puertes, I. R., Estrella, J. M., Viña, J. R. & Galbis, J. L. (1981) *Biochem. J.* **194**, 99–102

Watford, M., Lund, P. & Krebs, H. A. (1979) *Biochem. J.* **178**, 589–596

Whitehouse, S. & Randle, P. J. (1973) *Biochem. J.* **134**, 651–653

Whitehouse, S., Cooper, R. H. & Randle, P. J. (1974) *Biochem. J.* **141**, 761–774

Williamson, D. H. & Robinson, A. M. (1979) in *Cell Populations, Methodological Surveys (B) Biochemistry* (Reid, E., ed.), vol. 9, pp. 223–224, Ellis Horwood, Chichester

Williamson, D. H., Lopes-Vieira, O. & Walker, B. (1967) *Biochem. J.* **104**, 497–502

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

Windmueller, H. G. & Spaeth, A. E. (1975) *Arch. Biochem. Biophys.* **171**, 662–672

Windmueller, H. G. & Spaeth, A. E. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 177–181

Windmueller, H. G. & Spaeth, A. E. (1978) *J. Biol. Chem.* **253**, 69–76