Biochem. J. (1977) **164**, 349–355
Printed in Great Britain

The Activities of 2-Oxoglutarate Dehydrogenase and Pyruvate Dehydrogenase in Hearts and Mammary Glands from Ruminants and Non-Ruminants

By GRAHAM READ,* BERNARD CRABTREE and GRAHAM H. SMITH
Department of Animal Physiology and Nutrition, University of Leeds, Vicarage Terrace, Leeds LS5 3HL, U.K.

(*Received* 16 *November* 1976)

1. The activities of 2-oxoglutarate dehydrogenase (EC 1.2.4.2) were measured in hearts and mammary glands of rats, mice, rabbits, guinea pigs, cows, sheep, goats and in the flight muscles of several Hymenoptera. 2. The activity of 2-oxoglutarate dehydrogenase was similar to the maximum flux through the tricarboxylic acid cycle *in vivo*. Therefore measuring the activity of this enzyme may provide a simple method for estimating the maximum flux through the cycle for comparative investigations. 3. The activities of pyruvate dehydrogenase (EC 1.2.4.1) in mammalian hearts were similar to those of 2-oxoglutarate dehydrogenase, suggesting that in these tissues the tricarboxylic acid cycle can be supplied (under some conditions) by acetyl-CoA derived from pyruvate alone. 4. In the lactating mammary glands of the rat and mouse, the activities of pyruvate dehydrogenase exceeded those of 2-oxoglutarate dehydrogenase, reflecting a flux of pyruvate to acetyl-CoA for fatty acid synthesis in addition to that of oxidation via the tricarboxylic acid cycle. In ruminant mammary glands the activities of pyruvate dehydrogenase were similar to those of 2-oxoglutarate dehydrogenase, reflecting the absence of a significant flux of pyruvate to fatty acids in these tissues.

Although the tricarboxylic acid cycle is the major pathway for regenerating ATP under aerobic conditions in many tissues, it is difficult to determine its rate of operation *in vivo*. The indirect methods used hitherto are either very time-consuming or prone to serious errors. For example, Neely *et al.* (1972) have estimated the flux through the tricarboxylic acid cycle in the perfused rat heart indirectly from measurements of the uptake of glucose, O_2, the rate of glycolysis and changes in the content of endogenous fuels. Although this technique probably gives an accurate assessment of the rate of the cycle, it involves a considerable amount of work and can only be applied to isolated tissue preparations: it is not suitable for comparative investigations involving many different tissues. The flux through the cycle in mammary glands has been estimated from the rate of production of $^{14}CO_2$ from [6-^{14}C]glucose, but this method overestimates the flux if glucose carbon is cycled between hexose phosphate and triose phosphate (see Smith, 1971).

The maximum flux through several ATP-regenerating pathways in muscle has been estimated indirectly by measuring the activities of certain enzymes that catalyse irreversible (non-equilibrium) reactions *in vivo* (Crabtree & Newsholme, 1972*a*,*b*, 1975). This approach is most suitable for comparative investigations, since the enzyme activities can be measured relatively quickly, enabling the investigation of many tissues. However, not all enzymes catalysing irreversible reactions have maximum activities similar to the maximum flux through the pathways to which they belong (Crabtree & Newsholme, 1975), and this applies to previously reported activities of enzymes of the tricarboxylic acid cycle. Thus Alp *et al.* (1976) have shown that the activity of citrate synthase (EC 4.1.3.7) in muscle is usually severalfold greater than the maximum flux through the cycle *in vivo*, so that this enzyme cannot be used as an index of the maximum tricarboxylic acid-cycle flux *in vivo*. Further, although the activity of NAD-linked isocitrate dehydrogenase (EC 1.1.1.41) was only 2–3 times as great as the maximum flux in several insect flight muscles, in vertebrate muscles the high activity of the NADP-linked enzyme (EC 1.1.1.42), relative to the flux *in vivo*, and the possible reversibility of the isocitrate dehydrogenase reaction *in vivo* suggest that the reaction catalysed by NAD-linked isocitrate dehydrogenase is reversible in these latter muscles (see also Crabtree & Newsholme, 1975). If this is the case, the maximum activities of both the NAD- and the NADP-linked isocitrate dehydrogenases will be much greater than

* Present address: British-American Tobacco Ltd., Regents Park Road, Shirley, Southampton SO9 1PE, U.K.

the flux through the reactions *in vivo*, and neither can be used to estimate the maximum tricarboxylic acid-cycle flux. The activities of another enzyme of the tricarboxylic acid cycle, succinate dehydrogenase (EC 1.3.99.1), in several muscles were reported by Crabtree & Newsholme (1975): a comparison of these activities with the maximum fluxes *in vivo* given by Alp *et al.* (1976) shows that in several muscles (notably vertebrate heart) the activity of this enzyme is much greater than the maximum flux through the tricarboxylic acid cycle *in vivo*. Consequently, the activity of this enzyme is not a satisfactory index of the maximum rate of the cycle *in vivo*.

Since the reaction catalysed by 2-oxoglutarate dehydrogenase (EC 1.2.4.2) is strongly exergonic and is therefore likely to be irreversible *in vivo*, its maximum activity could be similar to the maximum tricarboxylic acid-cycle flux. The results in the present paper indicate that this is the case, and that the activity of this enzyme can be used to estimate the maximum flux through this pathway in vertebrate heart and mammary gland and in some insect flight muscles.

Materials and Methods

Chemicals and enzymes

All chemicals and coupling enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K., except for the following: EGTA and mercaptoethanol were obtained from Koch–Light Laboratories, Colnbrook, Bucks., U.K.; dichloroacetic acid, EDTA and all inorganic reagents were obtained from BDH, Poole, Dorset, U.K.; *p*-(*p*-aminophenylazo)benzenesulphonic acid was a gift from Dr. R. M. Denton of the Department of Biochemistry, University of Bristol; arylamine acetyltransferase was prepared from pigeon livers by the method of Tabor *et al.* (1953) as modified by Coore & Field (1974).

Sources of animals

Pregnant non-ruminant animals were obtained through the University of Leeds Medical School and were housed in this Department until required. Ruminant tissue was obtained from the local abattoir, except for goat mammary gland, which was obtained by biopsy from animals housed in this Department. Pigeons were obtained from the University of Leeds Department of Zoology. Insects were caught locally while foraging, and were used on the day of capture or after being kept alive overnight at 5°C.

Preparation of homogenates

Tissues were removed from the animals as soon as possible after death and, except for those obtained from the abattoir, were homogenized within 5min of removal: tissues obtained from the abattoir were transported to the laboratory in ice and were homogenized within 60min of collection. Mammalian hearts (from females) and mammary glands were cut into very small pieces with scissors (selecting those portions of the mammary gland that contained a reasonably high proportion of parenchymal as opposed to connective tissue) and were washed thoroughly with ice-cold 100mM-triethanolamine/KOH buffer, pH7.4, to remove as much blood and/or milk as possible. Insect flight muscles were removed after cooling the insect at 5°C and were homogenized without further treatment.

Samples (10–100mg) of tissue were homogenized manually in ground-glass homogenizers with 10vol. of medium. The homogenizing medium used when assaying 2-oxoglutarate dehydrogenase and phosphofructokinase consisted of 50mM-triethanolamine/KOH, 2mM-$MgCl_2$, 1mM-EDTA, 30mM-mercaptoethanol, 2M-glycerol and 1mM-2-oxoglutarate at pH7.4. The medium used when assaying pyruvate dehydrogenase consisted of 100mM-potassium phosphate, 16mM-$MgCl_2$, 1mM-pyruvate, 1mM-EGTA, 1mM-$CaCl_2$ and 1mM-dichloroacetate at pH7.8. Pyruvate, dichloroacetate and EGTA/Ca^{2+} (which buffers the Ca^{2+} concentration at approx. 10μM) were included to promote the activation of and minimize the inactivation of pyruvate dehydrogenase (see Whitehouse & Randle, 1973; Randle *et al.*, 1974; Whitehouse *et al.*, 1974). Preliminary experiments showed that incubating homogenates of rat heart and lactating mammary gland for up to 60min at 30°C did not increase the activity of pyruvate dehydrogenase.

Homogenates were sonicated for approx. 15s in a MSE 100W sonicator at maximum power, and those of insect flight muscle and mammalian heart muscle were assayed for activity within 5min of sonication: homogenates of mammary gland were centrifuged for approx. 1min at low speed (approx. 600*g*) to remove connective tissue and lessen turbidity in the cuvette. These treatments either had no effect or slightly increased the activities of the dehydrogenases in homogenates of bumble-bee flight muscle, rat heart and lactating mammary gland. Other methods for releasing latent mitochondrial enzymes, such as freezing and thawing the homogenate, were not investigated.

Enzyme assays

Enzyme activities were determined at 25°C using a recording spectrophotometer (Gilford model 252 attachment to Unicam SP.500) and are expressed as μmol of product formed/min per g wet wt. The assay procedures were assumed to be optimal for all the tissues investigated, although this was established only for some (see the Results and Discussion section; Crabtree & Newsholme, 1972*a*). The precautionary notes given by Crabtree & Newsholme (1972*a*) and Alp *et al.* (1976) about the use of the activities for

precise quantitative considerations apply to the activities reported here.

Assay of 2-oxoglutarate dehydrogenase. This enzyme was assayed by measuring the rate of reduction of NAD^+ at 340nm. The assay medium contained 90mM-Tris/HCl, 12mM-mercaptoethanol, 1mM-KCN, 0.5mM-CoA, 0.4mM-ADP, 2mM-NAD^+ and 0.8mM-2-oxoglutarate at pH7.4: volumes (1–20μl) of homogenate were added to 1ml of assay medium in a cuvette. The activity of 2-oxoglutarate dehydrogenase was obtained from the rate of change of A_{340} minus that of a control from which CoA was omitted. With homogenates of insect flight muscles there was a residual rate of NADH oxidation in the presence of cyanide which was not decreased by increasing the concentration of cyanide in the assay: this continuously reoxidized some of the NADH generated by 2-oxoglutarate dehydrogenase, leading to the underestimation of its activity. However, further investigations revealed that the residual NADH oxidation was saturated at very low concentrations of NADH (approx. 1μM), so that it should be operating at or near its maximum rate within the first minute of the 2-oxoglutarate dehydrogenase assay. Therefore the 2-oxoglutarate dehydrogenase activities in the insect flight-muscle homogenates were corrected by adding the residual rate of NADH oxidation, determined with a control assay containing 50μM-NADH in place of CoA. No cyanide-insensitive oxidation of NADH was observed with homogenates of mammalian tissues. The presence of ADP (0.4mM) was found to be necessary for the maximum activities of 2-oxoglutarate dehydrogenase in the insect flight muscles (see also Hansford, 1972).

Assay of pyruvate dehydrogenase. The assay for this enzyme was based on that described by Coore *et al.* (1971). The assay medium contained 100mM-Tris/HCl, 1mM-$MgCl_2$, 5mM-mercaptoethanol, 1mM-thiamin pyrophosphate, 1mM-NAD^+, 1mM-pyruvate, 0.08mM-CoA and 10μg of *p*-(*p*-aminophenylazo)-benzenesulphonic acid/ml at pH7.8: 0.1 unit of arylamine acetyltransferase and suitable volumes (2–20μl) of homogenate were added to 1ml of assay medium. The activity of pyruvate dehydrogenase was obtained from the rate of change of A_{460} minus that of a control from which pyruvate, CoA and NAD^+ were omitted. Another control, from which the homogenate was omitted, corrected for pyruvate dehydrogenase activity that contaminated some preparations of arylamine acetyltransferase.

Assay of phosphofructokinase. The assay for this enzyme was based on that described by Opie & Newsholme (1967). The assay medium contained 75mM-Tris/HCl, 7mM-$MgCl_2$, 200mM-KCl, 1mM-KCN, 0.17mM-NADH, 2mM-AMP, 1mM-ATP and 1mM-fructose 6-phosphate at pH8.2: aldolase (2 units), glycerol 3-phosphate dehydrogenase (2 units) and triose phosphate isomerase (10 units) were added to 1ml of assay medium and the reaction was initiated by adding 1–10μl of homogenate. The activity of phosphofructokinase was obtained from the rate of change of A_{340} minus that of a control from which fructose 6-phosphate, ATP and AMP were omitted.

Results and Discussion

Stability and some properties of 2-oxoglutarate dehydrogenase

The 2-oxoglutarate dehydrogenase activity in homogenates of insect flight muscles and rat heart was lost very rapidly unless both glycerol and 2-oxoglutarate were included in the homogenizing medium: this stabilizing effect of glycerol is similar to that reported for NAD-linked isocitrate dehydrogenase (Cox & Davies, 1967; Alp *et al.*, 1976). However, the enzyme was only moderately stable even with glycerol and 2-oxoglutarate present in the homogenate (up to 50% of the activity in bumble-bee flight muscle and rat heart was frequently lost within 20min of homogenization). This made it impossible to subject the homogenates to treatments (e.g. dialysis or gel filtration) that remove small molecules. Consequently, the kinetic properties reported here may have been influenced by effectors added with the homogenate.

With homogenates of bumble-bee (*Bombus terrestris*) flight muscle, rat heart and lactating mammary gland, the rate of NADH formation corresponding to the 2-oxoglutarate dehydrogenase activity required

Table 1. *K_m values for 2-oxoglutarate dehydrogenase*
Homogenates were prepared and the enzyme activity was measured as described in the Experimental section. Each K_m value was determined from a double-reciprocal plot (involving at least six points) of activity versus concentration in the presence of saturating concentrations of the other substrates and, except for the rat heart, in the presence of 0.4mM-ADP. The values given are the means of three determinations, with the range in parentheses. Because of the instability of the enzyme (see the Experimental section) it was not possible to determine the K_m values for all three substrates with a single homogenate, but sufficient time was always available for the determination of one K_m value.

Tissue	K_m (CoA) (μM)	K_m (NAD^+) (μM)	K_m (2-oxoglutarate) (μM)
Bumble-bee (*Bombus terrestris*) flight muscle	30 (20–40)	100 (95–110)	70 (70–75)
Queen wasp (*Vespa vulgaris*) flight muscle	30 (25–40)	90 (85–100)	70 (65–75)
Rat heart	25 (25–30)	100 (100–110)	60 (50–75)

the simultaneous presence of 2-oxoglutarate, CoA and NAD^+; the pH optimum for the enzyme in all three tissues was 7.4 in Tris/HCl buffer. The K_m values for each of the three substrates of 2-oxoglutarate dehydrogenase (at saturating concentrations of the others) were determined from double-reciprocal plots of activity versus substrate concentration, by using homogenates of bumble-bee and wasp flight muscles and rat heart (Table 1). The K_m values for 2-oxoglutarate are similar to those reported for the enzyme from rat heart (80 μM) by Williamson *et al.* (1973) and from blowfly flight muscle (90 μM) by Hansford (1972). However, the K_m values for CoA and NAD^+ are somewhat greater than those reported for rat heart (approx. 5 and 20 μM respectively) by Williamson *et al.* (1973), although these differences may be due to the use of crude homogenates in the present work. Nevertheless, the K_m values given in Table 1 indicate that the concentrations used in the routine assay are sufficient to saturate the enzyme.

2-Oxoglutarate dehydrogenase activity as an index of the rate of the tricarboxylic acid cycle in vivo

Table 2 compares the activities of 2-oxoglutarate dehydrogenase and the rate of the tricarboxylic acid cycle in several Hymenopteran flight muscles, in rat heart and lactating mammary gland and in lactating goat mammary gland. [Hymenopteran flight muscles were chosen for this comparison because they use only glucose as a fuel for flight and contain negligible activities of lactate dehydrogenase; thus all the pyruvate produced from glucose enters and is oxidized by the tricarboxylic acid cycle (for review see Crabtree & Newsholme, 1975). Since the maxi-

Table 2. *Comparison of 2-oxoglutarate dehydrogenase activities with the rate of the tricarboxylic acid cycle*

Enzyme activities were measured as described in the Experimental section. For the Hymenopteran flight muscles the rate of the tricarboxylic acid cycle was estimated as 2× phosphofructokinase activity (see the Results and Discussion section). For rat mammary gland, the rate of the cycle was calculated from the value of 20 μmol of CO_2 (=10 μmol of acetyl-CoA units oxidized)/h per 100 mg defatted dry wt. of parenchymal tissue at 35°C reported by Katz *et al.* (1974), assuming a Q_{10} of 2, that the parenchymal tissue constituted 50% of the total weight of the gland and that the wet wt./defatted dry wt. ratio of the tissue is 8 (see Katz *et al.*, 1974, pp. 7349 and 7353). For rat heart, the rate of the cycle was calculated from the value of 30.4 μmol of acetyl-CoA oxidized/min per g dry wt. at 37°C reported by Neely *et al.* (1972), assuming a Q_{10} of 2 and a wet wt./dry wt. ratio of 4. For goat mammary gland the rate of the cycle was calculated from the O_2 uptake of 23.4 ml/min per kg of udder (Linzell, 1960), assuming a body temperature of 37°C, a Q_{10} of 2 and that 1 μmol of acetyl-CoA produced from either glucose or fatty acids and oxidized by the tricarboxylic acid cycle is equivalent to the uptake of 3 μmol of O_2. The activities in insect flight muscles are those of individual insects, whereas those for the rat and goat are mean values from Table 3.

Species	Tissue	Acetyl-CoA or 2-oxoglutarate oxidized (μmol/min per g wet wt. at 25°C): Maximum rate of the tricarboxylic acid cycle	Acetyl-CoA or 2-oxoglutarate oxidized (μmol/min per g wet wt. at 25°C): 2-Oxoglutarate dehydrogenase activity	2-Oxoglutarate dehydrogenase activity / rate of tricarboxylic acid cycle
Queen bumble-bee (*Bombus terrestris*)	Flight muscle	38	48	1.3
Worker bumble-bee (*Bombus terrestris*)	Flight muscle	52	51	1
Queen bumble-bee (*Bombus hortorum*)	Flight muscle	36	41	1.1
Queen bumble-bee (*Bombus pratorum*)	Flight muscle	54	62	1.2
Worker bumble-bee (*Bombus pratorum*)	Flight muscle	90	65	0.7
		86	85	1
		72	52	0.7
Queen bumble-bee (*Bombus agrorum*)	Flight muscle	38	58	1.5
		38	59	1.5
Worker bumble-bee (*Bombus agrorum*)	Flight muscle	96	65	0.7
Queen wasp (*Vespa vulgaris*)	Flight muscle	60	64	1.1
		60	62	1
		98	71	0.7
Worker wasp (*Vespa vulgaris*)	Flight muscle	100	99	1
Worker honey-bee (*Apis mellifera*)	Flight muscle	30	26	0.9
Solitary bee (*Andrena trimmerana*)	Flight muscle	30	36	1.2
Cuckoo bumble-bee (*Psithyrus campestris*)	Flight muscle	42	47	1.1
Laboratory rat	Heart	3.8	6.7	1.8
Laboratory rat	Lactating mammary gland	0.05	0.11	2.2
Goat	Lactating mammary gland	0.18	0.12	0.7

mum flux of glucose to pyruvate can be estimated as the maximum activity of phosphofructokinase (Crabtree & Newsholme, 1972*a*), the maximum tricarboxylic acid-cycle flux in these flight muscles can be estimated as twice the activity of phosphofructokinase.] The results of the comparison between 2-oxoglutarate dehydrogenase activity and the rate of the tricarboxylic acid cycle (Table 2) show that the former provides a good assessment of the latter. Thus measuring the 2-oxoglutarate dehydrogenase activities of these tissues provides a simple method for estimating the maximum flux through the tricarboxylic acid cycle *in vivo*.

Table 3 presents the activities of 2-oxoglutarate dehydrogenase in the hearts and mammary glands of several mammals. The activity of this enzyme in the heart of any given species (and hence the maximum tricarboxylic acid-cycle flux) was much greater than that in the mammary gland, reflecting the lower oxidative capacity (on a tissue-weight basis) of the latter. The activities of 2-oxoglutarate dehydrogenase in mammary glands from the rat and guinea pig increased between pregnancy and lactation. Such an increase has been shown for several other mammary-gland enzyme activities, especially those involved in the increased biosynthesis that accompanies lactation (Gumaa *et al.*, 1973; Baldwin & Yang, 1974).

In the hearts, and more especially the lactating mammary glands, there was no tendency for the 2-oxoglutarate dehydrogenase activities (and hence the maximum rate of the tricarboxylic acid cycle) to decrease with an increasing body weight of the animal. Previous results had indicated a significant inverse relationship between body weight and the maximum tricarboxylic acid-cycle flux in heart (see Crabtree & Newsholme, 1975), but these were based on the activities of succinate dehydrogenase and NAD-linked isocitrate dehydrogenase, neither of which is a satisfactory index of the maximum rate of the cycle in vertebrate muscles (see the introduction). Since the tricarboxylic acid cycle in heart is responsible for regenerating much of the ATP for muscular activity under normal aerobic conditions, a decrease in maximum tricarboxylic acid-cycle flux (per unit weight of heart) with body weight would have been expected on theoretical grounds (see Crabtree & Newsholme, 1975). However, the physical fitness of an animal is also an important factor determining the oxidative capacity of, and hence the rate of the tricarboxylic acid cycle in, the muscles (Holloszy & Oscai, 1969; Baldwin *et al.*, 1972) and variations in this and/or some other factor superimposed on the effects caused by body weight could have produced the distribution in Table 3.

Table 3. *Activities of 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase in some mammalian hearts and mammary glands*

Enzyme activities were measured as described in the Experimental section and are presented as means, with the range in parentheses underneath: the numbers of determinations on separate animals are given in parentheses after the mean.

	Activity (μmol/min per g wet wt. at 25°C)	
	2-Oxoglutarate dehydrogenase	Pyruvate dehydrogenase
Hearts		
Species		
Mouse	3.5 (4) (2.2–4.9)	2.8 (4) (2.3–3.2)
Rat	6.7 (4) (5.3–7.7)	3.4 (6) (3.1–4)
Guinea pig	1.9 (4) (1.1–3.5)	1.25 (4) (1.1–1.4)
Rabbit	2.7 (4) (1.5–3.5)	2.5 (4) (2.2–2.7)
Pig	1.4 (3) (1.1–1.6)	1.5 (3) (1.4–1.6)
Sheep	1.2 (4) (1–1.3)	1.5 (4) (1.3–1.9)
Cow	1.2 (4) (0.9–1.4)	1.2 (4) (1–1.5)
Mammary glands		
Non ruminant species		
Mouse (7-day lactating)	0.13 (4) (0.1–0.2)	1.6 (4) (1.5–1.7)
Rat (20-day pregnant)	<0.05 (3)	0.2 (3) (0.15–0.25)
Rat (14-day lactating)	0.11 (6) (0.1–0.18)	0.55 (6) (0.42–0.7)
Guinea pig (30-day pregnant)	<0.05 (2)	<0.05 (2)
Guinea pig (7-day lactating)	0.14 (4) (0.1–0.2)	0.16 (4) (0.12–0.19)
Rabbit (14-day lactating)	0.15 (4) (0.1–0.2)	0.25 (4) (0.2–0.3)
Ruminant species		
Lactating goat	0.12 (4) (0.09–0.14)	0.13 (4) (0.11–0.14)
Lactating sheep	0.1 (4) (0.08–0.12)	0.1 (4) (0.09–0.12)
Lactating cow	0.17 (4) (0.12–0.24)	0.09 (4) (0.07–0.1)

Flux of pyruvate to acetyl-CoA and its oxidation via the tricarboxylic acid cycle

In rat adipose tissue the maximum activity of pyruvate dehydrogenase *in vitro* is similar to the maximum flux of pyruvate to acetyl-CoA *in vivo* (Wieland *et al.*, 1973), which suggests that the activity of this enzyme might be used to estimate and compare the maximum fluxes of pyruvate to acetyl-CoA in other tissues. Table 3 presents the activities of pyruvate dehydrogenase in mammalian hearts and mammary glands for comparison with those of 2-

oxoglutarate dehydrogenase, and hence with the maximum rate of the tricarboxylic acid cycle. Since pyruvate dehydrogenase exists in two enzymically interconvertible forms in mammalian tissues (see Wieland *et al.*, 1973; Coore & Field, 1974; Randle *et al.*, 1974), it is not possible to be certain that all the enzyme was extracted in the active form, although the homogenization medium was designed to favour the extraction of and conversion into this form (see the Materials and Methods section). However, the mean activity of pyruvate dehydrogenase in lactating rat mammary gland, 0.55 μmol/min per g (Table 3), is only slightly lower than the total activity reported by Coore & Field (1974) for this tissue (0.7 μmol/min per g, assuming that the activity is halved by a decrease in temperature of 10°C, i.e. a Q_{10} of 2). Also, the mean activity of this enzyme in rat heart, 3.4 μmol/min per g (Table 3), is only slightly lower than the total activity reported by Whitehouse & Randle (1973) for this tissue (4.5 μmol/min per g, assuming a Q_{10} of 2 and a wet wt./dry wt. ratio of 4). (The total activities were measured after complete activation with pyruvate dehydrogenase phosphatase.) Consequently, the procedure used in the present work appears to extract and assay most of the enzyme in its active form. It should also be noticed that in the hearts the distribution of the pyruvate dehydrogenase activities was very similar to that of the 2-oxoglutarate dehydrogenase activities (Table 3), which suggests that neither distribution is an artifact. Indeed, the similar activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in the heart of any given species (Table 3) suggest that this tissue can obtain all the acetyl-CoA for the tricarboxylic acid cycle from pyruvate, and hence from glucose. However, this contribution from pyruvate will be decreased when alternative fuels such as acetate, ketone bodies or long-chain fatty acids become increasingly available (see Randle *et al.*, 1966).

In contrast with heart, in which there is no significant synthesis of long-chain fatty acids *de novo*, the lactating mammary glands of several mammalian species use glycolytically produced pyruvate for the synthesis of long-chain fatty acids as well as for oxidation via the tricarboxylic acid cycle: the pathway for fatty acid synthesis from pyruvate involves the mitochondrial formation of citrate by pyruvate dehydrogenase and citrate synthase, transport of citrate into the cytoplasm and formation of cytoplasmic acetyl-CoA by ATP citrate lyase (for reviews, see Bauman & Davis, 1974, 1975). However, in ruminant mammary glands the activity of ATP citrate lyase is negligible, so that citrate and hence pyruvate cannot be used for fatty acid synthesis by these tissues (Hardwick, 1966; Bauman & Davis, 1974). The activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in most of the lactating mammary glands investigated (Table 3) reflect this difference between ruminant and non-ruminant tissue. Thus in lactating mammary glands from the rat and mouse the activity of pyruvate dehydrogenase is significantly greater than that of 2-oxoglutarate dehydrogenase (Table 3). Since the latter is involved only in the oxidative part of the tricarboxylic acid cycle, whereas the former is involved in both pyruvate oxidation and its conversion into fatty acids, the difference between the activities of these two enzymes may be an estimate of the maximum flux of pyruvate to acetyl-CoA for fat synthesis. Indeed, the difference between the mean activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in lactating rat mammary gland, 0.45 μmol/min per g, is similar to the rate of conversion of pyruvate into acetyl-CoA for fat synthesis in a cell preparation from this tissue, approx. 0.6 μmol/min per g wet wt. (Katz *et al.*, 1974; value corrected for temperature and weight as described in the legend of Table 2).

On the other hand, the activity of pyruvate dehydrogenase in the ruminant mammary glands was similar to or slightly lower than that of 2-oxoglutarate dehydrogenase, reflecting the absence from this tissue of a significant flux of pyruvate to fatty acids. However, the presence of similar activities of these two enzymes in ruminant mammary glands does suggest that these tissues can oxidize glycolytically produced pyruvate, and this has been shown for the goat by Hardwick (1966) and Smith & Taylor (1977).

Finally, in two non-ruminant mammary glands, those from the rabbit and guinea pig, the activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase were similar, as in ruminant mammary glands (Table 3). However, in rabbit and guinea-pig mammary glands the flux of glycolytically produced pyruvate to fatty acids is much lower than that in rat mammary gland (see Strong & Dils, 1972), so that any difference between the activities of the enzymes would probably have been too small to be significant in the present work. In this connexion it is noteworthy that the mean values for pyruvate dehydrogenase activity in lactating mammary glands (Table 3) decrease in the order rat > rabbit > guinea pig > cow, which is the same order as the rates of incorporation of glucose (and hence glycolytically produced pyruvate) into fatty acids (Strong & Dils, 1972).

We thank Professor A. D. Care and Dr. D. W. Pickard for taking biopsies from goat mammary glands, Dr. A. Chadwick for supplying the pigeons, Dr. R. M. Denton for a gift of *p*-(*p*-aminophenylazo)benzenesulphonic acid and for valuable advice on the preparation of arylamine acetyltransferase and Dr. E. A. Newsholme for helpful discussions and criticism of the manuscript. We also thank Miss A. E. Featherby for excellent technical assistance. The work was supported by the Agricultural Research Council.

References

Alp, P. R., Newsholme, E. A. & Zammit, V. (1976) *Biochem. J.* **154**, 689–700

Baldwin, K. M., Klinkerfuss, G. H., Terjung, R. L., Mole, P. A. & Holloszy, J. O. (1972) *Am. J. Physiol.* **222**, 373–378

Baldwin, R. L. & Yang, Y. T. (1974) in *Lactation* (Larson, B. L. & Smith, V. R., eds,), vol. 1, pp. 349–411, Academic Press, New York and London

Bauman, D. E. & Davis, C. L. (1974) in *Lactation* (Larson, B. L. & Smith, V. R., eds.), vol. 2, pp. 31–75, Academic Press, New York and London

Bauman, D. E. & Davis, C. L. (1975) in *Digestion and Metabolism in the Ruminant* (McDonald, I. W. & Warner, A. C. I., eds.), pp. 496–509, University of New England Publishing Unit, Armidale, Australia

Coore, H. G. & Field, B. (1974) *Biochem. J.* **142**, 87–95

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

Cox, G. F. & Davies, D. D. (1967) *Biochem. J.* **105**, 729–734

Crabtree, B. & Newsholme, E. A. (1972*a*) *Biochem. J.* **126**, 49–58

Crabtree, B. & Newsholme, E. A. (1972*b*) *Biochem. J.* **130**, 697–705

Crabtree, B. & Newsholme, E. A. (1975) in *Insect Muscle* (Usherwood, P. N. R., ed.), pp. 405–500, Academic Press, London and New York

Gumaa, K., Greenbaum, A. L. & McLean, P. (1973) *Eur. J. Biochem.* **34**, 188–198

Hansford, R. G. (1972) *FEBS Lett.* **21**, 139–141

Hardwick, D. C. (1966) *Biochem. J.* **99**, 228–231

Holloszy, J. O. & Oscai, L. B. (1969) *Arch. Biochem. Biophys.* **130**, 653–656

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

Linzell, J. L. (1960) *J. Physiol. (London)* **153**, 492–509

Neely, J. R., Denton, R. M., England, P. J. & Randle, P. J. (1972) *Biochem. J.* **128**, 147–159

Opie, L. H. & Newsholme, E. A. (1967) *Biochem. J.* **103**, 391–399

Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., Denton, R. M. & Pogson, C. I. (1966) *Recent Prog. Horm. Res.* **22**, 1–48

Randle, P. J., Denton, R. M., Pask, H. T. & Severson, D. L. (1974) *Biochem. Soc. Symp.* **39**, 75–88

Smith, G. H. (1971) *Proc. Nutr. Soc.* **30**, 265–272

Smith, G. H. & Taylor, D. J. (1977) *Symp. Zool. Soc. (London)* in the press

Strong, C. R. & Dils, R. (1972) *Comp. Biochem. Physiol. B* **43**, 643–652

Tabor, H., Mehler, A. H. & Stedtman, G. R. (1953) *J. Biol. Chem.* **204**, 127–138

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

Wieland, O. H., Siess, E. A., Loffler, G., Portzelt, C., Portenhauser, R., Hartmann, U. & Schirmann, A. (1973) *Symp. Soc. Exp. Biol.* **27**, 371–400

Williamson, J. R., Safer, B., LaNoue, K. F., Smith, C. M. & Walajtys, E. (1973) *Symp. Soc. Exp. Biol.* **27**, 241–281