Biochem. J. (1972) **130**, 697–705
Printed in Great Britain

The Activities of Lipases and Carnitine Palmitoyltransferase in Muscles from Vertebrates and Invertebrates

By B. CRABTREE and E. A. NEWSHOLME
Agricultural Research Council Unit of Insect Physiology, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

(*Received* 27 *June* 1972)

1. The activities of tri-, di- and mono-glyceride lipase and carnitine palmitoyltransferase were measured in homogenates of a variety of muscles. These activities were used to estimate the rate of utilization of glycerides and fatty acids by muscle. In muscles whose estimated rates of fat utilization can be compared with rates calculated for the intact muscle from such information as O_2 uptake, there is reasonable agreement between the estimated and calculated rates. 2. In all muscles investigated the maximum rates of hydrolysis of glycerides increase in the order triglyceride, diglyceride, monoglyceride. The activity of diglyceride lipase is highest in the flight muscles of insects such as the locust, waterbug and some moths and is lowest in the flight muscles of flies, bees and the wasp. These results are consistent with the utilization of diglyceride as a fuel for some insect flight muscles. 3. In many muscles from both vertebrates and invertebrates the activity of glycerol kinase is similar to that of lipase. It is concluded that in these muscles the metabolic role of glycerol kinase is the removal of glycerol produced during lipolysis. However, in some insect flight muscles the activity of glycerol kinase is much greater than that of lipase, which suggests a different role for glycerol kinase in these muscles.

Lipid is known to be an important fuel for muscular activity in some migratory birds and insects (Odum, 1965; Weis-Fogh, 1967), in some mammalian muscles, especially during starvation (Randle *et al.*, 1966) and in some moths that are unable to feed in the adult stage (Gilbert, 1967). Lipid is made available to the muscle in at least four forms: exogenous long-chain fatty acids, exogenous diglyceride (particularly in insects), exogenous triglyceride (as very-low-density lipoproteins in mammals) and endogenous triglyceride. In this investigation the catalytic activities of triglyceride, diglyceride and monoglyceride lipases as well as carnitine palmitoyltransferase have been measured in a variety of muscles. These activities are discussed in relation to the type of lipid and the rate at which it can be used by muscle.

Materials and Methods

Chemicals and enzymes

These were obtained from the sources given previously (see Newsholme & Taylor, 1969; Crabtree & Newsholme, 1972) except for the following: defatted bovine serum albumin (type F), diolein (grade II), monolein (grade II) and palmitoyl chloride (grade II) were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.; triolein (A grade) was obtained from Calbiochem Ltd., London W.1, U.K., and was purified by chromatography on Florisil (Carroll, 1961); Florisil (100–200 mesh) and L-carnitine were obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.; 3′:5′-cyclic AMP (adenosine 3′:5′-cyclic monophosphate) was obtained from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K.; polyvinyl alcohol, hydroxylamine, caffeine and all inorganic reagents were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K., and were of the highest purity available. Palmitoyl-L-carnitine was synthesized from L-carnitine and palmitoyl chloride by the method described by Bremer (1962).

Animals

The sources, ages and sexes of animals were as given by Crabtree & Newsholme (1972).

Preparation of muscle homogenates

Homogenates for the assay of lipase activity were prepared in 10vol. of 200mM-triethanolamine–KOH buffer containing 2% (w/v) defatted bovine serum albumin at a final pH of 7.5. When lipase activity was assayed by measuring the formation of glycerol with muscles in which the activity of glycerol kinase was very high (>2μmol/min per g), it was necessary to dialyse the homogenates for 60min at

5°C against the extraction medium to decrease the concentration of endogenous ATP. For the assay of carnitine palmitoyltransferase and glycerol kinase activities, homogenates were prepared in 10–50vol. of a medium consisting of 50mM-triethanolamine, 1mM-EDTA, 2mM-$MgCl_2$ and 30mM-2-mercaptoethanol at a final pH of 7.5. Preliminary experiments with homogenates of several insect flight muscles established that sonication of the homogenates did not increase the activity of any of the enzymes investigated. Unless otherwise stated all homogenates were prepared in ground-glass homogenizers at 0°C and were assayed for enzyme activity within 15min of preparation.

Enzyme assays

Triglyceride lipase activity was assayed by a method based on that described by Chino & Gilbert (1965). Emulsions were prepared by sonicating a mixture of 20mg of triolein/ml of 5% (w/v) polyvinyl alcohol solution (with an MSE 100W sonicator at maximum power) until no lipid droplets were visible to the naked eye. The emulsions were stable for up to 5h at room temperature (approx. 20°C). Samples (100μl) of emulsion were added to glass tubes and the lipase reaction was initiated by the addition of 100μl of homogenate. The tubes were stoppered with cotton-wool plugs and were incubated, with shaking, at 25°C for periods of 10–90min. The reactions were terminated by plunging each tube into a boiling-water bath, up to the level of the cotton-wool plug, for approx. 10s. Immediately after, the tubes were rapidly cooled by plunging into ice. Preliminary experiments established that this procedure completely inactivated the lipase and that there was no detectable alteration of the incubation volume. The heat-terminated extracts were filtered by drawing them into Pasteur pipettes through small plugs of cotton-wool placed in the stems of the pipettes. The plugs were removed with forceps, the stems were cleaned with cotton-wool and the extracts were subsequently ejected. Samples (50μl) of each extract were assayed for glycerol by the radiochemical method described by Newsholme & Taylor (1968), except that the concentration of [^{14}C]glycerol was 34μM. In some cases the incubation tubes were plunged into boiling water immediately after the addition of extract. These served as controls and they were used to provide the C_0 incubation tube for the assay of glycerol (see Newsholme & Taylor, 1968). By this method the presence of any activators and/or inhibitors of the commercial glycerol kinase in the assay for glycerol was taken into account.

Diglyceride lipase and monoglyceride lipase activities were each assayed in the same way as triglyceride lipase, except that the emulsions were prepared with 20mg of diolein or monolein/ml in place of the triolein. The emulsions of diglyceride were stable for several hours at room temperature, whereas emulsions of monoglyceride were stable for only approx. 60min. Since monolein is a solid at room temperature it was necessary to dissolve it in a small volume (approx. 0.1ml) of chloroform to prepare the emulsion; the chloroform was subsequently removed by passing a stream of N_2 through the emulsion.

Preliminary experiments established that the amount of emulsion in the lipase assay was sufficient to saturate the enzymes (50% saturation occurred with approx. 10μl of emulsion/0.2ml of incubation mixture). The production of glycerol from glyceride occurred only in the presence of homogenate and the amount of glycerol produced was directly proportional to both the time of incubation and the amount of homogenate added, within the ranges employed in the present investigations. Preliminary experiments also established that no glycerol was introduced or removed at the filtration stage. It was not possible to test directly whether the polyvinyl alcohol modified the lipase activity, since the glycerides did not form emulsions in the absence of the emulsifying agent. However, variations in the concentration of the alcohol from 0.5–5% (w/v) did not affect lipase activity, which suggests that the alcohol had no effect on the enzyme activity. The activity of triglyceride lipase in rat heart muscle (Table 2) is similar to that reported by Björntorp & Furman (1962), with a different assay method, and is also similar to the rate of release of glycerol by the isolated heart (Garland & Randle, 1964); the activities of the lipases in the flight muscles of moths (Table 2) are similar to those reported by Stevenson (1969). The activity of lipase, measured by the above-described technique, was similar to that measured by the production of ^{14}C-labelled fatty acids from an emulsion prepared with ^{14}C-labelled glyceride (B. Crabtree & E. A. Newsholme, unpublished work). These results suggest that the method provides a reasonable assessment of the maximum catalytic activity of lipase.

Carnitine palmitoyltransferase activity was assayed by a method based on that described by Beenakkers *et al.* (1967). In this assay palmitoylcarnitine reacts with CoA to produce palmitoyl-CoA, which in turn reacts with hydroxylamine to produce a hydroxamate. The amount of hydroxamate present is measured with $FeCl_3$ reagent (Lipmann & Tuttle, 1950). The assay medium contained 750mM-hydroxylamine, 4mM-CoA and 4mM-palmitoyl-L-carnitine at a final pH of 7.0. Samples (100μl) of homogenate were added to 0.2ml of assay medium to initiate the reaction and were incubated, with shaking, at 25°C for periods of 10–60min. Controls, from which palmitoylcarnitine was omitted, were also included. After a suitable period of incubation the reactions were terminated by

the addition of 1.5ml of 95% (v/v) ethanol to each incubation mixture. A volume (0.75ml) of a solution consisting of 28% (w/v) hydroxylamine, 4M-HCl, 3.5M-NaOH (equal parts by vol.), followed within 5min by 0.25ml of 24% (w/v) trichloroacetic acid and 0.25ml of 10% (w/v) $FeCl_3$, was then added to each incubation mixture. After centrifugation at 3400*g* for 10min the supernatants were clarified completely by drawing them into Pasteur pipettes through plugs of cotton-wool placed in the stem. This treatment was found to have no effect on the colour produced by the hydroxamate. The extinction of each solution was measured at 520nm in a Unicam SP.600 spectrophotometer. The molar extinction coefficient of the palmitoylhydroxamate complex was assumed to be 1×10^3 litre·mol^{-1}·cm^{-1} (Kornberg & Pricer, 1953). Preliminary experiments established that the colour was stable for up to 60min after development (fading occurred rapidly after 60min), that there was no direct reaction of palmitoylcarnitine and hydroxylamine under the assay conditions, and that the intensity of the colour was directly proportional to both the time of incubation and the amount of homogenate within the range used in these investigations. Relatively little work appears to have been done on the distribution of this enzyme in muscle, with assay methods other than the one described above; the assumption that the activities of this enzyme (Table 2) are maximal must therefore be regarded as tentative at the present time.

Glycerol kinase activity was assayed by the method described by Newsholme *et al.* (1967) and Newsholme & Taylor (1969).

All enzyme activities are expressed as μmol of product formed/min per g of fresh muscle at 25°C. In the case of lipase this product is glycerol, unless otherwise stated. The reported activities represent the mean of three or more determinations (with three animals or three separate pools of muscle from a larger number of animals). The variation between individual determinations of any enzyme in any given type of muscle was within 30% of the mean for all enzymes except triglyceride lipase; the variation for this enzyme was within 50% of the mean. Since, in the present work, no account has been taken of such factors as sex, season, age or diet on the enzyme activities, and since precise quantitative interpretations are not made from these activities, a conventional statistical presentation of the results was considered undesirable and unnecessary. Any use of the reported activities for precise quantitative analysis must be made with caution.

It is assumed that the assay conditions are optimal for the enzymes in all the muscles investigated. However, this point was established experimentally only with homogenates of locust, waterbug, fleshfly and hawk-moth flight muscles, and rat heart and pigeon pectoral muscle.

Results and Discussion

Some properties of triglyceride lipase from muscle

The pH optimum of both rat heart and locust flight muscle triglyceride lipase activity is approx. 7.5 (in phosphate or triethanolamine buffers). Inhibition of these enzymes by long-chain fatty acids was demonstrated and it was reversed by albumin. These properties are similar to those of the enzyme from other sources (see Björntorp & Furman, 1962; Biale *et al.*, 1968). The activity of the triglyceride lipase in extracts of either rat heart or locust flight muscle was unaffected by NaCl (0.8M), which suggests that the activity is not due to clearing-factor (lipoprotein) lipase (see Robinson, 1965). Activities of triglyceride lipase in extracts of rat heart and flight muscles of the locust, waterbug and fleshfly were unaffected by 0.1–1mM-3′:5′-cyclic AMP (in the presence of 5mM-ATP, 10mM-Mg^{2+} and 2mM-caffeine). This suggests either that the activities of the lipase in these muscles are not stimulated by the nucleotide or that the enzymes are present in the extracts in the activated form (see Corbin *et al.*, 1970).

Activities of lipase and carnitine palmitoyltransferase in relation to the rate of lipid utilization by muscle

In a previous paper an approach was outlined for obtaining reasonable quantitative assessments of the rate of utilization of fuels for muscle activity (Crabtree & Newsholme, 1972). This approach is based on the assumption that the maximum catalytic activity of an enzyme which catalyses a reaction far displaced from equilibrium in a metabolic pathway may be similar to the maximum rate of operation of that pathway in the cell. In the present investigations it has been assumed that the lipases and carnitine palmitoyltransferase catalyse reactions far displaced from equilibrium in the pathways for glyceride hydrolysis and fatty acid oxidation, respectively. This assumption is based on reports that these enzymes possess low maximum catalytic activities in relation to other enzymes of the pathways, and therefore catalyse reactions which are far displaced from equilibrium in the cell (see Krebs, 1963; Shepherd *et al.*, 1966; Beenakkers *et al.*, 1967; Biale *et al.*, 1968; Beenakkers, 1969). The designation of carnitine palmitoyltransferase as far displaced from equilibrium is speculative at the present time; however, support for the assumption that the activities of these enzymes indicate maximum rates of the pathways is provided from the information given in Table 1. The maximum rate of lipid utilization is calculated from the O_2 uptake of intact muscles during activity, and this rate is compared with the maximum activities of the enzymes measured *in vitro*. The activities of diglyceride lipase and the calculated rates of fatty acid oxidation are in reasonable agreement for the locust,

Table 1. *Rates of lipid utilization by intact muscles*

The rates of lipid utilization were calculated from published values of O_2 uptake by flying insects or pigeons or by the swimming trout, and they were obtained under conditions where fat was known to be the fuel for muscular activity (except for the trout). The metabolic rates were calculated by using information quoted by Crabtree & Newsholme (1972) plus the following: (*a*) Weis-Fogh (1952) obtained a value of 10ml of O_2/h per g of insect; (*b*) Zebe (1954) obtained a value of 50ml of O_2/h per g of insect, the flight muscles represent 17% of the body weight (Greenewalt, 1962) and the thoracic temperature is assumed to be 35°C; (*c*) Zebe (1954) obtained a value of 40ml of O_2/h per g of insect, the flight muscles represent 20% of the body weight (B. Crabtree & E. A. Newsholme, unpublished work) and the thoracic temperature is assumed to be 35°C; (*d*) Tucker (1968) reported a value of 11ml of O_2/h per g of bird, the pectoral muscles represent 20% of the body weight (Greenewalt, 1962) and the thoracic temperature is assumed to be 40°C (Welty, 1955); (*e*) Crass *et al.* (1969) obtained a value of 85μmol of fatty acid oxidized/h per g dry wt., the temperature was 37°C. It was assumed that the rate of each enzymic reaction doubles with a 10°C rise in temperature. For the conversion of O_2-uptake values, fat was assumed to be oxidized completely with 1μmol of C_{16} fatty acid being equivalent to the uptake of 23μmol of O_2; no allowance was made for oxidation of the glycerol produced by lipolysis. Lipase activities are expressed in terms of fatty acid production. The activities of carnitine palmitoyltransferase have been halved for comparison with calculated rates of lipid utilization; this adjustment is necessary since the enzyme occurs at two points on the pathway for fatty acid oxidation, on either side of the mitochondrial barrier to fatty acyl-CoA (see Greville & Tubbs, 1968).

Animal	Experimental conditions	Rate of lipid utilization required to account for the metabolic rate (μmol of C_{16} fatty acid/min per g of muscle at 25°C)	Enzyme activity (μmol of C_{16} fatty acid produced or utilized/min per g of muscle at 25°C)		
			Triglyceride lipase	Diglyceride lipase	0.5 × Carnitine palmitoyl-transferase
Locust (*Schistocerca gregaria*)	O_2 uptake of flying insect[a]	0.9	0.18	1.2	1.4
Peacock butterfly (*Vanessa io*)	O_2 uptake of flying insect[b]	4.8	0.12	1.8	0.9
Silver-Y moth (*Plusia gamma*)	O_2 uptake of flying insect[c]	3.2	0.12	0.9	0.5
Trout (*Salmo gairdneri*)	O_2 uptake during continuous swimming	0.4	0.06	—	0.1
Pigeon (*Columba livia*)	O_2 uptake during flight[d]	0.6	0.21	—	1.6
Rat heart	Utilization of exogenous fatty acid[e]	0.3	0.18	—	1.0

moth and butterfly (Table 1). This result is consistent with the use of diglyceride as a fuel for the flight muscles of these insects, demonstrated by more direct methods (Gilbert, 1967; Tietz, 1967; Mayer & Candy, 1969). The activities of carnitine palmitoyltransferase and the calculated rates of fatty acid oxidation are in reasonable agreement for rat heart, pigeon pectoral and red muscle of the trout (Table 1). However, it must be pointed out that although measurements of carnitine palmitoyltransferase activities may provide an indication of the maximum rate of fatty acid oxidation in some muscles, they do not distinguish between the use of exogenous fatty acid or endogenous triglyceride. Similarly, in insects, the activities of diglyceride lipase will not distinguish between the use of exogenous diglyceride or endogenous triglyceride.

Lipase activities in muscle

In agreement with previous work (Biale *et al.*, 1968; Stevenson, 1969) the activities of diglyceride lipase and monoglyceride lipase are usually five-to-ten-fold those of the triglyceride lipase in the same muscle (Table 2). These results support the conclusions of Denton & Randle (1967) that the removal of the first fatty acid from triglyceride is rate-limiting for lipolysis in muscle. In the moths the activity of monoglyceride lipase is approximately tenfold that of the diglyceride lipase, which is in agreement with the observations of Stevenson (1969). In some insect flight muscles the activity of diglyceride lipase may indicate the capacity for utilization of exogenous diglycerides (see the comparison of enzyme activities with calculated rates of fatty acid oxidation in Table 1). The highest activities of diglyceride lipase are found in the flight muscles of the locust, dragonfly, waterbug, butterflies and some moths (Table 2). This indicates that diglyceride is able to provide energy for a period of flight in these insects. The lowest activities of diglyceride lipase are found in the flight muscles of flies, bees and the cockroach (Table 2). From these activities and from published metabolic rates (see Crabtree & Newsholme, 1972), it can be calculated that the utilization of diglyceride in the latter insects could account for, at most, 7% of the metabolic rate during flight. These conclusions are in agreement with those of other investigators, who used different techniques, such as respiratory-quotient determinations and determinations of lipid content of insects (see Sacktor, 1965; Weis-Fogh, 1967). Triglyceride lipase activity was detected in all muscles investigated except those of the sea mussel and the dogfish (Table 2). Thus triglyceride may provide a reserve of fuel in most muscles, including those in which it does not serve as a major source of fuel for mechanical activity (e.g. vertebrate white muscle and the flight muscles of some insects). In such muscles it is possible that triglyceride serves as a fuel for the muscle under some conditions during rest (see Wigglesworth, 1949). The absence of triglyceride lipase activity from dogfish red muscle is surprising since this muscle is known to use lipid as a fuel during swimming (Bone, 1966). In vertebrate muscles the activity of triglyceride lipase is greater in the red than in the white muscles of any given species (Table 2). This result is consistent with a greater oxidative capacity of the red muscles and reflects their physiological role, namely the provision of continuous mechanical activity for relatively long periods of time (see Lawrie, 1953; Bilinski, 1963; Bone, 1966; George & Berger, 1966; Crabtree & Newsholme, 1972).

Carnitine palmitoyltransferase activities in muscle

In many muscles the activities of carnitine palmitoyltransferase and the lipases are similar, when expressed in units of fatty acid utilization or production (see Table 2). This suggests that these muscles could utilize exogenous fatty acids (if these are available) at approximately the same rate as endogenous triglyceride. However, in some muscles (e.g. the pectoral muscle of the pigeon, rat muscles) the activity of carnitine palmitoyltransferase is much greater than the activity of triglyceride lipase (Table 2), so that exogenous fatty acids may provide a greater proportion of the energy required for mechanical activity. If it is assumed that one-half the activity of carnitine palmitoyltransferase (see legend to Table 1) is an indication of the maximum capacity for the oxidation of fatty acids in any given muscle, this activity should be at least as great as the activity of the tri- or di-glyceride lipase (depending on whether the muscle uses endogenous triglyceride or exogenous diglyceride). In some insect flight muscles the activities of carnitine palmitoyltransferase are slightly lower than those of diglyceride lipase (exceptions include flight muscles of the locust and waterbug); however, in view of the cautionary notes given before, the differences may not be significant. A carnitine-independent pathway of fatty acid oxidation has been demonstrated in the flight muscles of a moth, *Prodenia* (Stevenson, 1968), and thus carnitine palmitoyltransferase might not be obligatory for fatty acid oxidation in some insect flight muscles.

Glycerol kinase activities in muscle

The activities of glycerol kinase (EC 2.7.1.30) in various muscles were reported by Newsholme & Taylor (1969). Some of the results obtained by these workers are given in Table 2 for comparison with the activities of the lipases. In some vertebrate muscles (e.g. avian pectoral muscles) and in many insect flight muscles (e.g. locust, dragonfly, waterbug, butterflies and moths) the activities of glycerol kinase are similar

Table 2. *Activities of tri-, di- and mono-glyceride lipases, carnitine palmitoyltransferase and glycerol kinase in various muscles*

Enzyme activities were determined as described in the Materials and Methods section; the number of determinations is given in parentheses when this was different from three. (a) is expressed as glycerol or glycerol phosphate production; (b) is expressed as fatty acyl-CoA production.

		Enzyme activity (μmol/min per g of fresh muscle)				
		(a) Lipase			(b) Carnitine palmitoyl-transferase	(a) Glycerol kinase
Animal	Muscle	Triglyceride	Diglyceride	Monoglyceride		
Mollusca						
Sea mussel (*Mytilus edulis*)	Posterior adductor	<0.005	<0.005	—	<0.01	0.1*
Insecta						
Odonata						
Dragonfly (*Anax imperator*)	Flight	0.05	0.8	—	—	0.8
Orthoptera						
Locust (*Locusta migratoria*)	Flight	0.07 (4)	0.6 (4)	—	3.6	0.5*
(*Schistocerca gregaria*)	Flight	0.06 (5)	0.7 (6)	0.6 (5)	2.8	0.5
Dictyoptera						
Cockroach (*Periplaneta americana*)	Flight	0.02	0.1	—	0.1	0.04*
Hemiptera						
Waterbug (*Lethocerus cordofanus*)	Flight	0.07 (5)	0.9 (5)	0.7 (4)	3.5	1.1*
Coleoptera						
Cockchafer (*Melolontha melolontha*)	Flight	0.03	0.3	1.9	0.1	1.2*
Hymenoptera						
Queen wasp (*Vespa vulgaris*)	Flight	0.05	0.2	—	—	9.2 (4)
Honey-bee (*Apis mellifera*)	Flight	0.04	0.3	0.4	0.2	1.5*
Bumble-bee (*Bombus hortorum*)	Flight	0.02	0.2	0.2	0.7 (4)	2.0
(*Bombus pratorum*)	Flight	0.03	0.2	0.2	—	5.5
Queen bumble-bee (*Bombus agrorum*)	Flight	—	0.3	0.5	—	6.8 (4)

Table 2 (*continued*).

Lepidoptera						
Small tortoiseshell butterfly (*Vanessa urticae*)	Flight	0.06	0.5	—	0.9	0.6*
Peacock butterfly (*Vanessa io*)	Flight	0.04	0.9	—	1.7	0.7
Red admiral butterfly (*Vanessa atalanta*)	Flight	0.05	0.6	—	—	0.5
Dusky-thorn moth (*Deuteronomos fuscantaria*)	Flight	0.05	0.7	—	—	0.7*
Silver-Y moth (*Plusia gamma*)	Flight	0.04	0.5	8.3	1.1	0.6
Yellow-underwing moth (*Noctua pronuba*)	Flight	0.05	0.6	6.4	1.1	0.7*
Poplar-hawk moth (*Laothoe populi*)	Flight	0.04	0.2	7.2	—	0.9
Diptera						
Fleshfly (*Sarcophaga barbata*)	Flight	0.04 (4)	0.3 (4)	—	0.3 (4)	0.2*
Blowfly (*Phormia terranova*)	Flight	—	0.2	—	0.2	2.1*
(*Calliphora erythrocephala*)	Flight	0.03 (4)	0.2 (4)	0.2	0.1	1.2*
Pisces						
Dogfish (*Scylliorhinus canicula*)	Red	<0.005 (4)	—	—	0.2	0.003*
	White	<0.005 (4)	—	—	0.05	<0.002*
Trout (*Salmo gairdneri*)	Red	0.02	—	—	0.2	<0.001
Silver eel (*Anguilla anguilla*)	Red	0.04	—	—	0.1	<0.001
	White	0.01	—	—	0.04	<0.001
Aves						
Sparrow (*Passer domesticus*)	Pectoral	0.02	—	—	—	0.01
Pigeon (*Columba livia*)	Pectoral	0.07 (4)	—	—	3.2	0.1*
Pheasant (*Phasianus colchicas*)	Pectoral	0.01	—	—	0.03	0.01*
Mammalia						
Rabbit (*Oryctolagus cuniculus*)	Semitendinosus (red)	0.03	—	—	0.5	0.01*
	Adductor longus (white)	0.01	—	—	0.06	0.003*
Rat	Heart	0.06 (7)	0.3 (5)	0.5 (5)	2.2 (4)	0.008*
	Quadriceps	0.01 (4)	—	—	1.9 (4)	0.003*

* Value from Newsholme & Taylor (1969).

Table 3. *Some properties of glycerol kinase from locust and bumble-bee flight muscle*

Experimental details were as described by Newsholme & Taylor (1969). This experiment is representative of three similar experiments.

	Locust* (*S. gregaria*)	Bumble-bee (*B. agrorum*)
(*a*) Kinetic properties		
K_m (glycerol) at saturating [ATP]	0.37mM	0.7mM
K_i (glycerol 3-phosphate) versus glycerol	0.4mM	0.4mM
(*b*) Intracellular distribution		
Cell fraction	Activity of fraction as percentage of activity in crude homogenate	
	Locust* (*S. gregaria*)	Bumble-bee (*B. agrorum*)
6000*g* pellet	66	46–65
6000*g* supernatant	32	25–35

* Values from Newsholme & Taylor (1969).

to the activities of the triglyceride lipase and diglyceride lipase, respectively. This suggests that the metabolic role of glycerol kinase in these muscles is the removal of glycerol produced by the hydrolysis of endogenous triglyceride or exogenous diglyceride. However, in other vertebrate muscles (e.g. rat heart) the activity of glycerol kinase is much lower than that of triglyceride lipase. This would explain the release of glycerol into the perfusion medium of an isolated rat heart preparation (Garland & Randle, 1964).

Newsholme & Taylor (1969) reported that the activities of glycerol kinase in the flight muscles of some insects (e.g. some flies, wasps and bees) are very high. The present investigations demonstrate that these high activities are unrelated to the hydrolysis of either tri- or di- or even mono-glycerides by the muscle. Consequently, the metabolic role of glycerol kinase in, for example, the flight muscles of the bumble-bee appears to be different from its role in the flight muscles of the locust. Nonetheless, despite this difference, the properties and distribution of the enzyme from bumble-bee flight muscle are similar to those of the enzyme from locust flight muscle (Table 3).

We thank Professor J. W. S. Pringle, F.R.S., for his interest and encouragement. B. C. was the recipient of a Medical Research Council Junior Research Fellowship.

References

Beenakkers, A. M. Th. (1969) *J. Insect Physiol.* **15**, 353
Beenakkers, A. M. Th., Dewaide, J. H., Henderson, P. T. & Lutgerhorst, A. (1967) *Comp. Biochem. Physiol.* **22**, 675
Biale, Y., Gorin, E. & Shafrir, E. (1968) *Biochim. Biophys. Acta* **152**, 28
Bilinski, E. (1963) *Can. J. Biochem. Physiol.* **41**, 107
Björntorp, P. & Furman, R. H. (1962) *Amer. J. Physiol.* **203**, 323
Bone, Q. (1966) *J. Mar. Biol. Ass. U.K.* **46**, 321
Bremer, J. (1962) *J. Biol. Chem.* **237**, 3628
Carroll, K. K. (1961) *J. Lipid Res.* **2**, 135
Chino, H. & Gilbert, L. I. (1965) *Anal. Biochem.* **10**, 395
Corbin, J. D., Reinmann, E. M., Walsh, P. A. & Krebs, E. G. (1970) *J. Biol. Chem.* **245**, 4849
Crabtree, B. & Newsholme, E. A. (1972) *Biochem. J.* **126**, 49
Crass, M. F., McCaskill, E. S. & Shipp, J. C. (1969) *Amer. J. Physiol.* **216**, 1569
Denton, R. M. & Randle, P. J. (1967) *Biochem. J.* **104**, 416
Garland, P. B. & Randle, P. J. (1964) *Biochem. J.* **93**, 678
George, J. C. & Berger, A. J. (1966) *Avian Myology*, Academic Press, New York and London
Gilbert, L. I. (1967) *Advan. Insect Physiol.* **4**, 69
Greenewalt, C. H. (1962) *Smithson. Misc. Collect.* **144**, no. 2
Greville, G. D. & Tubbs, P. K. (1968) *Essays Biochem.* **4**, 155
Kornberg, A. & Pricer, W. E. (1953) *J. Biol. Chem.* **204**, 329
Krebs, H. A. (1963) *Advan. Enzyme Regul.* **1**, 385
Lawrie, R. A. (1953) *Biochem. J.* **55**, 298
Lipmann, F. & Tuttle, L. C. (1950) *Biochim. Biophys. Acta* **4**, 301
Mayer, R. J. & Candy, D. J. (1969) *J. Insect Physiol.* **15**, 611
Newsholme, E. A. & Taylor, K. (1968) *Biochim. Biophys. Acta* **158**, 11
Newsholme, E. A. & Taylor, K. (1969) *Biochem. J.* **112**, 465
Newsholme, E. A., Robinson, J. & Taylor, K. (1967) *Biochim. Biophys. Acta* **132**, 338
Odum, E. P. (1965) *Adipose Tissue* 37
Randle, P. J., Garland, P. B., Hales, C. N., Newsholme, E. A., Denton, R. M. & Pogson, C. I. (1966) *Recent Progr. Horm. Res.* **22**, 1

Robinson, D. S. (1965) *Adipose Tissue* 295
Sacktor, B. (1965) in *Physiology of Insecta* (Rockstein, M., ed.), vol. 2, p. 483, Academic Press, New York and London
Shepherd, D., Yates, D. W. & Garland, P. B. (1966) *Biochem. J.* **98**, 3C
Stevenson, E. (1968) *Biochem. J.* **110**, 105
Stevenson, E. (1969) *J. Insect Physiol.* **15**, 1537
Tietz, A. (1967) *Eur. J. Biochem.* **2**, 236
Tucker, V. A. (1968) *J. Exp. Biol.* **48**, 67
Weis-Fogh, T. (1952) *Phil. Trans. Roy. Soc. London Ser. B* **237**, 1
Weis-Fogh, T. (1967) in *Insects and Physiology* (Beament, J. W. & Trehearne, J. E., eds.), p. 143, Oliver and Boyd, Edinburgh
Welty, C. (1955) *Sci. Amer.* **192**, 88
Wigglesworth, V. B. (1949) *J. Exp. Biol.* **26**, 150
Zebe, E. C. (1954) *Z. Vergl. Physiol.* **36**, 290