Derek became an established external staff member of the MRC! It was typical of Krebs to fail to notice this remarkable oversight and typical of Derek to do nothing about it! Further thoughts of moving never emerged.

One of several qualities that Derek brought to scientific research was a touch of common sense; indeed, he was a genius of common sense. It is perhaps fitting to finish on glutamine. Derek's first work with Krebs as a technician was to purify glutamine from root vegetables. It was essential for the work of Krebs on the enzymology of glutamine and its role in the body. Recently a workshop on glutamine, 'Glutamine 2000', was held in Oxford, in which international scientists presented papers on glutamine, several providing evidence that this simple amino acid is beneficial for patients in intensive care units and with severe systemic conditions. Fifty years of research on glutamine, started by Derek in Sheffield and culminating in an interactive meeting in Oxford on the biochemical, physiological and clinical importance of glutamine, is a fitting tribute to a first-class scientist.

References

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Travels with carnitine palmitoyltransferase I: from liver to germ cell with stops in between
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Abstract
Although malonyl-CoA was first recognized as a central component in the regulation of hepatic ketogenesis, its inhibitory effect on mitochondrial carnitine palmitoyltransferase I (CPT I) has come to be viewed as a key element in fuel sensing in a variety of body tissues. This broadening role of the malonyl-CoA/CPT I partnership in the control of glucose and fatty acid metabolism, as well as current understanding of structure–function relationships surrounding the CPT proteins, are outlined. The intriguing question of whether CPT I has an important function in sperm development is also addressed briefly.

Introduction
Because this symposium is being dedicated to the memory of Dr Derek Williamson, I should like to begin on a personal note by recounting the bitter-sweet occasion when our two paths first crossed at the scientific level. In 1968 I moved as a young postdoctoral fellow from the U.K. to Dallas, Texas, to join the laboratory of Dr Daniel Foster, who had just become interested in the question of how the liver regulates its production of ketone bodies from fatty acids. At the time, a plausible explanation for the greatly increased ketogenic capacity of livers from ketotic versus normal animals was that the former contain much higher levels of one or more of the enzymes in the 3-hydroxy-3-methylglutaryl-CoA cycle leading from acetyl-CoA to acetoacetate. Accordingly, we prepared liver homogenates from fed, fasted and alloxanized (ketoacidotic) rats, freeze-thawed them to disrupt the mitochondria, presented them with saturating levels of acetyl-CoA and measured the rate of production of acetoacetate. Accordingly, we prepared liver homogenates from fed, fasted and alloxanized (ketoacidotic) rats, freeze-thawed them to disrupt the mitochondria, presented them with saturating levels of acetyl-CoA and measured the rate of production of acetoacetate. To our surprise, all three liver preparations behaved similarly. Although the result was negative, we considered it important because it meant that primary control of the ketogenic process must be exerted elsewhere in the pathway. Accordingly, we quickly wrote up the manuscript and were just about to submit it for publication when I happened to open the latest issue of Biochemical Journal, only to find a paper by Williamson, Bates and Krebs [1] describing exactly the same findings! Naturally, this turn of events caused me considerable distress, but on the positive side it paved
the way for me to visit Oxford several months later and to meet Derek in person for the first time. On that and many subsequent occasions I was captivated by his unique combination of charm, sharp wit, insightful thinking and unusual modesty as we mulled over the enigmatic issue of fatty acid oxidation and its regulation. I know that these sentiments, together with immense admiration for Derek's major contributions to the field of metabolism, are shared by his numerous friends and colleagues. We will all miss him dearly.

Several years after recovering from the Oxford 'scoop', it finally dawned on us that control over the process of mitochondrial β-oxidation might be linked in some way to the opposing pathway of fatty acid synthesis. This ultimately led to the recognition in 1977 that carnitine palmitoyltransferase I (CPT I), the first step specific to fatty acid oxidation, is potently inhibited by malonyl-CoA, the product of the acetyl-CoA carboxylase (ACC) reaction and the first committed intermediate in the pathway of fatty acid synthesis [2]. It thus became clear that activation of hepatic fatty acid oxidation in ketotic states is, in large measure, an indirect result of the fall in malonyl-CoA concentration after elevation of the circulating glucagon-to-insulin ratio.

Current understanding of the CPT system

It is important to note that in the late 1970s our understanding of the mitochondrial CPT system was still quite rudimentary. A key unresolved and much debated question was whether CPT I and CPT II were the same or different proteins. In fact, it was only through application of the tools of molecular biology beginning a decade later that a clear-cut answer emerged. What we have learned from this and other initiatives, which have involved the work of many laboratories as reviewed in [3], can be summarized as follows.

1. CPT I (malonyl-CoA sensitive) and CPT II (malonyl-CoA insensitive) are indeed distinct proteins, the former tightly anchored in the mitochondrial outer membrane, the latter loosely associated with the inner membrane. Together with a carnitine-acylcarnitine translocase (also inner membrane) discovered independently by Pande [4] and Ramsay and Tubbs [5] in 1975, the CPT
enzymes function in the mitochondrial transport of long-chain fatty acids as illustrated in Figure 1.

(2) CPT II (approx. 70 kDa) is the product of a single gene located on chromosome 1p32 in humans.

(3) CPT I (approx. 88 kDa) contains an N-terminal region (not shared by CPT II) with two transmembrane domains that place it in the outer membrane, as depicted in Figure 1.

(4) CPT I exists as at least two isoforms, the liver (L) and muscle (M) variants, encoded by genes on human chromosome 11q13 and 22q13 respectively. They display profoundly different sensitivities to malonyl-CoA ($K_I \approx 0.02$ and 2 $\mu$M respectively) and affinities for carnitine ($K_m \approx 30$ and 500 $\mu$M respectively). L-CPT I is also much more sensitive than M-CPT I to the irreversible inhibitors tetradecylglycidyl-CoA and etomoxir-CoA (Et-CoA) [6]. The same is true of 2-bromo-palmitoyl-CoA (in the presence of carnitine) (V. Esser, S. Hajibashi, S. Kaka, L. Cayton, R. L. Dobbins, B. E. Stephenson and J. D. McGarry, unpublished work). None of these agents inhibit CPT II, except in competitive fashion at high concentrations. A curious feature of L-CPT I (at least in liver) is that its sensitivity to malonyl-CoA increases and decreases, respectively, with feeding and fasting, apparently because of changes in fluidity of the mitochondrial outer membrane [8]. Interestingly, M-CPT I of muscle mitochondria does not behave in this manner.

(5) Whereas CPT II is stable in detergent, CPT I loses activity when released from its membrane environment. However, we have recently succeeded in reconstituting a stable preparation of pure, recombinant rat L-CPT I in a liposome environment such that the enzyme regains catalytic activity and malonyl-CoA sensitivity [9].

(6) The molecular basis for the very different kinetic properties of L- and M-CPT I is still not fully understood, although new insights have come from studies on recombinant chimaeric constructs in which homologous regions of the two proteins have been interchanged [3,10]. As will be discussed subsequently by Victor Zammit, it appears that the widely divergent sensitivity to malonyl-CoA and affinity for carnitine between the L- and M-CPT I isoforms stem from subtle differences in their N-terminal, C-terminal and transmembrane domains and how these components interact with each other [10].

We suspect that malonyl-CoA interacts with the acyl-CoA-binding site on the enzyme through its CoA ester group and with a histidine residue (possibly His-5 and/or His-140) through its carboxy group. It seems likely that tetradecylglycidyl-CoA and Et-CoA also bind at the acyl-CoA site and that the epoxide ring on carbon 2 of these inhibitors forms a covalent link with a juxtaposed residue, causing irreversible loss of enzyme activity.

(7) Although liver and skeletal muscle produce exclusively the L and M forms of CPT I respectively, L-CPT I is found in many other tissues. Although less widespread in its distribution, M-CPT I is also strongly expressed in heart, brown fat and testes (see below). Furthermore, it is the dominant isoform of CPT I in white adipocytes of the rat, hamster and human, but surprisingly is absent from these cells in the mouse, which express only L-CPT I [11].

(8) Disease-causing mutations in the L-CPT I and CPT II genes are now being recognized with increasing frequency [12]. However, an inherited defect at the M-CPT I locus has yet to be reported.

### Expanding role of the malonyl-CoA/CPT I partnership

In 1983 we were surprised to find that malonyl-CoA is present in non-lipogenic tissues such as heart and skeletal muscle [13]. Moreover, its concentration in these sites was shown to fluctuate with feeding and fasting, just as in liver. Given the exquisite sensitivity of M-CPT I to inhibition by malonyl-CoA, the implication was that the malonyl-CoA/CPT I interaction is also at work in non-hepatic tissues, where it presumably functions in a capacity unrelated to the control of ketogenesis. In recent years this notion has gained considerable support, as reviewed by Ruderman et al. [14]. Thus malonyl-CoA in muscle cells is now believed to constitute a fuel sensor involved in cross-talk between glucose and fatty acid metabolism. When glucose is the primary oxidative fuel, pyruvate dehydrogenase is active and malonyl-CoA levels rise because of the abundance of citrate, which serves as a precursor of cytosolic acetyl-CoA and as an activator of ACC, the primary isof orm of which in muscle tissue is the 280 kDa $\beta$-variant. Under these conditions fatty acid oxidation is suppressed by the action of malonyl-CoA on M-CPT I. Conversely, when glucose utilization is limited (starvation, diabetes), malonyl-CoA levels fall and the oxidation of non-esterified fatty acids is enhanced. The concomitant elevation in mitochondrial acetyl-CoA content further limits glucose utilization, as described by Randle et al. [15].
An important new development in this area is the finding that ACCβ, unlike ACCα, contains an N-terminal extension that anchors it into the mitochondrial outer membrane, perhaps in the vicinity of CPT I [16]. This has interesting implications for the concentration of malonyl-CoA seen by CPT I versus that in the whole cell. Also coming to the fore is the role of AMP-activated kinase in regulating the malonyl-CoA concentration within muscle cells. It seems that this enzyme might have a dual role to bring about acute lowering of the malonyl-CoA pool under conditions of exercise and/or hypoxia. First, activation of the kinase after a decrease in the ATP-to-AMP ratio is known to inhibit ACC activity. In addition, evidence is mounting that AMP-activated kinase can also phosphorylate and activate malonyl-CoA decarboxylase, an enzyme that is thought to function in concert with ACC for the turnover of malonyl-CoA in muscle cells [17, 18].

It is possible that malonyl-CoA might also be a player in pancreatic β-cell function, as proposed by Prentki et al. some 10 years ago [19]. The thinking here is that after a rise in its circulating concentration, glucose is rapidly converted in the β-cell to malonyl-CoA (just as in liver) with the result that CPT I activity is suppressed, causing an increase in the cytosolic concentration of long-chain acyl-CoA, which in turn acts as a signal for insulin secretion (mechanism unclear). Certainly this model has received some experimental support. However, as reviewed in [20, 21], not all studies are in agreement with it and we believe that more definitive experiments will be required before firm conclusions can be drawn.

A recent and particularly provocative suggestion regarding the fuel sensing role of malonyl-CoA is that it might also operate in the central nervous system as a component of appetite control [22]. This idea sprang from the unexpected finding that treatment of mice with cerulenin or a compound known as C75, both inhibitors of fatty acid synthase (FAS), caused them to stop eating. Not only did tetradecyloxyfuroic acid, an inhibitor of ACC, fail to cause appetite suppression, but it also largely offset the effect of the FAS inhibitors on food intake. Because the actions of these compounds were seen whether they were administered systemically or intracerebroventricularly, and because the mRNA species for ACC and FAS were found in the hypothalamus, the authors propose that malonyl-CoA (whose concentration is expected to rise and fall after the suppression of FAS and ACC respectively) acts as a central anorectic signal, possibly through suppression of CPT I activity in neurons of the arcuate nucleus [22]. Although this concept has attractive features, it also invites some scepticism, as noted elsewhere [23]. Undoubtedly, attempts to support or refute the model are now continuing in numerous laboratories.

Finally, a question of considerable current interest to us is what M-CPT I is doing in the testis. We know that within this organ M-CPT I expression is restricted to the germ cell and begins in robust fashion at the meiotic stage of spermatogenesis [24]. What is not clear is whether the process of fatty acid oxidation has an essential role in sperm development and, if so, what this might be. If it is important, what might this mean in terms of paternal transmission of a faulty M-CPT I gene? By extension, if an individual with inherited, heterozygous M-CPT I deficiency were to be found, must the defective gene have been maternal? Would homozygosity for this condition be possible? If mutations at this locus can be genetically transmitted, will heart and skeletal muscle function be compromised? In the hope of answering some of these questions, we and others are attempting to produce a mouse line with a testis-specific knock-out of the M-CPT I gene. Also under way in several laboratories are efforts to understand how the L-CPT I and M-CPT I genes are regulated in various (patho)physiological states, an area that will be addressed by other contributors to this symposium.

In conclusion, progress in elucidation of the CPT system and the physiological significance of the interaction between malonyl-CoA and CPT I is now moving at an exponential pace despite a rather sluggish start. Only some of the exciting developments in the field have been touched on here. Some of these have brought surprises. Predictably, others will follow as we learn more about the aetiology of disease states such as obesity and type 2 diabetes in which abnormalities of lipid metabolism, possibly including dysregulation at the level of CPT I, seem to be key underlying factors.

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References
Carnitine palmitoyl transferase I and the control of myocardial β-oxidation flux

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Abstract
Carnitine palmitoyltransferase I is assumed to be rate limiting for β-oxidation in all tissues. However, the concentration of malonyl-CoA in heart and muscle is high and is enough to completely inhibit β-oxidation if this assumption is correct. In this review, we consider whether: (i) there is a malonyl-CoA-insensitive carnitine palmitoyltransferase I activity; (ii) the measured malonyl-CoA concentration in the heart is physiologically meaningful; and (iii) carnitine palmitoyltransferase I is rate-limiting for β-oxidation in the heart.

Introduction
Carnitine palmitoyl transferase I (CPT I), a transmembrane enzyme of the mitochondrial outer membrane, catalyses the transfer of an acyl moiety from a long-chain acyl-CoA ester to carnitine to form a long-chain acyl-carnitine ester, which can then enter the mitochondrion and undergo β-oxidation [1, 2]. The enzyme is a potential site for the regulation of β-oxidation flux via its physiological inhibitor, malonyl-CoA [2, 3] and is widely assumed to be the rate-limiting step in the β-oxidation of long-chain fatty acids in the heart and other tissues [1–3]. However, the concentration of malonyl-CoA in the heart is estimated to be in the range 1–10 μM [3–7]. This greatly exceeds the IC50 of heart CPT I for malonyl-CoA [8], so it is difficult to see how β-oxidation proceeds in cardiac tissue if CPT I activity is rate-limiting for β-oxidation. A similar situation exists in skeletal muscle, in which the IC50 for malonyl-CoA is even lower.

Among the possibilities to account for this observation are the following: (1) there is a malonyl-CoA-insensitive CPT I activity in the outer mitochondrial membrane (2) much of the measured malonyl-CoA is intramitochondrial or bound and therefore not available to inhibit.