some evidence for coupling of influx and efflux of glutamine across the muscle membrane, although at present we do not know the extent to which the two processes share the same transporter or reaction mechanism.

In our view, the balance of the evidence at present available does not support the existence of metabolically-important substrate or translocation cycles involving glutamine in skeletal muscle, at least in the form described by Parry-Billings & Newsholme.

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Some evidence for the existence of substrate cycles and their utility *in vivo*

The concept of substrate cycles for improving sensitivity in control was first proposed by Newsholme & Gevers in 1967, extended by Newsholme & Crabtree in 1970, and the quantitative basis of this suggested improvement, and the potential for heat generation, were established by Newsholme & Crabtree in 1976. A historical account of the development of the concept of cycling up to that time is given by Newsholme (1976). On the basis of publications since then, a sample of which is given below, we consider there is considerable evidence to support the original suggestion of utility rather than futility of such cycles and hence the statement by Rennie *et al.* (1992) that "the whole question of the general applicability and utility of intracellular substrate cycles should be challenged" is somewhat out-of-date.

First, it is important to appreciate that the original concept encompassed *both* intracellular and extracellular cycles. Thus the *extracellular* triacylglycerol/fatty acid cycle between liver and adipose tissue was discussed by Newsholme & Crabtree (1976) and it was *hypothesized* that it could play a role in "maintaining the concentrations of a fuel (fatty acid) despite large variations in its rate of utilisation". Strong support for this hypothesis is provided in the paper cited by Rennie *et al.* (1992), that of Wolfe and co-workers (1990).

Second, in contrast to the statement of Rennie *et al.* (1992), Wolfe and co-workers did report a rate of the *intracellular* triacylglycerol/fatty acid substrate cycle in man: "the rate of intracellular cycling was 1.3 μmol·kg^{-1}·min^{-1} at rest, which was 20% of the total fatty acid released". Indeed this may represent a minimum rate of cycling since Wolfe *et al.* (1990) assume that adipose tissue contains *no* glycerol kinase: this is not the case; see Robinson & Newsholme (1967). Furthermore, Wolfe *et al.* (1987*a*) demonstrated that the *intracellular* cycle is increased 15-fold in adipose tissue of patients suffering from severe burn injury and that this one cycle could contribute significantly to the increased thermogenesis in these patients. Wolfe *et al.* (1987*b*,*c*) provide evidence that infusion of glucose in man increases the rate of this cycle in adipose tissue and such changes are consistent with changes in the sensitivity of lipolysis. Other workers have also reported significant rates of the *intracellular* triacylglyerol/fatty acid cycle in adipose tissue of man *in vivo* (Coppack *et al.*, 1989, 1990) and *in vitro* (Leibel & Hirsch, 1985; Hammond & Johnson, 1987).

Third, it was proposed by Newsholme (1980) that enhanced activity of substrate cycles after exercise might explain some if not a high proportion of the oxygen debt (now known as recovery oxygen): the work of Bahr *et al.* (1990) in man has shown that, at 3 h after cessation of prolonged exercise, 50% of the recovery oxygen could be accounted for by the increased rate of the tricylglycerol/fatty acid substrate cycle.

Fourth, a direct method for measuring the rate of the intracellular triacylglycerol/fatty acid substrate cycle in adipose tissue *in vivo* in experimental animals by using tritiated water was established by Brooks *et al.* (1983). This technique has been used to show that not only does this cycle exist in adipose tissue of mouse, rat and hamster *in vivo* but that the rate can be increased by catecholamines, by glucose administration and after exercise (Brooks *et al.*, 1983; Dobbin, 1986; Mattacks & Pond, 1988; Tagliaferro *et al.*, 1990); it is known that the rate of this cycle is increased *in vivo* in man by catecholamines (Wolfe *et al.*, 1987*a*), by glucose (Wolfe *et al.*, 1987*b*) and by exercise (Wolfe *et al.*, 1990); it is also increased after exercise in several adipose tissue depots, particularly intermuscular depots in the hamster (Mattacks & Pond, 1988) and it is decreased in the adipose tissue of the rat by lactation (Hansson *et al.*, 1987).

Fifth, although a considerable amount of work has been done on the triacylglycerol/fatty acid cycle, rates of other substrate cycles both *in vitro* and *in vivo* have been demonstrated. For example, flight in the death's head hawk moth (*Acherontia atropos*) increased the rate of glucose/glucose 6-phosphate cycle by 60-fold (Surholt & Newsholme, 1983). In the bumble bee (*Bombus terrestris*), during the warm-up period prior to flight, the rate of the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in the flight muscle *in vivo* is increased by more than 2000-fold and it is shown that this cycle is responsible for most of the heat generation in this period (Surholt *et al.*, 1990). This result was predicted by the work of Newsholme *et al.* as early as 1972, in which the ecological importance of this warming up mechanism for pollination on cold spring days was emphasized. Studies *in vitro* with isolated incubated epitrochlearis muscle of the rat demonstrated that catecholamines could increase the rate of this

cycle by 20-fold (Challiss *et al.*, 1984*a*,*b*), a finding consistent with the proposed role of cycles in improving sensitivity of the flux through the phosphofructokinase reaction (Newsholme & Crabtree, 1976). Using ^{13}C-n.m.r. it has been possible to demonstrate the existence of a glycogen/glucose-1-phosphate cycle in liver of the rat *in vivo* (Barrett & Shulman, 1991) which is consistent with the finding of significant rates of this cycle in the isolated incubated soleus muscle of the rat (Challiss *et al.*, 1987).

Sixth, other substrate cycles have also been reported in man. Hers (1976) demonstrated the existence of the glucose/glucose 6-phosphate cycle in the liver of man by following the decrease in the ^{3}H/^{14}C ratio in 2-[^{3}H,^{14}C]glucose in the blood of normal subjects, a change which did not occur in patients with hepatic glucose 6-phosphatase deficiency. The rate of this cycle plus that of fructose 6-phosphate/fructose bisphosphate has been shown to decrease markedly in the hypothyroid state *in vivo* in man (Shulman *et al.*, 1985).

We fully appreciate the need for caution in the extrapolation of biochemical findings to the physiological situation (see Newsholme & Crabtree, 1981); indeed we believe we emphasized caution in our letter concerning the proposed glutamine cycles in skeletal muscle. In 1972 we proposed the *hypothesis* that bumble bee flight muscle fructose bisphosphate was involved in a substrate cycle "for the generation of heat during short periods of rest" (Newsholme *et al.*, 1972). This hypothesis was tested by Surholt *et al.* (1991) nearly 20 years later and strong evidence in support is reported. We hope someone will take up the challenge for the glutamine cycles in muscle since, if glutamine is important for the immune system, such cycles may not be physiologically or indeed clinically unimportant. We hope it takes less than 20 years.

Except perhaps for the work on thermogenesis of Surholt *et al.* (1991), Bahr *et al.* (1990) and Wolfe *et al.* (1987*a*) these findings do not prove a particular role for substrate cycles. The evidence that they play a role in control is still circumstantial, e.g. the rates of cycles are increased under conditions when increased sensitivity is known to be required, and mathematical models support this role. The same pattern of circumstantial evidence is used to support the view that interconversion cycles (covalent modification) play a role in improving sensitivity in control (Koshland *et al.*, 1982) but no *direct* quantitative physiological tests for this improvement have ever been carried out.

We accept that detailed knowledge of all the reactants of the processes of glutamine transport are required for calculation of the equilibrium nature. Our calculations and other evidence support the view that both processes are non-equilibrium [see p. 104 of Newsholme & Crabtree (1976) for division between equilibrium and non-equilibrium reactions, or see Rolleston (1972)] and, therefore, cannot be catalysed by the *same* transporter. Whether both processes provide the basis for a translocation cycle is still, however, hypothetical, as I believe we were at pains to point out in our original letter.

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Agonist-evoked Ca^{2+} entry in human platelets

In a recent report, Garcia-Sancho and colleagues asserted that agonist-induced Ca^{2+} influx into human platelets is secondary to the emptying of intracellular Ca^{2+} stores [1]. This hypothesis is based on the findings that thrombin-evoked Mn^{2+} entry lags behind the release of Ca^{2+} from intracellular stores, and that inhibitors of cytochrome *P*-450, effective in reducing store-related Mn^{2+} entry in other cell types [2,3], also inhibit agonist-evoked Mn^{2+} entry in platelets. Our own work supports the existence of a store-regulated Ca^{2+} entry pathway in platelets. However, our data do not (as incorrectly stated by Garcia-Sancho and coworkers) support the notion that such an entry pathway can account for the full response to agonists other than ADP. Additionally, our work indicates that caution is needed when interpreting data obtained using Mn^{2+} as a tracer for Ca^{2+} entry, and in the use of cytochrome *P*-450 inhibitors.

We have shown using stopped-flow fluorimetry that ADP evokes a biphasic elevation in $[Ca^{2+}]_i$ [4]. Both phases of $[Ca^{2+}]_i$ rise are associated with Mn^{2+} and therefore, we assume, Ca^{2+} entry. The first phase of entry commences without measurable delay and appears to be conducted by an ADP-receptor-operated non-selective cation channel which we have identified electrophysiologically [5,6]. ADP-evoked currents are conducted not