stimulates glutamine release at the expense of tissue glutamate while decreasing or having little effect on alanine release (Ruderman & Lund, 1972; Chang & Goldberg, 1978). In the perfused rat hindlimb total glutamine production by resting muscle is over 4-fold greater than ammonia production, but the addition of methionine sulfoximine (a glutamine synthetase inhibitor) reverses this pattern (Goodman & Lowenstein, 1977). However, during muscular contraction, when ammonia production increases markedly, there is no change in muscle glutamine production, either during contraction or the recovery phase (Goodman & Lowenstein, 1977; Meyer & Terjung, 1979). In resting muscle the purine nucleotide cycle may turn over at a low rate so as to effect amino acid deamination, and ammonia so-generated is largely removed by glutamine formation. During contraction, the fall in intracellular ATP concentration (and in that of glutamate due to transamination to form alanine) will inhibit glutamine synthetase and prevent glutamine production.

**Muscle alanine and glutamine synthesis during starvation**

It has been argued elsewhere that for muscle alanine (via hepatic gluconeogenesis) and glutamine (via renal gluconeogenesis) to contribute to the net body glucose requirement in gluconeogenic situations such as starvation, diabetes etc. the carbon for their synthesis in muscle must originate from other amino acids (Snell, 1979, 1980). Increased alanine production by muscle in starved rats has been demonstrated in vivo (Blackshear et al., 1974; Ruderman et al., 1977), in the perfused hindlimb (Goodman et al., 1978) and in isolated epitrochlaris (Karl et al., 1976), diaphragm (Snell & Duff, 1979) and extensor digitorum longus (D. A. Duff & K. Snell, unpublished work) muscles. In contrast, the release of glutamine is either unchanged or diminished during starvation (Karl et al., 1976; MacDonald et al., 1976; Goodman et al., 1978). In any case it is not clear by what pathway the total carbon for glutamate (for glutamine synthesis) could be provided from other amino acids without invoking additional tricarboxylic acid-cycle inputs, such as by 'malic' enzyme activity in diaphragm, soleus or extensor digitorum longus muscles (Snell & Duff, 1979; D. A. Duff & K. Snell, unpublished work) declines during adaptation to starvation, whereas phosphoenolpyruvate carboxykinase activity is increased (Fig. 2a). Inhibition of phosphoenolpyruvate carboxykinase activity by 1 mM-mercaptoalkanethiol in diaphragm (by 74%) and in extensor digitorum longus (by 94%) muscles decreased the valine-stimulated increase in alanine production by the muscles from starved rats in vitro by 60 and 50% respectively (Snell & Duff, 1977; D. A. Duff & K. Snell, unpublished work). Taken together these data implicate the route of alanine synthesis de novo via phosphoenolpyruvate carboxykinase in skeletal muscle. Alanine synthesized in this manner would constitute a true gluconeogenic substrate in terms of whole-body glucose homeostasis rather than simply a recycled carbon precursor (such as lactate).

We are grateful to the Wellcome Trust for generous financial support.


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**Role of the glycine-cleavage system in glycine and serine metabolism in various organs**

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Several routes have been suggested for the catabolism of glycine (Meister, 1965), but now the most prominent pathway of glycine catabolism in various vertebrates has been shown to be the direct cleavage of glycine to form methylenetetrahydrofolate, CO₂ and ammonia, followed by the further oxidation of methylenetetrahydrofolate to CO₂, possibly by the sequential action of methylenetetrahydrofolate dehydrogenase, cyclo-hydrolase and 10-formyltetrahydrofolate-NADP⁺ oxidoreductase (Yoshida & Kikuchi, 1970; Kikuchi, 1973).

The glycine-cleavage system, or so-called glycine synthase (EC 2.1.2.10), consists of four protein components, which have tentatively been named P-protein (a pyridoxal phosphate-containing protein), H-protein (a lipocalic acid-containing protein, initially called hydrogen-carrier protein), T-protein (a protein catalysing the tetrahydrofolate-dependent step of the reaction).
and L-protein (a lipoamide dehydrogenase). The glycine-cleavage system in animals is confined to mitochondria possibly as an enzyme complex that is loosely bound to the mitochondrial inner membrane. The reaction is completely reversible, and in both glycine cleavage and glycine synthesis an aminomethyl moiety bound to the pyridoxic acid of H-protein represents an intermediate that is subsequently degraded to, or can be formed from, methylenetetrahydrofolate and ammonia by the action of T-protein (Motokawa & Kikuchi, 1974; Kochi & Kikuchi, 1976). A tentative scheme for the overall reaction of the reversible glycine cleavage is shown in Fig. 1.

One of the most characteristic properties of the glycine-cleavage reaction is that, although P-protein should belong to a class of pyridoxic phosphate-dependent amino acid dehydrogenases, P-protein requires H-protein to catalyse the decarboxylation of glycine significantly. Moreover, P-protein alone is able, though slightly, to catalyse the exchange of glycine with CO₂, and the exchange activity catalysed by P-protein is greatly increased when H-protein is also present. The suspected intimate functional association of P-protein and H-protein was studied by using P-protein and H-protein preparations that had been purified to apparent homogeneity (Kikuchi, 1976). For instance, the glycine-cleavage activity was about 10% of that of the H-protein (K., 1974). Kochi & Kikuchi, 1976). Nevertheless the H-protein from the patient exhibited a high glycine-cleavage activity as compared with the control H-protein (K., 1974) and was found to be more active than the patient's P-protein (K., 1974). Kochi & Kikuchi, 1976). Recently we had an opportunity to examine in more detail the liver and brain specimens from a non-ketotic hyperglycaemia patient and found that the primary lesion in the glycine-cleavage system in this patient was an anomaly of H-protein in that the purified H-protein was apparently devoid of pyridoxic acid (G. Kikuchi, K. Hiraga, H. Kochi & K. Hayasaka, unpublished work). Nevertheless the H-protein from the patient definitely stimulated glycine decarboxylation as well as the glycine-CO₂ exchange reaction catalysed by the purified chicken liver P-protein, although the specific activity of the purified H-protein in stimulating the P-protein-catalysed glycine-CO₂ exchange was only about 5% of that of the H-
The interrelationship between glutamine and alanine in the intestine

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The epithelial cells of the small intestine can receive substrates for metabolism from two separate sources, the mesenteric blood and the intestinal lumen (Parsons, 1979). The first source, from blood passing through capillaries at the base of the epithelial cells, is probably important under all physiological circumstances, but must be particularly so in the post-absorptive animal, when no substrates are available from the absorption of products of the digestion of food from the lumen.

Metabolism of glutamine to alanine by tissue slices of the small intestine was reported by Finch & Hird (1960), and it was probably thought merely that glutamine, like glutamate and aspartate (Neame & Wiseman, 1957), was transaminated during its absorption from the lumen of the intestine. The importance of the intestinal tract as a major site for the metabolism of glutamine has become apparent only recently, with the demonstration that the intestine could take up glutamine from the blood (Matsutaka et al., 1973; Windmueller & Spaeth, 1974), and with the realization that the non-hepatic splanchic bed was a major source of the glutamine used by the intestine of post-absorptive animals (Ruderman & Lund, 1972).

Site of glutamine metabolism

The major site of metabolism of glutamine in the non-hepatic splanchic bed is the mucosa of the small intestine. Metabolism of glutamine from both the vascular perfusate and from the lumen has been demonstrated with the vascularly perfused jejunum and ileum in vitro (Hanson & Parsons, 1977a) and with the jejunum in vivo (Windmueller & Spaeth, 1975). Metabolism of glutamine occurs at high rates in epithelial cells isolated from the small intestine (Baverel & Lund, 1979; Watford et al., 1979a), and the mucosa of the small intestine has the highest specific activity of glutaminase (Fig. 1) of any region of the gastrointestinal tract, that in the muscle layers of the small intestine, by contrast, being particularly low (Pinkus & Windmueller, 1977).

Glutamine as a respiratory substrate

Addition of glutamine stimulates the consumption of O2 by rabbit ileum (Neptune, 1965) and by isolated epithelial cells from the rat small intestine (Towler et al., 1978; Watford et al., 1979a), and 55% of the 14C from [14C]glutamine is found to be incorporated into CO2 by rat jejunum in vivo (Windmueller & Spaeth, 1978). Glutamine is therefore a respiratory fuel for the small-intestinal mucosa, and its combustion makes a major contribution (35%) to the total CO2 produced by the jejunum of rats deprived of food overnight (Windmueller & Spaeth, 1978).

Production of alanine and NH3 from glutamine

The metabolism of glutamine stimulates the production of alanine and NH3 by a variety of preparations of rat small intestine (Windmueller & Spaeth, 1972; Hanson & Parsons, 1977a; Watford et al., 1979a). The low activity of glutamine transaminase (EC 2.6.1.15) relative to that of glutaminase (Fig. 1) suggests that most glutamine is converted into glutamate by glutaminase. Production of NH3 ranges from 6% to 124% of the rate of glutamine utilization (Windmueller & Spaeth, 1978; Baverel & Lund, 1979; Watford et al., 1979a; P. J. Hanson & D. S. Parsons, unpublished work), which suggests that relatively little glutamate is then metabolized by glutamate dehydrogenase (Fig. 1) (Volman-Mitchell & Parsons, 1974) under normal circumstances. Rather it would seem that glutamate is transaminated with pyruvate by alanine aminotransferase to produce alanine and 2-oxoglutarate (Fig. 1). Little alanine amino- transferase activity (5—10%) is found in the particulate fraction of rat small-intestinal mucosa (Volman-Mitchell & Parsons, 1974), and negligible (0.27 ± 1.6%) activity is associated with mitochondria in the mucosa of the small intestine of mice (P. Sethi & P. J. Hanson, unpublished work; results corrected for contamination of the particulate cell fractions by cytosolic alanine aminotransferase). Transamination of glutamate with pyruvate may therefore be localized within the cytosol (see below).