Amino acid metabolism in the piglet

2.* Influence of fasting on plasma free amino acid concentration and in vivo oxidation of methionine, isoleucine and threonine

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1. The influence of a 24 h fast on the concentrations of free amino acids in the plasma, and upon the oxidation rates of methionine, isoleucine and threonine was studied (using early weaned, 4-week-old piglets which were receiving a semi-purified diet.

2. There was no change in the total concentration of the essential amino acids as a result of the 24 h fast: the concentration of the branched-chain amino acids increased, but the effect of this was offset by decreases in the concentrations of arginine, histidine, lysine, methionine and phenylalanine. There was a reduction in the concentration of the non-essential amino acids.

3. The piglets received infusions of L-[1-^{14}C]methionine, L-[U-^{14}C]isoleucine and L-[U-^{14}C]-threonine, and the recovery of the label in carbon dioxide was determined. Less than 5 % of the activity from methionine was recovered in the CO_2 from the fed piglets, whereas 12 % was recovered from the fasted piglets. The corresponding values with threonine were 11 and 19 % but there was no effect of fasting on the recovery of the label from isoleucine in CO_2.

4. The initial dilution of a single dose of a labelled amino acid infused into the bloodstream depends on the plasma concentration of the amino acid. Nutritional regimens may effect the free amino acid concentration in the plasma. Thus comparisons based upon direct determination of activity recovered in CO_2 from the labelled dose of an amino acid with animals on different nutritional regimens could be misleading, unless the differences in the concentrations of the amino acid in the plasma are considered.

The influence of dietary balance on amino acid metabolism has been studied by measuring growth, nitrogen balance and plasma amino acid concentrations as response criteria. More recently measurement of the oxidation of labelled amino acids has been proposed for this purpose (Brooks, Owens & Garrigus, 1972). Neale & Waterlow (1974) used labelled amino acids to study the adaptation of rats to a low-protein diet. On the basis of the uptake of the label by the tissues they concluded that the rats on the protein-free diet were conserving the essential amino acids, but the results of their studies of the recovery of label in the expired carbon dioxide did not confirm this finding.

The general hypothesis on which studies of either amino acid levels in the plasma, or amino acid oxidation are based is that if an amino acid is limiting (or deficient) in the diet, the main proportion will be used for protein synthesis. Thus only a small proportion of the intake will remain, either to accumulate in the plasma or to be oxidized to CO_2. Increasing the supply of the amino acid to a point which exceeds the need for protein synthesis would allow increases in the concentration in the plasma, and in the amount oxidized to CO_2. These increases should be a function of the excess of the intake over the amount required for protein synthesis. This relationship was found between dietary lysine intake, and both plasma lysine concentration,

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Table 1. *Composition (g/kg) of experimental diets fed to piglets*

Ingredients

Maize starch*	465·0
Isolated soya-bean protein†	360·0
Cellulose‡	100·0
Maize oil	20·0
Limestone (380 g calcium/kg)	8·0
Dicalcium phosphate (200 g Ca, 200 g phosphorus/kg)	24·0
Vitamin mixture§	5·0
Mineral mixture ‖	10·0
Salt (sodium chloride)	3·0
DL-methionine	5·0

* 'Pearl Starch'; St Lawrence Starch Co. Ltd., Port Credit, Ontario, Canada.
† 'Promosoy-100'; Central Soy Co. Inc., Chicago, Illinois, USA.
‡ 'Alpha-Floc'; Brown & Co. Inc., Berlin, New Hampshire, USA.
§ Supplied (mg/kg diet): nicotinic acid 44, calcium D-pantothenate 28·4, riboflavin 6, pyridoxine 3·6, thiamin hydrochloride 3, pteroylmonoglutamic acid 2, D-biotin 0·2, choline chloride 1000, DL-α-tocopheryl acetate 15, phylloquinone 0·2, retinyl acetate 0·8, cholecalciferol 0·011, cyanocobalamin 0·044, ethoxyquin 500.
‖ Supplied (mg/kg diet): potassium (as carbonate) 1000, magnesium (as sulphate) 400, iron (as sulphate) 125, zinc (as sulphate) 100, manganese (as sulphate) 20, copper (as sulphate) 6, selenium as sodium selinte) 0·1.

and the recovery of ^{14}C in CO_2 after an infusion of ^{14}C-labelled lysine in rats (Brooks *et al.* 1972) and in sheep (Brooks, Owens, Brown & Garrigus, 1973). However, New-port, Chavez, Horney & Bayley (1976) using young pigs did not find a similar relation-ship between the methionine intake and the recovery of ^{14}C in CO_2 after an infusion of ^{14}C-labelled methionine.

These three studies were made using diets containing graded levels of the amino acid being studied, thus the higher intake of the amino acid would be expected to increase both the plasma concentration and the amount of the amino acid being oxidized. In the present study, changes in the concentration of amino acid in the plasma were induced by fasting for 24 h rather than by feeding graded levels of amino acid. The recoveries of ^{14}C in CO_2 after infusions of ^{14}C-labelled amino acids were estimated in the fed and fasted piglets to determine how they were affected by the plasma concentration of the amino acid being studied. The essential amino acids selected for this study were: isoleucine as an example of the branched-chain amino acids, methionine as an example of an amino acid whose concentration in the plasma decreased during fasting, and threonine whose concentration in the plasma was not greatly influenced by fasting (Atkinson & Bayley, 1973).

<center>EXPERIMENTAL</center>

The experiments were done using Yorkshire piglets which were weaned between 17 and 21 d of age and transferred to individual cages in the laboratory. They received a semi-purified diet (Table 1) *ad lib.* and water was freely available. The piglets were fitted with catheters which passed through the jugular vein into the vena cava to permit blood sampling and intravenous infusion of the labelled amino acids. Recovery of the label in CO_2 from the ^{14}C-labelled amino acids was measured by placing the piglets in

Plexiglass chambers for 6 h. Air was drawn from these at 15 l/min and passed through a series of traps containing organic solvents to absorb the CO_2. The amount of radio-activity in the solvent was measured by liquid-scintillation counting. Further details of these procedures are given by Newport *et al.* (1976).

Samples of the isolated soya-bean-protein concentrate were hydrolysed under N_2 in a sealed tube with 6 M-hydrochloric acid at 110° for 24 h and the hydrolysate filtered and evaporated to dryness at 45°. The sample was redissolved in sodium citrate buffer (pH 2), and the amino acids determined by ion-exchange chromato-graphy (TSM Amino Acid AutoAnalyzer with retrofit modification; Technicon Instrument Corp., Tarrytown, New York, USA). These analyses indicated that the experimental diet contained (g/kg): 8 methionine, 9·5 isoleucine, 8 threonine.

Effect of fasting on plasma amino acid concentration

The determinations were made with 4-week-old piglets which had received the experimental diet from 3 weeks of age. Blood samples were taken through the catheters at 10.00 hours from each of five 4-week-old piglets which were consuming 177 g food/d. The piglets were then fasted (with access to water) for 24 h and a further sample taken. Each sample of blood was transferred to heparinized tubes, centrifuged and the plasma deproteinized with an equal volume of sulphosalicylic acid solution (90 g/l). The deproteinized supernatant fraction of the plasma was evaporated to dryness under reduced pressure at 45°, and was then suspended in sodium citrate buffer, pH 2, and frozen. The amino acids in these samples were determined by ion-exchange chromatography, as described previously.

Oxidation of amino acids

The following amino acids were purchased from Amersham-Searle Ltd, Don Mills, Ontario: L-[1-^{14}C]methionine (62 mCi/mmol), L-[U-^{14}C]isoleucine (243 mCi/mmol), and L-[U-^{14}C]threonine (332 mCi/mmol). They were diluted to an appropriate con-centration in saline solution (9 g sodium chloride/l) for administration in 1 ml. The doses of labelled methionine and isoleucine were 2 μCi but for threonine were only 1 μCi.

Two chambers were available enabling the determination to be made with pairs of pigs: food was withdrawn from the pig designated to fast at 10.00 hours, but the pig designated as 'fed' continued to have *ad lib.* access to the food. The next day at 10.00 hours both the fed and the fasted pigs were placed in the chambers where neither food nor water was available. The tracer dose was infused, the catheter flushed with saline and the chamber sealed. The temperature inside the chambers increased from room temperature (24°) to 30° in the 6 h experimental period. The concentration of CO_2 in the exhaust air was usually 1% and never exceeded 2%.

The procedure for collecting $^{14}CO_2$ was verified by infusing 1 μCi NaH$^{14}CO_3$ into a pig; 90% of the activity was recovered in the organic solvents during the 6 h collection period, with more than 50% of the activity being collected within 30 min of the in-fusion. The samples were counted with an average efficiency of 70%.

Table 2. *Influence of a 24 h fast on the concentration (μmol/l) of free amino acids (FAA) in the plasma of early weaned, 4-wk-old piglets receiving a semi-purified diet*

(Mean values with their standard errors for five fed pigs which weighed 5.3 ± 0.3 kg, and four fasted pigs which weighed 4.7 ± 0.4 kg)

| | FAA concentration | | | | Change in FAA concentration | |
| | Fed | | Fasted | | | |
Essential amino acids	Mean	SE	Mean	SE	Absolute	Relative*
Arginine	121	12	57	4	−64	−53
Histidine	56	7	37	3	−19	−34
Isoleucine	102	5	118	3	+16	+16
Leucine	91	6	155	9	+64	+70
Lysine	117	10	96	9	−21	−18
Methionine	71	6	21	7	−50	−70
Cystine	22	2	27	4	+5	+23
Phenylalanine	71	6	42	3	−29	−61
Threonine	52	3	63	3	+11	+21
Valine	148	13	240	5	+92	+62
Total	851		856		+5	+1
Non-essential amino acids						
Alanine	279	21	111	14	−168	−60
α-Aminobutyric acid	20	4	39	4	+19	+95
Aspartic acid	21	1	10	1	−11	−52
Asparagine	177	17	40	3	−137	−77
Glutamic acid	231	37	96	9	−135	−58
Glycine	534	32	265	10	−269	−50
Ornithine	86	8	40	5	−46	−54
Serine	85	9	41	4	−34	−40
Tyrosine	47	5	33	2	−16	−30
Total	1480		685		−795	−54
Ratio, total essential:total non-essential amino acids	0.67		1.25			

* Amino acid concentrations of fed pigs equivalent to 100.

Statistical procedures

The standard errors of each mean were calculated and the significance of the results was assessed using Student's *t* test as described by Steel & Torrie (1960).

RESULTS

Fasting the 4-week-old piglets resulted in significant decreases in the concentrations of arginine, histidine, methionine, and phenylalanine. (Table 2.) There were no significant changes in the concentrations of lysine and cystine, but the concentrations of isoleucine, leucine and valine increased significantly, so that over all, there was no change in the total concentration of essential amino acids as a result of fasting. In contrast there was a marked decrease in the total concentration of non-essential amino acids: the concentrations of alanine, asparagine, glutamic acid and glycine being halved by the fast.

Less than 5 % of the activity administered in the dose of [^{14}C]methionine was re-
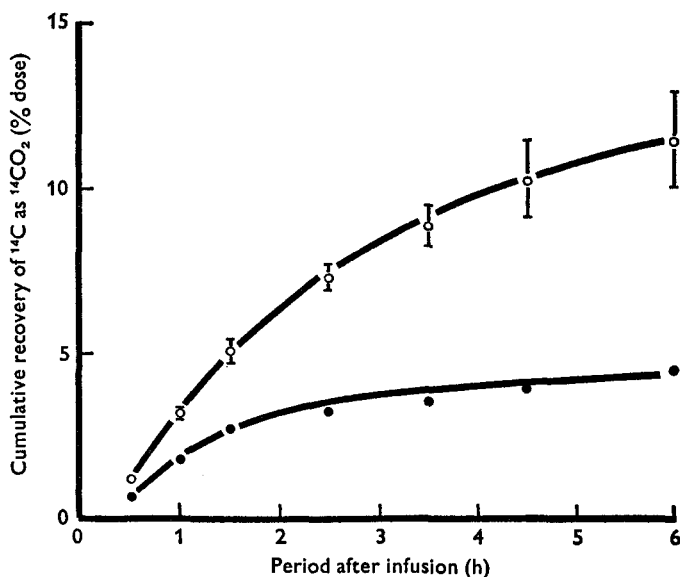
Fig. 1. Cumulative recovery of ^{14}C as $^{14}CO_2$ in the 6 h period after the infusion of a dose of 2 μCi L-[1-^{14}C]methionine. Five pigs, mean body-weight 6·3 kg, which had been fed until the infusion (●—●), and five pigs, mean body-weight 5·5 kg, which had been fasted for 24 h before the infusion (○—○). Standard errors, represented by vertical bars, for the fed animals increased from 0·01 to 0·23 % dose from the 1st to the 6th hour of the experiment.
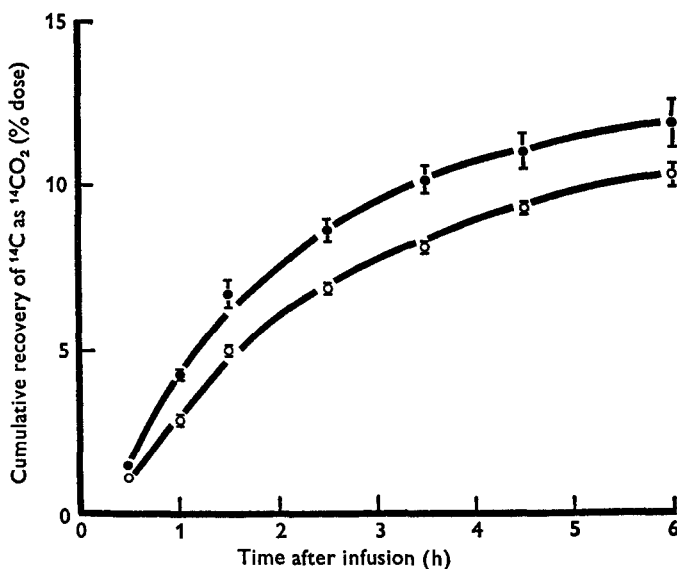

Fig. 2. Cumulative recovery of ^{14}C as $^{14}CO_2$ in 6 h period after the infusion of a dose of 2 μCi L-[U-^{14}C]isoleucine. Seven pigs, mean body-weight 6·2 kg, which had been fed until the infusion (●—●), and seven pigs, mean body-weight 6·0 kg, which had been fasted for 24 h before the infusion (○—○). Standard errors are represented by vertical bars.
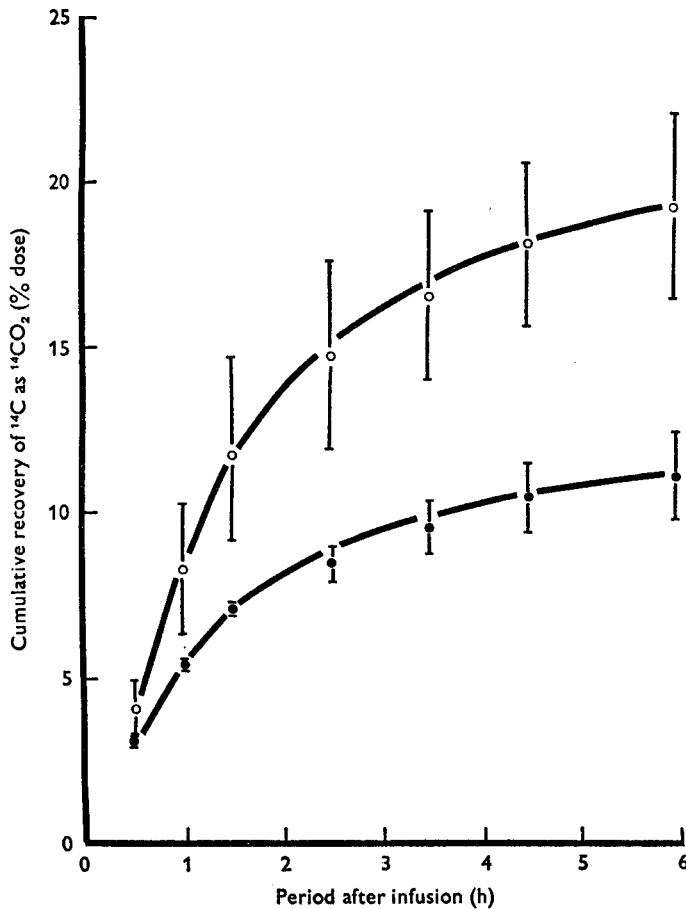
Fig. 3. Cumulative recovery of ^{14}C as $^{14}CO_2$ in 6 h after the infusion of a dose of 1 μCi L-[U-^{14}C]threonine. Five pigs, mean body-weight 6·6 kg, which had been fed until the infusion (●—●), and five pigs, mean body-weight 5·5 kg, which had been fasted for 24 h before the infusion (○—○). Standard errors are represented by vertical bars.

covered in the CO_2 produced by the piglets which had been fed until the infusion (Fig. 1); most of this activity was recovered in the first 2 h. The piglets which had been fasted for the 24 h before infusion continued to release $^{14}CO_2$ throughout the 6 h experimental period. Thus, 12 % of the activity administered in the [^{14}C]methionine had been recovered at the end of the experimental period. The shape of the $^{14}CO_2$ recovery v. time after infusion curve suggested that if the experimental period had been extended the activity recovered would have increased for the fasted piglets, but for the fed piglets there would have been little effect. In contrast, the recoveries of activity as $^{14}CO_2$ from the [^{14}C]isoleucine dose were similar for both the fed and fasted piglets (Fig. 2); both groups continued to produce $^{14}CO_2$ throughout the 6 h experimental period. The pattern of the recoveries of $^{14}CO_2$ from the [^{14}C]threonine dose (Fig. 3) was similar to that for [^{14}C]methionine, however, in this instance the output from the fed piglets did not reach a plateau until 4 h after the infusion.

Table 3. *Recovery (% dose) of ^{14}C as $^{14}CO_2$ in the 6 h period after the infusion of 2 μCi* L-[1-^{14}C]methionine or L-[U-^{14}C]isoleucine or 1 μCi L-[U-^{14}C]threonine to piglets which *had been either fed or fasted in the 24 h before the infusion*

	L-methionine		L-isoleucine		L-threonine	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
No. of animals	5	5	7	7	5	5
Body-wt (kg)	6·3	5·5	6·2	6·0	6·6	5·5
Recovery of ^{14}C as $^{14}CO_2$ (% dose): mean	4·5	11·6	11·8	10·3	11·0	19·2
SE	0·23	3·04	1·39	0·43	2·75	5·57
Statistical significance of effect of nutritional regimen:* P	< 0·01		NS		< 0·01	

NS, not significant.

* Student's t test with 8 df for L-methionine and L-threonine, and 12 df for L-isoleucine.

The total recoveries of the activity within the 6 h after the infusion are summarized in Table 3. The increases in the recoveries of the label from [^{14}C]methionine and [^{14}C]threonine as a result of fasting were highly significant, but there was no significant effect of fasting on the recovery of the label from [^{14}C]isoleucine. The standard errors of the $^{14}CO_2$ recoveries from [^{14}C] methionine and [^{14}C]threonine were increased by fasting, indicating a much greater variation in the metabolism of these amino acids in the fasted than in the fed pigs.

DISCUSSION

Fasting the 4-week-old piglets caused marked changes in the concentrations of the amino acids in the plasma: values for total essential:total non-essential amino acid concentrations doubled, this is similar to the finding of Grimble & Whitehead (1969), who maintained pigs on a low-protein diet from 4 weeks of age. They found that the pigs became anorexic after 10 weeks and stopped growing, and values for total essential:total non-essential amino acid concentrations in serum doubled. The concentrations of some essential amino acids decreased as a result of the 24 h fast, but this effect was offset by the increase in the concentrations of the branched-chain amino acids. This finding is different from those of Richardson, Hale & Ritchey (1965) and of Typpo, Meade, Nordstrom & Stockland (1970), who found no significant changes in the concentrations of these amino acids during fasting in pigs. In contrast, Shimada & Zimmerman (1973) found that the concentration of the branched-chain amino acids decreased during a 24 h fast in 10-d-old piglets.

Only a small part of the body pool of free amino acids is present in the plasma (Young, 1970), but it is generally assumed that alterations in the concentrations of amino acids in the plasma reflect the changes taking place in the free amino acid concentrations in other tissues (Adibi, 1971), although Atkinson (1976) found that this is not the situation in young pigs for glutamic acid, glutamine, serine and lysine.

Changes in the concentration of an amino acid in the plasma, such as those induced by fasting in the present study could be particularly important in the interpretation of the recovery of ^{14}C in CO_2 after the infusion of ^{14}C-labelled amino acids. The plasma

Table 4. *Comparative values for plasma amino acid concentrations ($\mu mol/l$), ^{14}C recovered as $^{14}CO_2$ (% dose) and oxidation rate of plasma amino acids ($\mu mol/l$) in the 6 h period after a single dose of ^{14}C-labelled amino acid infused in fed and 24 h-fasted piglets*

(Mean values for five pigs for L-methionine and L-threonine, and for seven pigs for L-isoleucine: values relative to the respective 'fed' values (taken as 100) are given in parentheses)

| | Fed | | Fasted |
Amino acid infused	Determined value	Adjusted* value	Determined value
L-[1-^{14}C]methionine			
Plasma concentration	71 (100)	21	21 (30)
^{14}C recovered as $^{14}CO_2$	4·5	15·1 (100)	11·6 (77)
Plasma methionine oxidized† ($\mu mol/l$)	3·2	—	2·4
L-[U-^{14}C]isoleucine			
Plasma concentration	102 (100)	118	118 (116)
^{14}C recovered as $^{14}CO_2$	11·8	10·2 (100)	10·3 (101)
Plasma isoleucine oxidized† ($\mu mol/l$)	12·0	—	12·2
L-[U-^{14}C]threonine			
Plasma concentration	52 (100)	63	63 (121)
^{14}C recovered as $^{14}CO_2$	11·0	9·1 (100)	19·2 (211)
Plasma threonine oxidized† ($\mu mol/l$)	5·7	—	12·1

* Values adjusted to the values which would have been obtained if the initial dilutions of label in plasma had been uniform (for details, see below).

† $\dfrac{^{14}CO_2 \text{ recovered (\%)}}{100} \times$ plasma amino acid concentration.

free amino acid pool is the first 'functionally homogeneous compartmental content of the body' (Christensen, 1964) to be instantaneously labelled when a tracer dose of an amino acid is infused into the bloodstream of an animal. Sketcher & James (1974) considered the influence of changing pool size on the interpretation of results from amino acid oxidation studies in animals where the digesta is making an intermittent contribution to the body pool of free amino acids. Their conclusion was that this precluded any measure of the absolute rate of oxidation of an amino acid. However, in the present study differences in plasma amino acid concentrations were induced by fasting and neither the fed nor the fasted pigs were consuming any food during the course of the 6 h experimental period.

The recoveries of $^{14}CO_2$ from the tracer doses of ^{14}C-labelled methionine, isoleucine and threonine should be determined in relation to the concentrations of the amino acids being studied in the plasma of the fed and fasted piglets. The methionine dose was infused into fed pigs in which the plasma contained 71 $\mu mol/l$ whereas in the fasted piglets the corresponding concentration was only 21 $\mu mol/l$ and thus the initial dilutions of the label would be different. Using this difference in initial dilution as a factor, the recoveries obtained for label in CO_2 from the fed pigs can be adjusted to what it would have been, if the initial dilutions had been uniform. Alternatively the comparison of activity administered with that recovered could be considered simply as a measure of the oxidation rate of the amino acid in the blood plasma (Table 4).

Since the differences in concentrations of isoleucine and threonine are less marked than for methionine, adjustment of their recoveries had little effect on the proportion of the label recovered from these two amino acids in the expired CO_2.

The adjusted value indicated that fasting reduces the rate at which methionine is oxidized, which is consistent with the relatively lower proportion of methionine provided by the endogenous source, procine muscle protein (Altman & Dittmer, 1968), than in the exogenous source of protein provided through the diet used in this study. In contrast, fasting increased the catabolism of threonine, the second most abundant essential amino acid in porcine muscle. This amino acid has been found to be poorly conserved in rats even when a threonine-free diet is given (Yamashita & Ashida, 1970). Isoleucine oxidation was not influenced by the imposition of a 24 h fast; this amino acid, along with leucine and valine are oxidized in the muscle rather than in the liver, as are methionine and threonine (Elwyn, 1970), and the metabolism of muscle is less susceptible to changes in nutritional status than is the liver. Similarly, even though the recoveries of the label in CO_2 from both ^{14}C-labelled isoleucine and threonine were equal in the fed pigs, it cannot be concluded that both amino acids were being oxidized at the same rate, because the concentration of isoleucine in the plasma was twice that of threonine.

The results presented in this report suggest that direct measurements of ^{14}C recovered in the expired CO_2 as a proportion of the dose of ^{14}C-labelled amino acid may be misleading when applied to nutritional regimens which alter the pool size of the free amino acid being studied. It is suggested that the changes in plasma concentrations be included in the interpretation as a first step in overcoming the shortcomings in the single-dose amino acid oxidation technique (Neale & Waterlow, 1974; Sketcher & James, 1974).

REFERENCES

Adibi, S. A. (1971). *Am. J. Physiol.* **221**, 829.
Altman, P. L. & Dittmer, D. S. (1968). *Metabolism.* Bethesda, Maryland: Federation of American Societies for Experimental Biology.
Atkinson, J. L. (1976). Nutrient utilization during post natal development of the pig. Ph.D. Thesis, University of Guelph, Ontario, Canada.
Atkinson, J. L. & Bayley, H. S. (1973). *Fedn Proc. Fedn Am. Socs exp. Biol.* **32**, 910.
Brooks, I. M., Owens, F. N., Brown, R. E. & Garrigus, U. S. (1973). *J. Anim. Sci.* **36**, 965.
Brooks, I. M., Owens, F. N. & Garrigus, U. S. (1972). *J. Nutr.* **102**, 27.
Christensen, H. N. (1964). In *Mammalian Protein Metabolism*, vol. 1, p. 105, [H. N. Munro, editor]. New York: Academic Press.
Elwyn, D. H. (1970). In *Mammalian Protein Metabolism*, vol. 4, p. 525, [H. N. Munro, editor]. New York: Academic Press.
Grimble, R. F. & Whitehead, R. G. (1969). *Br. J. Nutr.* **23**, 791.
Neale, R. J. & Waterlow, J. C. (1974). *Br. J. Nutr.* **32**, 257.
Newport, M. J., Chavez, E. R., Horney, F. D. & Bayley, H. S. (1976). *Br. J. Nutr.* **36**, 87.
Richardson, L. R., Hale, F. & Ritchey, S. J. (1965). *J. Anim. Sci.* **24**, 368.
Shimada, M. A. & Zimmerman, D. R. (1973). *J. Anim. Sci.* **36**, 245.
Sketcher, R. D. & James, W. P. T. (1974). *Br. J. Nutr.* **32**, 615.

Steel, R. G. D. & Torrie, J. H. (1960). *Principles and Procedures of Statistics.* New York: McGraw Hill Book Co. Inc.
Typpo, J. T., Meade, R. J., Nordstrom, J. W. & Stockland, W. L. (1970). *J. Anim. Sci.* **31**, 885.
Yamashita, K. & Ashida, K. (1970). *J. Nutr.* **99**, 267.
Young, V. R. (1970). In *Mammalian Protein Metabolism*, vol. 4, p. 585, [H. N. Munro, editor]. New York: Academic Press.