The catabolism of valine in the malnourished rat. Studies in vivo and in vitro with different labelled forms of valine

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1. The catabolism of valine was estimated in vivo by measurement of the production of labelled CO_2 for 2 h after the oral administration of either [U-^{14}C]valine or [1-^{14}C]valine. It was also estimated in vitro in homogenates of liver and muscle incubated with labelled valine. Experiments were performed in rats given diets providing either 215 g (HP) or 25 g (LP) protein per kg diet.

2. The proportion of [U-^{14}C]valine excreted as $^{14}CO_2$ was not reduced in rats given the LP diet for 16 d but the excretion of $^{14}CO_2$ from [1-^{14}C]valine was reduced by 40% in these animals. When rats were transferred from the HP diet to the LP diet there was a reduction in the excretion of $^{14}CO_2$ from [1-^{14}C]valine; when the diet was changed from LP to HP output of $^{14}CO_2$ increased to control values.

3. Homogenates of muscle and liver catabolized valine to CO_2. Both liver and muscle from rats fed on the LP diet catabolized less [1-^{14}C]valine than tissues from control animals.

4. Valine aminotransferase activity was higher in muscle than in liver, and did not change in tissues from rats fed on the LP diet. In these animals 2-ketoisovaleric acid dehydrogenase activity was reduced in both liver and muscle.

5. The production of $^{14}CO_2$ was lower with [U-^{14}C]valine as the substrate than with [1-^{14}C]-valine and there was no difference between tissues from rats fed on the HP and LP diets.

6. The results with [1-^{14}C]valine suggest that both liver and muscle from protein-depleted rats catabolize valine at a reduced rate. The reason for the discrepancy between these results and those with [U-^{14}C]valine is not clear. It is concluded that the results with [U-^{14}C]valine in vitro are affected by dilution of the label before the formation of $^{14}CO_2$, but that this does not hold in vivo.

The excretion of nitrogen in the urine varies with the protein content of the diet in man and in the rat (cf. Chan, 1968; Neale, 1971). These changes in nitrogen excretion are accompanied by alterations in the activities of enzymes of the urea cycle (Schimke, 1962; Stephen & Waterlow, 1968; Das & James, 1972). Similarly, several studies of the excretion of the carbon of essential amino acids have suggested that their carbon catabolism is reduced in animals given diets low in protein or lacking one essential amino acid (for example, Kim & Miller, 1969; McFarlane & von Holt, 1969*a*; Yamashita & Ashida, 1969; Aquilar, Harper & Benevenga, 1972). There are, however, many problems in the interpretation of experiments in which the labelling of respiratory CO_2 is estimated after the administration of labelled amino acids (discussed by Kim & Miller (1969) and Neale & Waterlow (1974)) and there are several reports in which experiments with malnourished animals have provided no evidence for conservation of the carbon of essential amino acids (Neale, 1971, 1972; Neale & Waterlow, 1974).

The branched-chain amino acids are unusual among the essential amino acids in that they are catabolized to a significant extent by extrahepatic tissues (Miller, 1962) and in particular by skeletal muscle (Manchester, 1965; Pain & Manchester, 1970;

Neale, 1972; Odessey & Goldberg, 1972). It is well established that the concentrations of the branched-chain amino acids, and in particular valine, are much reduced in the plasma of malnourished children (Arroyave, Wilson, de Funes & Béhar, 1962; Grimble & Whitehead, 1971), and it has been proposed by Neale (1972) that one of the major reasons for this is the continuing high rate of catabolism of these amino acids by muscle. The finding of increased activity of branched-chain aminotransferase in the skeletal muscle of malnourished rats (Mimura, Yamada & Swenseid, 1968) supports this conclusion. On the other hand, McFarlane & von Holt (1969*a*) found that adult rats which had been fed on a low-protein diet for a long time catabolized less leucine to CO_2 than age-matched controls. These authors subsequently demonstrated a reduction in the activity of 2-ketoisocaproic acid dehydrogenase in mitochondria obtained from the livers of malnourished rats (McFarlane & von Holt, 1969*b*). This result was later confirmed for all three branched-chain keto-acid dehydrogenases by Wohlhueter & Harper (1970).

Apart from the experiments of Mimura *et al.* (1968) and some results for kidney and heart muscle reported by Wohlhueter & Harper (1970), most attention has been paid to the catabolism of the branched-chain amino acids by liver. Indeed, Wohlhueter & Harper (1970) were unable to find any branched-chain keto-acid dehydrogenase activity in homogenates of rat skeletal muscle, a finding which is surprising in view of the ability of this tissue to catabolize the branched-chain amino acids to CO_2.

In previous experiments in vivo different labelled forms of leucine and valine have been used (McFarlane & von Holt, 1969*a*; Neale, 1971, 1972), and it is possible that the different results obtained in these two studies were the result of different positions of the ^{14}C-label in the amino acids. The experiments reported in this paper were designed in part to test this possibility by the comparison of results obtained with uniformly labelled valine ([U-^{14}C]valine) and with valine labelled only in the carboxylic acid group ([1-^{14}C]valine). They were also designed to test the catabolism of valine in vitro in homogenates prepared from liver and muscle and, in particular, to investigate the ability of skeletal muscle to reduce the rate at which it catabolizes valine in response to the prolonged ingestion of a diet inadequate in protein. The weanling rat model described by Flores, Sierralta & Monckeberg (1970), and used by Neale (1971), was chosen as we are primarily concerned in this Unit with the general problem of the malnourished child.

METHODS

Chemicals

2-Ketoglutaric acid, ATP, CoA and NAD were purchased from the Sigma Chemical Co. (St Louis, Miss., USA), phenylethylamine from Packard Instruments (Downers Grove, Ill., USA), diphenyloxazole (PPO) from Beckman Instruments (Fullerton, Calif., USA) and all other chemicals from British Drug Houses (Poole, Dorset). Uniformly labelled L-valine was purchased from the Radiochemical Centre (Amersham, Bucks.) and carboxyl-labelled L-valine from Calatomic (San Diego, Calif., USA).

Table 1. *Composition (g/kg dry weight) of the synthetic diets given to weanling rats*

Diet	HP	LP
Casein	200	10
Glucose	150	150
Wheat flour	150	150
Maize starch	290	480
Vegetable lard	150	150
Minerals*	50	50
Vitamins†	10	10

* Purchased from Glaxo Research Ltd.
† Flores *et al.* (1970).

Animals and diets

Inbred litter-mate Sprague–Dawley rats were used. They were weaned when 21 d old onto one of the synthetic diets shown in Table 1. These diets provided 215 g of protein (HP) and 25 g protein (LP) per kg diet. Some rats were given the HP diet for 16 d and were then given the LP diet for a further 7 d. Other animals were given the LP diet for 16 d and then the HP diet for 7 d. The excretion of $^{14}CO_2$ after the oral administration of [1-^{14}C]valine was tested in these animals at weaning and at 16 d and 23 d after weaning.

In vivo experiments

The rats were given by tube a single dose of 5 μCi of either [U-^{14}C]valine or [1-^{14}C]valine per 100 g body-weight. The amino acids were administered in casein hydrolysate supplying a total of 10 μmol valine per 100 g body-weight. The experiments were performed late at night without previous fasting of the animals. Immediately after the administration of the amino acid mixture, the rats were placed in glass tubes (200 mm × 100 mm diameter) which were tightly sealed. Humid CO_2-free air was drawn past the animals at a rate of approximately 500 ml/chamber per min and the expired CO_2 collected in 20 ml 2 M-KOH. The CO_2 traps were changed every hour and extracted more than 98% of the labelled CO_2 as judged by the recovery of labelled CO_2 produced by acid digestion of $NaH^{14}CO_3$ within the chambers. Most experiments were continued for 2 h.

At the end of the collection period the labelled CO_2 was liberated from the KOH traps by acidification and collected in 0·3 ml phenylethylamine.

The CO_2 content of the traps was estimated gravimetrically with barium hydroxide.

In vitro experiments

In general, the procedure of McFarlane & von Holt (1969*b*) was adopted. Rats were killed by cervical dislocation at between 07.30 and 09.00 hours without previous fasting. Homogenates of liver and mixed muscle (thigh, abdominal wall and diaphragm) (1:10, w/w) were prepared in buffer A (0·25 mol sucrose, 50 mmol KCl and 5 mmol $MgCl_2$ in 1 l 50 mM-Sorensen's phosphate buffer, pH 7·6). The livers were homogenized in a motor-driven Teflon–glass homogenizer. The muscles were homogenized by a sequence of hand-mincing, preliminary homogenization in a M.S.E. blendor (Hospital and Laboratory Supplies, London) and final homogenization in an all-glass

Duall tissue grinder (Kontes Glass Co., Vineland, NJ, USA). All operations were performed at 0–4°. The homogenates were centrifuged at 2000 *g* for 5 min at 5° and the supernatant fractions taken for subsequent incubation.

The incubation mixture normally contained in a volume of 1 ml:0·8 ml homogenate, CoA (1 μmol), ATP (5 μmol), labelled L-valine (1 μmol), either uniformly labelled (957 d/min per nmol) or [1-^{14}C]valine (252 d/min per nmol), and 2-ketoglutarate (1 μmol). All additions were made in buffer A. CoA and ATP were the only cofactors which were found to be absolutely necessary for the optimum production of labelled CO_2; in particular NAD and Ca^{2+} did not increase the production of labelled CO_2, in contrast to the observations of Wohlhueter & Harper (1970). The incubations were done in rimless 25 ml centrifuge tubes and were normally for 1 h at 37° under an atmosphere of oxygen. CO_2 was collected in 0·1 ml phenylethylamine on folded strips of paper contained in small beakers suspended from the no. 33 Subaseal stoppers (A. Gallenkamp & Co. Ltd, London) which were used to seal the tubes. The pH of the medium was initially 7·5 and fell to 7·1–7·2 after 1 h of incubation.

The reaction was stopped by the injection of 2 ml 2 N-H_2SO_4 through the cap, the tubes being then incubated for a further hour at 37°. At the end of the incubation the traps were removed. When the incubation mixture contained [1-^{14}C]valine a fresh trap was suspended from the stopper; the tube was resealed and the contents were treated with 4 ml of a saturated solution of ceric sulphate in 4 N-H_2SO_4 in order to liberate the labelled CO_2 from the carboxyl group of 2-ketoisovaleric acid (KIV).

Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) against bovine serum albumin as standard.

Measurement of radioactivity

The radioactivity in the phenylethylamine traps was estimated by scintillation counting in a Beckman LS 150 scintillation spectrometer. The filter-papers containing the phenylethylamine together with methanol washings were placed in a scintillant consisting of a mixture of methanol (200 ml), toluene (800 ml) and PPO (6 g). Correction for quenching was by the external standard channels ratio method and the mean counting efficiency was 40%.

Expression of results

The method of McFarlane & Von Holt (1969*b*) allows the separate estimation of the activities of 2-ketoisovaleric acid dehydrogenase (reaction 2) and valine 2-ketoglutarate aminotransferase (reaction 1). The reactions are:

(1) Valine 2-ketoglutarate aminotransferase

$$\text{valine} + \text{2-ketoglutarate} \rightleftharpoons \text{2-ketoisovalerate} + \text{glutamate}.$$

(2) 2-ketoisovaleric acid dehydrogenase

$$\text{2-ketoisovalerate} + \text{CoASH} + \text{NAD}^+ \rightarrow \text{isobutyrylCoA} + \text{CO}_2 + \text{NADH} + \text{H}^+.$$

$$\downarrow$$

$$\text{succinylCoA}$$

Table 2. *Body-weights and the weights of some tissues of rats fed on the HP (215 g protein/kg) or LP (25 g protein/kg) diet for 16 d from weaning*

(Mean values with their standard errors for six rats/group)

Diet	Body-wt (g)	Liver wt (g/kg body-wt)	Muscle wt (g/kg body-wt)		
			Gastrocnemius	Quadriceps	Diaphragm
HP	107 ± 6	53·7 ± 0·4	4·3 ± 0·1	10·9 ± 0·8	3·1 ± 0·4
LP	43 ± 3	58·4 ± 0·6	3·4 ± 0·1***	10·1 ± 0·5	2·9 ± 0·2

*** Statistically different from value for HP group ($P < 0·01$).

Table 3. *Fraction of an orally administered dose of either [U-^{14}C]valine or [1-^{14}C]valine excreted as CO_2 by control or protein-depleted rats 1 and 2 h after the administration of the amino acids, measured at weaning and again in the same rats 16 d later*

(Mean values with their standard errors for eight animals/group)

Diet†	Labelled amino acid	Time of experiment	Proportion excreted as CO_2 (ratio, activity : activity of dose × 1000)	
			In 1st hour	In 2 h
HP	[U-^{14}C]valine	Weaning	75·8 ± 6·6	140·1 ± 11·0
LP		Weaning	80·8 ± 10·2	137·2 ± 18·1
HP		+16 d	55·8 ± 1·8	114·5 ± 9·3
LP		+16 d	84·6 ± 8·8**	104·9 ± 9·3
HP	[1-^{14}C]valine	Weaning	65·5 ± 14·8	114·9 ± 4·1
LP		Weaning	62·9 ± 2·9	105·5 ± 6·3
HP		+16 d	68·5 ± 3·9	108·1 ± 6·3
LP		+16 d	46·1 ± 5·8***	66·6 ± 7·0**

Statistically different from values for HP group: ** $P < 0·02$; *** $P < 0·01$.
† HP, 215 g protein/kg diet; LP, 25 g protein/kg diet.

The results that were obtained in vitro are expressed as nmol valine catabolized/mg protein per h by relating the radioactivity in the CO_2 to the specific radioactivity of the valine which was added to the incubation mixture. In an incubation system which contains the amount of tissue normally used, the dilution of the exogenous valine by valine contained within the tissue will be of the order of 1–2%, as the tissue will contribute some 10–20 nmol valine (Reeds, unpublished observation). All results are expressed as the means with their standard errors and the statistical significance of differences between means was estimated by Student's *t* test (Diem, 1971). Values of *P* less than 0·05 have been regarded as statistically significant.

Rats that had been kept on the low-protein diet are referred to synonymously as 'protein-depleted' or 'malnourished'.

RESULTS

In vivo

After 16 d of feeding on the synthetic diets the HP animals had approximately doubled in body-weight, whereas the LP rats had lost about 10 g in weight (Table 2). The malnourished rats showed a relative increase in liver weight and a relative decrease

Table 4. *Fraction of an orally administered dose of* [1-^{14}C]*valine excreted as* CO_2 1 *and* 2 *h after administration of the amino acid in rats at weaning, after the ingestion of the HP* (215 *g protein/kg*) *or LP* (25 *g protein/kg*) *diet for* 16 *d, and after the subsequent ingestion of the LP or HP diet, respectively, for a further* 7 *d. In each group successive tests were made at the intervals shown*

(Mean values with their standard errors for the number of animals shown in parentheses)

Diet	Period of feeding (d)	Proportion excreted as CO_2 (ratio, activity:activity of dose × 1000) In 1st hour	In 2 h
Weaning		55·3 ± 12·1 (6)	104·0 ± 6·3 (6)
HP	16	68·9 ± 5·2 (6)	101·9 ± 8·0 (6)
then LP	7	43·3 ± 9·1 (6)***	71·8 ± 9·6 (6)***
Weaning		62·1 ± 2·9 (8)	105·5 ± 9·6 (8)
LP	16	46·1 ± 8·4 (8)	66·6 ± 7·0 (8)
then HP	7	67·1 ± 8·4 (8)***	100·1 ± 12·2 (8)*

Statistically different from value for same animals on day 16: * $P < 0.05$; *** $P < 0.01$.

in the weights of three muscles taken for analysis. With the exception of the change in the weight of the gastrocnemius muscle, the differences were not significant.

The fraction of a single orally administered dose of uniformly labelled valine which was excreted as CO_2 during 2 h after administration was the same in both groups of animals, whether measured at weaning or after the ingestion of the diets for 16 d (Table 3). The malnourished rats excreted a higher proportion of the dose in the 1st hour and less during the 2nd hour than did the control animals. Control animals excreted a similar fraction of a dose of [1-^{14}C]valine as CO_2 (Table 3). However, malnourished animals excreted a lower proportion of a dose of [1-^{14}C]valine as CO_2 than either control rats given [1-^{14}C]valine or malnourished rats given [U-^{14}C]valine.

The results of the experiments in which different diets were given to HP or LP animals at 16 d after weaning are shown in Table 4. In rats which had been given the HP diet for 16 d the subsequent ingestion of the LP diet for a further 7 d was associated with a lower excretion of the label of [1-^{14}C]valine as CO_2 on day 23, when compared with the values obtained in the same animals on day 16. Conversely, in the rats which had been given the LP diet for 16 d the ingestion of the HP diet was associated with an increased excretion of label as CO_2. These partly rehabilitated animals excreted a proportion of the label similar to that excreted by the control animals shown in Table 3.

Although malnourished rats did not excrete a lower proportion of a dose of uniformly labelled valine, they excreted significantly ($P < 0.01$) more CO_2 (12·0 ± 0·5(8) mmol/100 g body-weight per h) at a lower specific activity than did control animals (9·6 ± 0·6 (8) mmol/100 g body-weight per h).

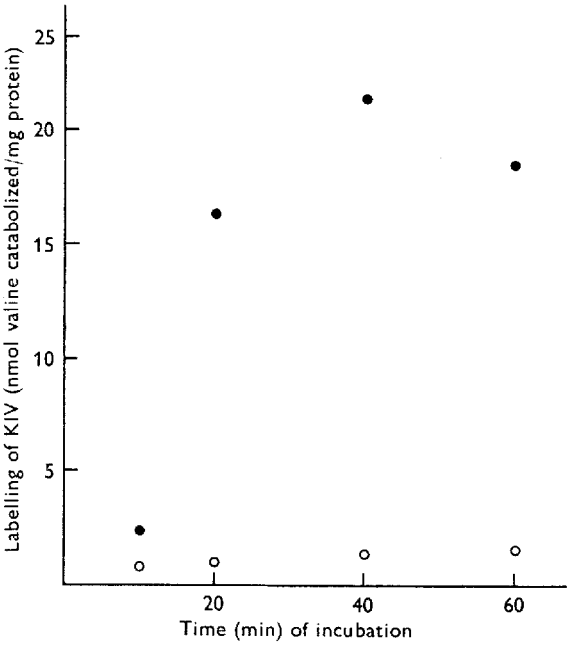

Fig. 1. Effect of time of incubation upon the labelling of 2-ketoisovaleric acid (KIV) from a substrate of [1-^{14}C]valine by liver (○) or muscle (●) homogenates from well-nourished rats. Each point represents the mean of two experiments.

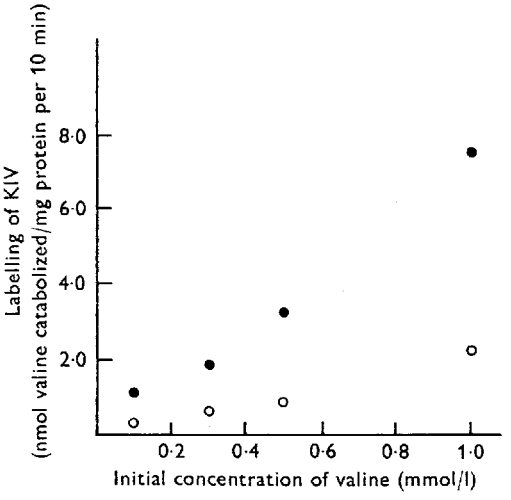

Fig. 2. Effect of the initial concentration of valine upon the labelling of 2-ketoisovaleric acid (KIV) from a substrate of [1-^{14}C]valine by liver (○) or muscle (●) homogenates from well-nourished rats. Incubations were for 10 min and the points represent the means of three experiments.

Table 5. *2-Ketoisovaleric acid dehydrogenase (2-KIVDH) and valine 2-ketoglutarate aminotransferase (VAT) activities in homogenates of liver and muscle from control and protein-depleted rats*

(Mean values and their standard errors for the number of observations shown in parentheses)

Diet†	Tissue	Activity (nmol valine catabolized/mg protein per h) 2-KIVDH	VAT
HP	Liver	1·93 ± 0·15 (11)	2·30 ± 0·21 (11)
LP	Liver	1·38 ± 0·11 (10)***	2·08 ± 0·21 (10)
HP	Muscle	1·87 ± 0·25 (11)	18·15 ± 3·53 (11)
LP	Muscle	1·27 ± 0·12 (10)*	22·20 ± 4·63 (10)

Statistically different from values for HP group: * $P < 0.05$; *** $P < 0.01$.
† HP, 215 g protein/kg diet; LP, 25 g protein/kg diet.

Table 6. *Fraction of newly synthesized 2-ketoisovaleric acid (KIV) decarboxylated by liver and muscle homogenates from control and protein-depleted rats*

(Mean values with their standard errors for the number of observations shown in parentheses)

Diet†	Tissue	Decarboxylation of KIV (ratio, activity in CO_2 : activity in CO_2 + KIV)
HP	Liver	0·84 ± 0·02 (11)
LP	Liver	0·66 ± 0·04 (10)****
HP	Muscle	0·10 ± 0·01 (11)
LP	Muscle	0·05 ± 0·01 (10)***

Statistically different from values for HP group: *** $P < 0.01$; **** $P < 0.001$.
† HP, 215 g protein/kg diet; LP, 25 g protein/kg diet.

In vitro

The formation of $^{14}CO_2$ from [1-^{14}C]valine was linear with respect to time of incubation in both liver and muscle homogenates. This indicates that the decarboxylation of KIV was proceeding at a constant rate. However, the amount of label remaining in KIV in muscle homogenates from control and malnourished rats and in liver homogenates from malnourished rats had reached a more or less constant level after 20 min of incubation (Fig. 1). This shows that the aminotransferase reaction had reached equilibrium, since the product (KIV) was not being oxidized as quickly as it was formed. Therefore the method estimates KIV dehydrogenase (2-KIVDH) with some accuracy, but it estimates only net valine aminotransferase activity. In all the experiments the estimated activity of valine 2-ketoglutarate aminotransferase was much higher in muscle than in liver (Fig. 1, Table 5). This was confirmed in experiments in which shorter times of incubation were used (Fig. 2).

Both liver and muscle homogenates from rats which had been given the LP diet labelled CO_2 to a lesser extent when incubated with [1-^{14}C]valine than did homogenates from control animals. This must be attributed to a reduction in the activity of 2-KIVDH as the activities of valine 2-ketoglutarate aminotransferase were similar

Table 7. *Formation of labelled CO_2 from a substrate of [U-^{14}C]valine by muscle and liver homogenates from control and protein-depleted rats*

(Mean values with their standard errors for the number of observations shown in parentheses)

Diet*	Tissue	Apparent valine catabolism (nmol/mg protein per h)
HP	Liver	0·18 ± 0·01 (10)
LP	Liver	0·15 ± 0·02 (10)
HP	Muscle	0·35 ± 0·04 (10)
LP	Muscle	0·34 ± 0·02 (10)

* HP, 215 g protein/kg diet; LP, 25 g protein/kg diet.

in tissue homogenates from both control and malnourished rats (Table 5). The relative changes in the two enzymes led to increased labelling of the KIV in the tissue homogenates from the protein-depleted animals, and as a consequence a lower proportion of the labelled KIV was decarboxylated (Table 6). This reduction was most marked in homogenates from muscle of malnourished rats.

When homogenates are incubated in the presence of uniformly labelled valine one cannot predict what proportion of the label in CO_2 has come from C 1 or from C 2–C 5, and therefore it is not possible to interpret the results in terms of the amount of valine catabolized. However, once the rate-limiting step of decarboxylation has been passed oxidation of the remaining carbons should proceed rapidly provided that the mitochondria are intact. Therefore the results of incubation with [U-^{14}C]valine have been calculated on the assumption that $^{14}CO_2$ is derived from all the carbons of valine. The values obtained are shown in Table 7. Comparison with Table 5 shows that the apparent catabolism was much lower with [U-^{14}C]valine than with [1-^{14}C]valine and that this was not affected by the protein level of the diet. This difference once again illustrates the difficulty of interpreting results obtained with the uniformly labelled compound.

DISCUSSION

When rats were given diets low in protein there was a significant reduction, compared with control animals, in the excretion of $^{14}CO_2$ after a test dose of [1-^{14}C]valine. This suggests an adaptive change in the rate of valine catabolism. This finding is borne out by results which were obtained with [1-^{14}C]valine in vitro: both in liver and in muscle there was a significant reduction in the activity of 2-KIVDH. There was, however, no change in aminotransferase activity. The activity of 2-KIVDH per mg protein is essentially the same in the two tissues. Since the total amount of protein in muscle is about eight times that in liver, the adaptive change in the muscle enzyme is of great importance for the economy of the whole animal.

The same changes in enzyme activity have previously been obtained in liver by both McFarlane & von Holt (1969*b*) and Wohlhueter & Harper (1970). The present results extend these observations, which were obtained with mitochondria and post-mitochondrial supernatant fractions, to a system where the substrate for the branched-

chain keto-acid dehydrogenase is formed during the course of the incubation. Neither of these previous studies nor the present results have confirmed the finding of Mimura *et al.* (1968) of grossly elevated branched-chain aminotransferase activity in malnourished rats. Results essentially similar to ours have recently been obtained with leucine (Sketcher, Fern & James, 1974).

However, with uniformly labelled valine there was no difference between protein-depleted and control animals in the output of $^{14}CO_2$, either in vivo or in vitro. In the present experiments valine was given intragastrically. The results therefore are in agreement with those of Neale (1971). He showed that the nutritional status of the animal did not affect the output of $^{14}CO_2$ from [U-^{14}C]valine when the amino acid was given by the intragastric route but there was a small, but not statistically significant, reduction when it was given by the intravenous route. This was attributed to the fact that valine is catabolized mainly by muscle and when it is given intravenously it is taken up by muscle before it reaches the liver.

We now have to consider what explanation there can be for the different behaviour of the two types of valine when given by stomach tube. If present ideas on the catabolic pathway of valine are correct (Meister, 1965), 80% of the carbon of uniformly labelled valine has to be metabolized by the enzymes of the tricarboxylic acid cycle before it can enter the bicarbonate pool and thence respiratory CO_2. If the fractional excretion of $^{14}CO_2$ after the administration of [1-^{14}C]valine is in any way a realistic estimate of the formation of succinate from valine, then about 5 μmol valine are catabolized in 2 h. The tricarboxylic acid cycle is turning over at about 6 μmol/min in liver alone (Heath & Threlfall, 1968) and thus the formation of $^{14}CO_2$ from [U-^{14}C]valine must result largely from reactions that are not rate-limiting for the catabolism of valine. Under these circumstances the output of $^{14}CO_2$ should reflect the rate of entry of label into the tricarboxylic acid cycle.

Once label has reached the level of succinate it will be considerably diluted in the large pools of fumarate and malate (LaNoue, Nicklas & Williamson, 1970), and will be taken up into aspartate and glutamate and thence into protein. It will also be incorporated into fatty acids and glucose. Therefore the label from [U-^{14}C]valine that has reached the level of succinate will be diluted to a high degree before it reaches reactions which will yield labelled CO_2, and it is not surprising that any adaptive change in the initial catabolic steps should be obscured. It should be noted also that the protein-depleted animals excreted more CO_2 at a lower specific activity than the controls, an effect observed also by Neale & Waterlow (1974). As they point out, an adaptive change could also be obscured by the greater total excretion of CO_2 in the malnourished rats. This also implies that the catabolism of valine was making a smaller contribution to the total CO_2 formation.

If the preceding argument is correct, that with [U-^{14}C]valine some of the non-carboxyl label will be retained in protein, glucose and fat, then it follows that an estimate of valine catabolism with [1-^{14}C]valine will always be higher and should never be lower than an estimate with [U-^{14}C]valine. This was, in fact, found in vitro, when the apparent catabolism of valine was lower with the uniformly labelled amino acid than with the carboxyl-labelled form. However, it was not so in vivo, where in

weanling rats and protein-depleted rats the estimate of valine catabolism with [1-^{14}C]-valine was lower than with uniformly labelled valine.

There could be two explanations for these paradoxical results which have been obtained in vivo:

(1) The decarboxylation of KIV is not rate-limiting for the catabolism of valine. The conclusion that the activities of the dehydrogenases are rate-limiting for the catabolism of the branched-chain amino acids is based on the observation that both in vivo and in vitro there is a build-up of label in the keto acid produced by the transamination step (McFarlane & von Holt, 1969*a*, *b*); that the enzymes catalyse essentially irreversible reactions (Meister, 1965); and that they are at low activity and vary with the nutritional status of the animals (McFarlane & von Holt, 1969*b*; Wohlhueter & Harper, 1970). Although these points provide good evidence for the conclusion, in some instances the distribution of the enzyme in tissue homogenates does not correlate with the observed rates of catabolism of branched-chain amino acids by intact-tissue preparations. Thus Wohlhueter & Harper (1970) found that the activities of the branched-chain keto-acid dehydrogenases were highest in liver mitochondria, yet Mortimore & Mondon (1970) could find little or no catabolism of [1-^{14}C]valine by perfused liver. Conversely, although rapid rates of catabolism of branched-chain amino acids have been observed in isolated whole-muscle preparations (Manchester, 1965; Odessey & Goldberg, 1972), Wohlhueter & Harper (1970) were unable to detect any branched-chain keto-acid dehydrogenases in skeletal muscle homogenates. Sketcher *et al.* (1974), however, have demonstrated that the conditions of homogenization are critical for the demonstration of branched-chain 2-keto-acid dehydrogenase activity, and that polytron homogenization (the technique used by Wohlhueter & Harper (1970) in muscle) destroys the activity of the enzymes, presumably secondarily to the disruption of the mitochondria.

The results described in the present paper demonstrate the presence of 2-KIVDH in skeletal muscle homogenates, but the activity was no higher than that found in liver, and was low when one considers the high activity of the aminotransferase in muscle. This discrepancy could be resolved if the principal site for branched-chain amino acid transamination is muscle but that the keto acids pass to the liver to be metabolized further. Evidence for this proposition is lacking and is the subject of present investigations. Since valine is a gluconeogenic amino acid, metabolism of the keto acid must occur to some extent in liver or kidney.

(2) The second explanation is that labelled CO_2 formed by the decarboxylation of KIV enters the bicarbonate pool at a slower rate than labelled CO_2 formed by the tricarboxylic acid cycle, or alternatively is preferentially reutilized in CO_2-fixation reactions. If this is so then an important distinction should be made between the rate of catabolism of valine and the rate of excretion of $^{14}CO_2$ in an experiment lasting for 2 h. The explanation is difficult to test. Although the turnover of the bicarbonate pool is not normally regarded as being rate-limiting in measurements of the labelling of respiratory CO_2, estimates of this turnover have given half-lives ranging from 12 min in the rat (Millward, 1970) to 40 min in the dog (Steele, Altzuler, Wall, Dunn & de Bodo, 1959). It is difficult to see how CO_2 released from the decarboxylation of KIV

and from the tricarboxylic acid cycle can mix at grossly different rates with the bicarbonate pool, since both reactions occur within the mitochondrion. Rates of CO_2 fixation from different sources of bicarbonate are unknown and again are the subject of present investigations.

The present experiments provide some evidence for the proposition that the protein-depleted rat catabolizes valine at a decreased rate and that this adaptive phenomenon occurs in both liver and muscle. The results do, however, emphasize the difficulties not only of interpreting experiments based upon the measurement of the labelling of respiratory CO_2, but also of comparing the results obtained from experiments in the whole animal with those obtained from experiments in isolated tissues or homogenates.

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