Regulation of Branched-Chain Amino Acid Oxidation in Isolated Muscles, Nerves and Aortas of Rats

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1. The oxidation of the three branched-chain amino acids was regulated in parallel fashion in rat tissues studied *in vitro*. 2. With 0.1 mM-[1-¹⁴C]isoleucine as substrate in the presence of 5.5 mM-glucose, ¹⁴CO₂ production was 0.6 μmol/2h per g in the aorta, 0.3 in peripheral nerve, 0.2 in muscle and 0.13 in spinal cord. 3. The ratio ¹⁴C oxidized/¹⁴C incorporated into proteins with 0.1 mM-[1-¹⁴C]leucine was 1.3 in hemidiaphragms, 3.3 in sciatic nerve and 1.0 in nerves undergoing Wallerian degeneration. Leucine oxidation decreased only slightly during degeneration, but protein synthesis doubled. 4. Hemidiaphragms incubated with [1-¹⁴C]leucine or 4-methyl-2-oxo[1-¹⁴C]pentanoate increased ¹⁴CO₂ production 7–9-fold as substrate concentration was increased from 0.1 to 0.5 mM; under the same conditions ¹⁴CO₂ production by nerves increased only 2–3-fold. 5. 2-Oxoglutarate stimulated the oxidation of the branched-chain amino acids by muscles and peripheral nerves and the oxidation of 4-methyl-2-oxopentanoate by hemidiaphragms but not by nerves. 6. Octanoate (0.1–1.0 mM) markedly stimulated the oxidation of branched-chain amino acids and of 4-methyl-2-oxopentanoate in hemidiaphragms, but inhibited oxidation of both by peripheral nerves and spinal cord. In aortas, oxidation of isoleucine (the only substance tested) was inhibited by octanoate. 7. The effects of octanoate and 2-oxoglutarate on leucine oxidation by hemidiaphragms were additive at low concentrations. When maximally stimulating concentrations of either agent were used, addition of the other was ineffective. 8. Pyruvate inhibited the oxidation of branched-chain amino acids and 4-methyl-2-oxopentanoate in all tissues tested. 9. Insulin did not affect the oxidation of 4-methyl-2-oxopentanoate by muscles or nerves. 10. The oxidative decarboxylation of the branched-chain α-oxo acids is suggested as a regulatory site of branched-chain amino acid oxidation. Differences in regulation between muscle on the one hand, and nerve and aorta on the other, are discussed.

The branched-chain amino acids, leucine, isoleucine and valine, are the only essential amino acids that are degraded mainly extrahepatically, primarily by muscles (Miller, 1962; Manchester, 1965; Odessey & Goldberg, 1972; Buse *et al.*, 1972). Several tissues besides skeletal muscles are known to oxidize branched-chain amino acids; these include the heart (Buse *et al.*, 1972), liver, kidney, brain, placenta and adipose tissues of various animal species (Dancis *et al.*, 1969; Odessey & Goldberg, 1972) and human leucocytes and fibroblasts in culture (Dancis *et al.*, 1969). The oxidation of branched-chain amino acids by skeletal muscle and heart is under metabolic (Odessey & Goldberg, 1972; Buse *et al.*, 1972) and hormonal regulation (Manchester, 1965; Buse *et al.*, 1973a) and is stimulated by starvation (Goldberg & Odessey, 1972; Buse *et al.*, 1973a). Certain fatty acids stimulate the oxidation of branched-chain amino acids by rat diaphragm and heart *in vitro*; the

most potent ones, octanoate and hexanoate, stimulate at 0.5 mM. Glucose and pyruvate inhibit branched-chain amino acid oxidation by heart and to a lesser degree by diaphragm, whereas glucagon and adrenaline stimulate under certain conditions *in vitro* (Buse *et al.*, 1972, 1973a).

In mammalian tissues the oxidation of the three branched-chain amino acids follows a common path for the first three steps (for review see Dancis & Levitz, 1972). Regulation is thought to occur at one of the first two reactions, transamination or the oxidative decarboxylation of the resulting branched-chain α-oxo acids (Manchester, 1965; Odessey & Goldberg, 1972; Buse *et al.*, 1972).

The circulating concentrations of branched-chain amino acids are increased during fasting (Felig *et al.*, 1969; Adibi, 1971; Meikle & Klain, 1972) and insulin deficiency (Carlsten *et al.*, 1966; Scharff & Wool, 1966) in humans and rats. Isolated hemi-

diaphragms and sciatic nerves from diabetic rats appear to oxidize [$1-^{14}\text{C}$]leucine faster than those from controls (Herlong *et al.*, 1974). Regulation of the catabolism of branched-chain amino acids at the periphery may play a role in homeostasis.

The present paper describes two lines of investigation: studies on the identification of the step at which branched-chain amino acid oxidation by muscles is regulated, and extension of these studies to the isolated peripheral nerve, spinal cord and aorta. The latter tissues were selected because of the involvement of peripheral nerves and blood vessels in the 'complications' of diabetes.

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Materials and Methods

Male rats of the Wistar strain were killed by decapitation with a guillotine (Harvard Apparatus Co., Dover, Mass., U.S.A.). The animals had free access to food (Wayne Lab-Blocks, Allied Mills Inc., Chicago, Ill., U.S.A.) and water up to the time of the experiments. Rats weighing 120–220 g were used; in individual experiments the weight of the animals was as uniform as possible, usually within ± 10 g of the mean.

The technique for dissecting and incubating hemidiaphragms has been described (Buse & Buse, 1967). Both sciatic nerves along with their major branches were dissected free of the connective tissue and stripped of perineurium. They were weighed and incubated in the same manner as the diaphragms. In some experiments the two brachial plexuses were also dissected and incubated with the sciatic nerves. In experiments with isolated aortas, the descending aorta was dissected from the arch to the bifurcation. The aorta was opened via a longitudinal incision. The adventitia and periaortic tissues were stripped off manually. Aortic strips weighing 10–15 mg were incubated in the same manner as the diaphragms. The tissues were incubated in a Dubnoff metabolic shaker (42 cycles/min). The medium was Gey & Gey's (1936) balanced salt solution with the appropriate additions, equilibrated with $\text{O}_2 + \text{CO}_2$ (95:5), pH 7.4, at 37.5°C .

A paired experimental design was used in every study, e.g. one hemidiaphragm or nerve of each rat was incubated in the control medium, and its pair in the experimental medium. For the spinal cord and the aorta the tissues of each animal were divided into halves and distributed between the experimental and control media.

After preincubation for 1 h, to stabilize metabolism under conditions *in vitro*, the tissues were gently

blotted and transferred to vessels containing 1–4 ml of fresh medium and incubated for 2 h, or as indicated in the text. For spinal cords preincubation was omitted. The volume and composition of preincubation and incubation media were identical, except that the radioactive tracer and insulin were added to the latter only. The incubation flasks were sealed with rubber stoppers equipped with sleeves. Small plastic cups were suspended from the stoppers over the medium. At the end of the incubation 0.5 ml of 20% (v/v) H_2SO_4 was injected into the medium and 0.5 ml of Hyamine into the plastic cups; incubation was continued for an additional 1 h. This procedure resulted in a 95% recovery of $^{14}\text{CO}_2$ (Buse *et al.*, 1972). The small vials containing Hyamine were placed in counting vials containing 14 ml of toluene scintillation mixture (Buse & Buse, 1967) and counted for radioactivity in a liquid-scintillation spectrometer (Packard Corp., La Grange, Ill., U.S.A.). Portions (50 μl) of incubation medium were obtained before incubation, and in some instances also after incubation, and were counted for radioactivity in 1 ml of Hyamine and 14 ml of scintillation mixture. Counting efficiency was 80–85%. Sufficient counts were obtained to keep the error of counting below $\pm 1\%$. Quenching was monitored by external standardization and internal standards when appropriate. Counts were corrected to d.p.m.

In experiments where the protein and DNA concentrations as well as the incorporation of leucine into proteins were measured, 0.5 or 1 ml of 20% (w/v) trichloroacetic acid was injected into the incubation vessels at the end of incubation, depending on the volume of the medium. The $^{14}\text{CO}_2$ generated was trapped in Hyamine and the precipitated tissues were stored at 4°C for later determination of protein and DNA. The precipitated samples were homogenized and washed with cold 5% trichloroacetic acid, cold ethanol and ethanol-diethyl ether (3:1, v/v). The residue was warmed to 80°C and DNA extracted with 5% trichloroacetic acid at 72°C (Hoffert & White, 1968). DNA in the supernatant was determined colorimetrically with indole by the method of Hubbard *et al.* (1970).

The precipitate was washed with ethanol, then with di-isopropyl ether and dried. Protein was digested in 1 M-NaOH and samples were removed for protein determination by the method of Lowry *et al.* (1951) with bovine serum albumin standards. Another portion was solubilized in Biosolve TM (Beckman Instruments Inc., Palo Alto, Calif., U.S.A.) and counted for radioactivity in a liquid-scintillation counter.

The radioactivity recovered in $^{14}\text{CO}_2$ or in protein was related to the concentration of radioactivity in the medium before incubation, and expressed as nmol of the ^{14}C -labelled substrate. The values were

related to the weight or protein content of the tissues. Means $\pm$ S.E.M. are tabulated. The significance of the difference between means was tested by Student's *t* test. The paired *t* test was used whenever it was applicable. Significance was ascribed if *P* was less than 0.05.

Reagents

All chemicals used were of the highest purity obtainable commercially. Substrates, (L-amino acids, 2-oxoglutarate, pyruvate, CoA and thiamin pyrophosphate) were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), and from Calbiochem Corp. (La Jolla, Calif., U.S.A.). Hyamine hydroxide (1M, in methanol) and ^{14}C -labelled substrates were purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.). 4-Methyl-2-oxo[^{14}C]pentanoate was prepared in our laboratory from [1- ^{14}C]leucine (sp. radioactivity 45mCi/mmol) to which carrier leucine was added. The technique of Meister (1953), was adapted for small amounts of starting material. The resulting compound was identified by t.l.c. and spectrophotometry of the 2-oxo acid hydrazones (Dancis *et al.*, 1963; Aki & Ichihara, 1970). The specific radioactivity of the starting material and of the resulting crystallized product was identical and of the order of 0.03 mCi/mmol. The i.r. spectrum was consistent with the structure of 4-methyl-2-

oxopentanoate. Bovine insulin (crystallized five times) was a gift of Eli Lilly Research Laboratories (Indianapolis, Ind., U.S.A.).

Results

Effect of 2-oxoglutarate on leucine oxidation by rat diaphragms

The amino-group acceptor of the branched-chain aminotransferase in muscles is 2-oxoglutarate. The effect of 2-oxoglutarate on $^{14}\text{CO}_2$ production from [1- ^{14}C]leucine by isolated hemidiaphragms was measured by using three concentrations of leucine (0.1, 0.5 and 1.0mM) and three concentrations of 2-oxoglutarate (1.5 and 10mM; Table 1). Increasing the concentration of leucine in the medium from 0.1 to 0.5mM caused an approximately eightfold increase in $^{14}\text{CO}_2$ production, suggesting that at low concentrations of substrate, which include the physiological range, leucine may stimulate its own oxidation. Similar observations were made when isoleucine oxidation by rat hemidiaphragms was studied (Buse *et al.*, 1972). However, the observed relationship between $^{14}\text{CO}_2$ production and substrate concentration may represent dilution of [^{14}C]leucine by the intracellular leucine pool, which may be significant at low concentrations of leucine in the medium.

Table 1. *Effect of 2-oxoglutarate on L-leucine oxidation by rat hemidiaphragms*

Paired hemidiaphragms were preincubated for 1 h in 4 ml of Gey & Gey's (1936) balanced salt solution containing 5.5mM-glucose and [^{14}C]leucine with or without added sodium 2-oxoglutarate. Conditions were: temperature 37°C; pH 7.4; gas phase $\text{O}_2 + \text{CO}_2$ (95:5). Incubation lasted 2 h in media that were identical with the preincubation media, except for the addition of the ^{14}C tracer (0.02 $\mu\text{Ci/ml}$) during incubation. One member of each pair of hemidiaphragms was incubated in the control medium and the other with added 2-oxoglutarate. $^{14}\text{CO}_2$ production was expressed as nmol of [^{14}C]leucine metabolized and calculated as follows: (d.p.m. in $^{14}\text{CO}_2/\text{d.p.m. per ml of initial medium}) \times (\text{nmol of leucine/ml of medium})$. Means of four observations $\pm$ S.E.M. are tabulated. *P* was calculated by paired Student's *t* test.

[1- ^{14}C]Leucine (mM)	2-Oxoglutarate (mM)	$^{14}\text{CO}_2$ produced (nmol/2h per g)	Change %	<i>P</i>
0.1	0	183 $\pm$ 18.8		
	1	293 $\pm$ 36.7	60.1 $\pm$ 13.2	<0.05
	0	237 $\pm$ 12.5		
	5	359 $\pm$ 21.7	51.5 $\pm$ 9.5	<0.02
	0	207 $\pm$ 27.8		
	10	412 $\pm$ 32.3	99.0 $\pm$ 13.4	<0.01
0.5	0	1663 $\pm$ 22.7		
	1	2006 $\pm$ 56.3	20.6 $\pm$ 3.4	<0.01
	0	1636 $\pm$ 60.8		
	5	2510 $\pm$ 73.8	53.4 $\pm$ 6.5	<0.01
	0	1485 $\pm$ 444.5		
	10	2414 $\pm$ 525.8	62.6 $\pm$ 7.2	<0.01
1.0	0	3489 $\pm$ 100.2		
	1	3738 $\pm$ 156.8	7.1 $\pm$ 3.3	<0.1
	0	3600 $\pm$ 119.8		
	5	4705 $\pm$ 138.2	30.7 $\pm$ 1.9	<0.01
	0	2176 $\pm$ 543.8		
	10	3532 $\pm$ 631.2	62.3 $\pm$ 5.0	<0.01

Table 2. *Effect of octanoate and 2-oxoglutarate on the oxidation of L-leucine by rat hemidiaphragms*

Rat hemidiaphragms were preincubated for 1 h and incubated for 2 h as described in Table 1. All media contained 5.5 mM-glucose. Octanoate and 2-oxoglutarate were added as the sodium salts. $^{14}\text{CO}_2$ production was expressed as nmol of [^{14}C]leucine metabolized (see Table 1). The values are means $\pm$ S.E.M. The number of paired observations is indicated in parentheses. *P* was calculated by paired *t* test. N.S., Not significant.

[1- ^{14}C]Leucine (mM)	Octanoate (mM)	2-Oxoglutarate (mM)	$^{14}\text{CO}_2$ produced (nmol/2h per g)	<i>P</i>
0.1	0	0	202 $\pm$ 19.4	<0.01
	1	0	405 $\pm$ 13.2 (7)	
0.1	0	0	202 $\pm$ 20.6	<0.02
	0.1	0	317 $\pm$ 38.5 (11)	
0.1	0	1	298 $\pm$ 15.1	<0.01
	1	1	439 $\pm$ 24.3 (9)	
0.1	0	1	280 $\pm$ 19.8	<0.02
	0.1	1	463 $\pm$ 51.5 (11)	
0.5	0	0	1427 $\pm$ 116.0	<0.01
	0	5	2184 $\pm$ 146.0 (8)	
0.5	0	5	2023 $\pm$ 86.0	N.S.
	1	5	2156 $\pm$ 112.0 (8)	

Increasing the concentration of leucine from 0.5 to 1.0 mM resulted in an approximately twofold increase in leucine oxidation.

2-Oxoglutarate stimulated $^{14}\text{CO}_2$ production from leucine significantly at each leucine concentration studied. When leucine was 0.1 mM the stimulatory effect obtained with 1 or 5 mM-2-oxoglutarate was of the same magnitude; both increased the rate of leucine oxidation by 50–60%. In the presence of 0.5 mM-leucine, increasing 2-oxoglutarate from 1 to 5 mM increased the stimulatory effect from 20 to 50% of the basal rate. The addition of 10 mM-2-oxoglutarate did not cause further stimulation.

The interaction of octanoate and 2-oxoglutarate was studied in an effort to determine whether or not they acted at different sites. Table 2 shows that when hemidiaphragms were incubated with 0.1 mM-leucine, the addition of 0.1 mM-octanoate stimulated leucine oxidation by approx. 50%, and the addition of 1 mM-octanoate essentially doubled the rate of leucine oxidation. When these data are compared with those in Table 1 it appears that octanoate is approximately ten times as potent as 2-oxoglutarate as a stimulator of leucine oxidation. When 1 mM-2-oxoglutarate was added with 0.1 mM-octanoate, the effect of the two agents on leucine oxidation was additive, and was significantly greater than that observed with either agent at these submaximum concentrations. However, when 1 mM-2-oxoglutarate was added to 1 mM-octanoate, no further stimulation was observed.

In the presence of 0.5 mM-leucine, 5 mM-2-oxoglutarate caused the maximum stimulation of leucine oxidation that could be achieved with this agent, although leucine oxidation could be stimulated further by increasing the substrate concentration (Table 1). The addition of 1 mM-octanoate to 5 mM-

2-oxoglutarate caused no further stimulation (Table 2). These results are compatible with the hypothesis that 2-oxoglutarate and octanoate stimulate leucine oxidation by hemidiaphragms at a common site.

Effect of different agents on the oxidation of 4-methyl-2-oxopentanoate by hemidiaphragms (Table 3)

The fact that 2-oxoglutarate stimulated leucine oxidation suggested that transamination may be the rate-limiting step that regulates leucine oxidation by muscle. The removal of the amino group from leucine yields 4-methyl-2-oxopentanoate. The effect of 2-oxoglutarate on $^{14}\text{CO}_2$ production from 4-methyl-2-oxo[1- ^{14}C]pentanoate was tested, to ascertain whether or not 2-oxoglutarate stimulated leucine oxidation beyond transamination. At comparable substrate concentrations 4-methyl-2-oxopentanoate was oxidized 2.5–3 times as fast as leucine by hemidiaphragms. At low concentrations of 4-methyl-2-oxopentanoate the stimulatory effect of increasing the substrate concentration was noted, as was previously seen with leucine. Thus increasing the concentration of 4-methyl-2-oxopentanoate from 0.1 to 0.5 mM resulted in an approximately ninefold acceleration of $^{14}\text{CO}_2$ production from this substrate. At low concentrations of 4-methyl-2-oxopentanoate the addition of 5 mM-oxoglutarate stimulated 4-methyl-2-oxopentanoate oxidation significantly, by 47% of the basal rate in the presence of 0.05 mM and 33% in the presence of 0.1 mM-4-methyl-2-oxopentanoate. When the concentration of 4-methyl-2-oxopentanoate was 0.5 mM or higher the addition of 2-oxoglutarate did not stimulate oxidation significantly. The kinetics of oxidation of leucine and 4-methyl-2-oxopentanoate by rat hemidiaphragms and the

Table 3. *Effect of octanoate, 2-oxoglutarate, pyruvate and insulin on the oxidation of 4-methyl-2-oxo[1-¹⁴C]pentanoate by rat hemidiaphragms*

Rat hemidiaphragms were preincubated for 1 h and incubated for 2 h as described in Table 1. All media contained 5.5 mM-glucose. Organic acids were added as their sodium salts. Bovine insulin, recrystallized five times (Eli Lilly), was used. ¹⁴CO₂ production was expressed as nmol of ¹⁴C-labelled substrate oxidized (see Table 1). The values are means $\pm$ S.E.M. The number of paired observations is indicated in parentheses. *P* was calculated by paired *t* test. N.S., Not significant.

4-Methyl-2-oxopentanoate (mM)	Additions	(mM)	¹⁴ CO ₂ produced (nmol/2h per g)	<i>P</i>
0.05	2-Oxoglutarate	0	261 $\pm$ 27.2	<0.02
		5	384 $\pm$ 25.6 (4)	
0.1	2-Oxoglutarate	0	497 $\pm$ 71.5	<0.01
		5	656 $\pm$ 70.0 (8)	
0.5	2-Oxoglutarate	0	4647 $\pm$ 287.9	N.S.
		5	4919 $\pm$ 248.7 (10)	
1.0	2-Oxoglutarate	0	8648 $\pm$ 496.0	N.S.
		5	8425 $\pm$ 389.4 (10)	
2.0	2-Oxoglutarate	0	10119 $\pm$ 785.0	N.S.
		5	10978 $\pm$ 862.0	
0.1	Octanoate	0	497 $\pm$ 46.4	<0.01
		1	872 $\pm$ 40.8 (9)	
0.1	Pyruvate	0	574 $\pm$ 85.3	<0.01
		5	348 $\pm$ 54.5 (6)	
0.5	Pyruvate	0	4316 $\pm$ 488.2	<0.01
		5	3728 $\pm$ 138.1 (6)	
0.1	Insulin	0	664 $\pm$ 68.7	N.S.
		1 munit/ml	654 $\pm$ 44.4 (11)	

Table 4. *Effect of glucose and pyruvate on the oxidation of 0.1 mM-L-[¹⁴C]leucine by isolated sciatic nerves of the rat*

Paired sciatic nerves were preincubated for 1 h and incubated for 2 h with 0.1 mM-leucine and the additions indicated. The [¹⁴C]leucine tracer was added during incubation only. The conditions of the experiment were as described in Table 1, except that only 1 ml of medium was used for nerves. Pyruvate was added as the sodium salt. ¹⁴CO₂ production was expressed as nmol of [¹⁴C]leucine oxidized (see Table 1). Means $\pm$ S.E.M. are shown; the numbers of paired observations are indicated in parentheses. *P* was calculated by paired *t* test. N.S., Not significant.

Glucose (mM)	Pyruvate (mM)	¹⁴ CO ₂ produced (nmol/2h per g)	<i>P</i>
0	0	328.3 $\pm$ 17.5	
16.5	0	452.0 $\pm$ 42.8 (7)	<0.025
0	0	303.4 $\pm$ 20.4	
5.5	0	379.8 $\pm$ 18.7 (4)	<0.05
5.5	0	403.6 $\pm$ 27.9	
16.5	0	383.1 $\pm$ 14.1 (4)	N.S.
0	0	425.9 $\pm$ 10.1	
0	11	272.1 $\pm$ 13.9 (6)	<0.001
5.5	0	407.2 $\pm$ 27.1	
5.5	5	285.0 $\pm$ 13.7 (7)	<0.01

effect of 5 mM-2-oxoglutarate on this process are compared in Fig. 1.

The effects of octanoate, pyruvate and insulin on 4-methyl-2-oxopentanoate oxidation by rat diaphragms (Table 3) were identical with those described with regard to the oxidation of leucine (Buse & Buse, 1967; Buse *et al.*, 1972). Octanoate (1 mM) added in the presence of glucose approximately doubled ¹⁴CO₂ production from 0.1 mM-4-methyl-2-oxo-[1-¹⁴C]pentanoate. Pyruvate (5 mM), added in the

presence of glucose, inhibited the oxidation of the branched-chain oxo acid by approx. 40%. The addition of insulin did not affect the oxidation of 4-methyl-2-oxopentanoate.

Effect of glucose and pyruvate on leucine oxidation by nerves

We have shown that leucine oxidation by isolated rat hemidiaphragms proceeds linearly with time during 2 h of incubation (Buse *et al.*, 1972). ¹⁴CO₂

Fig. 1. $^{14}\text{CO}_2$ production by rat hemidiaphragms during incubation with increasing concentration of 4-methyl-2-oxo-[1- ^{14}C]pentanoate or L-[1- ^{14}C]leucine with or without added 5mm-2-oxoglutarate

Hemidiaphragms were preincubated for 1 h and incubated for 2 h as described in Tables 1 and 3. Glucose (5.5 mM) was in all media with 4-methyl-2-oxo[1- ^{14}C]pentanoate (●), [1- ^{14}C]leucine (▲), 4-methyl-2-oxo[1- ^{14}C]pentanoate + 5mm-2-oxoglutarate (○), [1- ^{14}C]leucine + 5mm-2-oxoglutarate (△). Each point is the mean of at least four observations. Stimulation by 2-oxoglutarate was significant ($P < 0.02$) at low concentrations of 4-methyl-2-oxopentanoate (0.05–0.10 mM) and at all concentrations of leucine tested (0.1, 0.5 and 1.0 mM). The inset shows $^{14}\text{CO}_2$ production with low concentrations of ^{14}C -labelled substrates.

production by isolated sciatic nerves incubated with 0.1 mM-[1- ^{14}C]leucine, with or without added 16.5 mM-glucose, is illustrated in Fig. 2. Sciatic nerves oxidized leucine at a slightly higher rate than diaphragms. When glucose was included in the medium leucine oxidation was linear with time for 2 h of observation. When glucose was omitted, leucine oxidation proceeded linearly for 90 min, but reached a plateau during the next 30 min of incubation. During the first 90 min, the rate of leucine oxidation was essentially identical in the presence or absence of glucose. Glucose has been reported to cause a mild but significant inhibition of branched-chain amino acid oxidation by rat hemidiaphragms and hearts

(Odessey & Goldberg, 1972; Buse *et al.*, 1972). This effect was not observed in sciatic nerves. The effect of glucose was not dose related, since it was essentially identical when either 5.5 or 16.5 mM-glucose was used (Table 4).

Substitution of glucose by pyruvate as the energy-yielding substrate did not achieve the same effect. Nerves incubated with pyruvate for 2 h oxidized 36% less leucine ($P < 0.001$) than did nerves incubated with leucine alone. The inhibitory effect of pyruvate on leucine oxidation by nerves was observed in the presence or the absence of glucose. Sciatic nerves incubated with 5.5 mM-glucose and 5 mM-pyruvate oxidized 30% less leucine ($P < 0.01$) than nerves incubated with glucose alone.

To determine whether or not the leucine molecule was completely oxidized by diaphragm and nerves, $^{14}\text{CO}_2$ production from [1- ^{14}C]leucine and [U- ^{14}C]leucine was compared during incubation with 0.1 and 0.5 mM-leucine (Table 5). In diaphragm $^{14}\text{CO}_2$ production from [U- ^{14}C]leucine was approximately 40% of that observed during incubation with [1- ^{14}C]leucine at the same substrate concentration.

Fig. 2. Time-course of L-leucine oxidation by sciatic nerves *in vitro*

Rat sciatic nerves were preincubated for 1 h in 1 ml of balanced salt solution containing 0.1 mM-leucine with 16.5 mM-glucose (○), or without glucose (●). They were then transferred to media of identical composition except for the addition of 0.02 μCi of [1- ^{14}C]leucine and incubated for the time-periods indicated. The conditions of incubation are described in the Materials and Methods section and Table 1. Each point is the mean of four to seven measurements $\pm$ S.E.M.

Table 5. Oxidation of L-leucine by hemidiaphragms, peripheral nerves and spinal cords of the rat

Muscles and nerves were preincubated for 1 h and incubated for 2 h in balanced-salt solution containing 5.5 mM-glucose and 0.1 or 0.5 mM-leucine as described in Table 1. The ^{14}C -labelled tracers were added during incubation only. Spinal cords were not preincubated. One hemidiaphragm, sciatic nerve and brachial plexus of each rat were incubated with $[1\text{-}^{14}\text{C}]\text{leucine}$, the other with $[\text{U-}^{14}\text{C}]\text{leucine}$. The sciatic nerve and brachial plexus were placed together into 1 ml of medium. The spinal cord of each rat was divided longitudinally, and each half was placed into a tared flask containing 1 ml of medium and incubated for 2 h. $^{14}\text{CO}_2$ production was expressed as nmol of $^{14}\text{C}]\text{leucine}$ metabolized; for the calculation see Table 1. The values are means of six observations $\pm$ S.E.M.

Label (mM)	$^{14}\text{CO}_2$ produced (nmol/2h per g)		
	Hemidiaphragms	Nerves	Spinal cord
1- ^{14}C (0.1)	280.5 $\pm$ 27.5	307.9 $\pm$ 27.8	126.6 $\pm$ 23.7
U- ^{14}C (0.1)	120.4 $\pm$ 13.3	63.4 $\pm$ 2.8	20.2 $\pm$ 3.2
1- ^{14}C (0.5)	1939.8 $\pm$ 128.3	913.8 $\pm$ 78.1	260.7 $\pm$ 35.9
U- ^{14}C (0.5)	790.4 $\pm$ 66.2	192.8 $\pm$ 23.9	69.8 $\pm$ 12.0

Increasing the concentration of leucine from 0.1 to 0.5 mM increased leucine oxidation by hemidiaphragms sevenfold, irrespective of the tracer used, whereas the ratio of $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{leucine}$ to that from $[1\text{-}^{14}\text{C}]\text{leucine}$ remained unchanged. Peripheral nerve and spinal cord yielded only about one-fifth as much $^{14}\text{CO}_2$ during incubation with $[\text{U-}^{14}\text{C}]\text{leucine}$ as with $[1\text{-}^{14}\text{C}]\text{leucine}$; this ratio remained constant during incubation with 0.1 or 0.5 mM-leucine. In contrast with hemidiaphragms, a fivefold increase in the leucine concentration of the medium resulted only in a 2–3-fold increase in leucine oxidation by isolated peripheral nerves and spinal cords.

Odessey & Goldberg (1972) compared $^{14}\text{CO}_2$ production from leucine that was labelled either uniformly or in the C-1 position, during incubation of hemidiaphragms obtained from small (60–90 g) rats. They reported that the ratio of $^{14}\text{CO}_2$ produced from $[\text{U-}^{14}\text{C}]\text{leucine}$ to that from $[1\text{-}^{14}\text{C}]\text{leucine}$ was approx. 0.6. This ratio was considered compatible with complete oxidation of the leucine molecule, since the complete degradation of $[\text{U-}^{14}\text{C}]\text{leucine}$ produces many radioactive intermediates (e.g. $^{14}\text{C}]\text{-acetate}$ or $^{14}\text{C}]\text{-acetoacetate}$), which are diluted in the cell by a large amount of non-radioactive material. Incorporation of ^{14}C from $[\text{U-}^{14}\text{C}]\text{leucine}$ into fat by hemidiaphragms was 2% of that incorporated into proteins, and less than 4% of that recovered as $^{14}\text{CO}_2$. In our studies, the ratio of ^{14}C from uniformly labelled leucine incorporated into lipids to that into proteins by hemidiaphragms was 0.04 (L. W. Stillway, D. A. Weigand & M. G. Buse, unpublished work), whereas the ratio of $^{14}\text{C}]\text{leucine}$ oxidized to that incorporated into proteins was 1.32 (Table 8). The somewhat smaller ratio of $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{leucine}$ to that from $[1\text{-}^{14}\text{C}]\text{leucine}$ by muscles in the present study may be related to the use of older animals, resulting in slower turnover of the intermediate pools. The data suggest that in nerves a

significant portion of the breakdown products of leucine is not oxidized. This supports the observation that leucine is a significant precursor of lipid synthesis by peripheral nerves *in vitro* (Stillway *et al.*, 1974).

Effects of 2-oxoglutarate and octanoate on the oxidation of branched-chain amino acids by hemidiaphragms, nerves, spinal cord and aorta (Table 6)

Previous work from this laboratory indicated that in isolated hemidiaphragms and perfused hearts, the oxidation of the three branched-chain amino acids is regulated in a parallel fashion (Buse *et al.*, 1972, 1973a). The data in Table 6 show that 2-oxoglutarate stimulated the oxidation of all three branched-chain amino acids by hemidiaphragms, and that the stimulatory effect was of the same order of magnitude for the three substrates.

In isolated nerves and spinal cords the effect of 2-oxoglutarate was tested only with isoleucine as substrate; 5 mM-2-oxoglutarate stimulated $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{isoleucine}$ by peripheral nerves significantly; however, no effect of 2-oxoglutarate on isoleucine oxidation by isolated spinal cords was observed.

The effect of octanoate on the oxidation of leucine and isoleucine in peripheral nerves was the opposite of that observed in hemidiaphragms. Whereas the addition of 1 mM-octanoate produced an approximate doubling of the oxidation of 0.1 mM-isoleucine by hemidiaphragms, in sciatic nerves octanoate caused significant inhibition of $^{14}\text{CO}_2$ production from 0.1 mM- $[1\text{-}^{14}\text{C}]\text{isoleucine}$. Octanoate also inhibited leucine oxidation by sciatic nerves by approx. 35% of the basal rate when 0.1 or 0.03 mM-leucine was used as substrate.

Aortic strips were very efficient in oxidizing isoleucine; when calculated on the basis of weight, the aorta oxidized twice as much isoleucine as muscle or peripheral nerve. In contrast with muscle, and as

Table 6. *Effect of 2-oxoglutarate and octanoate on the oxidation of branched-chain L-amino acids by different tissues in the rat*

Tissues were preincubated and incubated as described in Tables 1 and 5 in media containing 5.5 mM-glucose. In some of the experiments with nerves, only sciatic nerves were used. In others, sciatic nerves and brachial plexus were incubated together; since the results were identical they were pooled. $^{14}\text{CO}_2$ production was expressed as nmol of ^{14}C -labelled substrate metabolized (see Table 1). Octanoate and 2-oxoglutarate were added to the media as the sodium salts. Means $\pm$ S.E.M. are tabulated. The number of paired observations are indicated in parentheses. *P* was calculated by paired *t* test. N.S., Not significant.

^{14}C -labelled substrate	Concn. (mM)	Tissues	Additions	Concn. (mM)	$^{14}\text{CO}_2$ produced (nmol/2h per g)	<i>P</i>
[1- ^{14}C]Valine	0.1	Hemidiaphragm	2-Oxoglutarate	0	194 $\pm$ 11.3	<0.001
				5	296 $\pm$ 18.5 (7)	
[U- ^{14}C]Valine	0.1	Hemidiaphragm	2-Oxoglutarate	0	66 $\pm$ 5.2	<0.001
				5	113 $\pm$ 9.5 (8)	
[1- ^{14}C]Isoleucine	0.1	Hemidiaphragm	2-Oxoglutarate	0	229 $\pm$ 14.3	<0.001
				5	359 $\pm$ 17.8 (10)	
		Nerve	2-Oxoglutarate	0	253 $\pm$ 16.7	<0.025
				5	318 $\pm$ 17.6 (11)	
		Spinal cord	2-Oxoglutarate	0	133 $\pm$ 22.5	N.S.
				5	145 $\pm$ 6.6 (12)	
		Hemidiaphragm	Octanoate	0	212 $\pm$ 18.9	<0.001
				1	409 $\pm$ 21.5 (6)	
		Nerve	Octanoate	0	299 $\pm$ 20.4	<0.0025
				1	236 $\pm$ 15.4 (14)	
		Aorta	Octanoate	0	596 $\pm$ 54.2	<0.001
				1	316 $\pm$ 23.0 (14)	
[1- ^{14}C]Leucine	0.1	Nerve	Octanoate	0	408 $\pm$ 17.4	<0.005
				1	266 $\pm$ 8.4 (14)	
	0.03	Nerve	Octanoate	0	225 $\pm$ 20.8	<0.025
				1	153 $\pm$ 7.3 (4)	

in nerve, the addition of 1 mM-octanoate significantly inhibited isoleucine oxidation by the aorta.

Since the cofactors involved in the oxidative decarboxylation of the branched-chain α -oxo acids may have been rate-limiting in nerves, the effect of CoA and thiamin pyrophosphate were tested in pilot experiments, which are not included in the Tables. The addition of 1 mM-CoA did not affect the oxidation of 0.1 mM-leucine by isolated nerves incubated in the presence of 5.5 mM-glucose; nor was the inhibitory effect of octanoate on leucine oxidation affected by the addition of 1 mM-CoA to the medium. Thiamin pyrophosphate (1 mM) added to a medium containing 5.5 mM-glucose and 0.1 mM-4-methyl-2-oxo[1- ^{14}C]-pentanoate did not affect the oxidation of the latter substrate by sciatic nerves.

Oxidation of 4-methyl-2-oxopentanoate by nerves and spinal cords (Table 7)

At equimolar concentrations of substrate, peripheral nerves and spinal cords appeared to oxidize 4-methyl-2-oxopentanoate slightly faster than they oxidize leucine. Increasing the concentration of 4-methyl-2-oxo[1- ^{14}C]pentanoate from 0.1 to 0.5 mM

caused only a 2–3-fold increase in $^{14}\text{CO}_2$ production. Pyruvate and octanoate inhibited 4-methyl-2-oxopentanoate oxidation by nerves, decreasing it to less than one-half of the basal rate. The effect of pyruvate on the oxidation of 4-methyl-2-oxopentanoate by spinal cords was essentially identical with that observed in nerves. The addition of 5 mM-2-oxoglutarate to nerves or spinal cords did not significantly affect the oxidation of the branched-chain oxo acid by these tissues at the substrate concentrations tested (0.1 and 0.5 mM). Insulin (1 munit/ml) did not affect 4-methyl-2-oxopentanoate oxidation by nerves.

Comparison of leucine metabolism by hemidiaphragms and sciatic nerves and the effect of Wallerian degeneration

Table 8 demonstrates that oxidation of leucine to CO_2 is a quantitatively important pathway of leucine metabolism in muscles and peripheral nerves. Since incorporation into protein and oxidation to CO_2 are main metabolic fates of the first carbon atom of leucine in muscle (Odessey & Goldberg, 1972), the data in Table 8 suggest that diaphragms oxidize approx. 60% of their leucine uptake. In isolated intact

Table 7. *Effect of 2-oxoglutarate, pyruvate, insulin and octanoate on the oxidation of 4-methyl-2-oxo[1-¹⁴C]pentanoate by isolated peripheral nerves and spinal cords of the rat*

Peripheral nerves were preincubated for 1 h and incubated for 2 h in balanced salt solution containing 5.5 mM-glucose, 4-methyl-2-oxopentanoate (0.1 or 0.5 mM) and the additions indicated in the Table. The conditions were as described in Tables 1 and 5. Organic acids were added as their sodium salts. 4-Ethyl-2-oxo[¹⁴C]pentanoate was added during incubation only. In the experiment marked with * incubation lasted only 45 min and in that marked ** 90 min; the ¹⁴CO₂ produced during 45 or 90 min is recorded. Spinal cords were not preincubated and were incubated for 2 h. ¹⁴CO₂ production was expressed as nmol of 4-methyl-2-oxo[¹⁴C]pentanoate metabolized (see Table 1). Means $\pm$ S.E.M. are tabulated. The numbers of paired observations are indicated in parentheses. *P* was calculated by paired *t* test. N.S., Not significant.

Tissues	4-Methyl-2-oxo-pentanoate (mM)	Additions	Concn. (mM)	¹⁴ CO ₂ produced (nmol/2h per g)	<i>P</i>
Nerves (sciatic + brachial)	0.1	2-Oxoglutarate	0	596 $\pm$ 34.5	N.S.
			5	633 $\pm$ 54.5 (6)	
	0.5	2-Oxoglutarate	0	1240 $\pm$ 80.9	N.S.
			5	1481 $\pm$ 146.2 (6)	
Spinal cord	0.1	2-Oxoglutarate	0	118 $\pm$ 45.3	N.S.
			5	116 $\pm$ 23.7 (6)	
	0.5	2-Oxoglutarate	0	459 $\pm$ 24.5	N.S.
			5	478 $\pm$ 82.9 (6)	
Nerves (sciatic + brachial)	0.1	Pyruvate	0	649 $\pm$ 73.1	<0.01
			5	334 $\pm$ 47.0 (6)	
	0.5	Pyruvate	0	1308 $\pm$ 166.2	<0.01
			5	588 $\pm$ 130.0 (6)	
Spinal cord	0.1	Pyruvate	0	162 $\pm$ 11.3	<0.01
			5	99 $\pm$ 18.4 (6)	
	0.5	Pyruvate	0	569 $\pm$ 57.3	<0.01
			5	324 $\pm$ 61.5 (6)	
Nerves (sciatic)	0.1	Insulin	0	849 $\pm$ 57.4	N.S.
			1 munit/ml	899 $\pm$ 50.2 (6)	
Nerves (sciatic)	0.1	Octanoate	0	267 $\pm$ 35*	<0.025
			1	165 $\pm$ 23 (4)	
	0.1	Octanoate	0	581 $\pm$ 45**	0.05
			1	260 $\pm$ 54 (3)	

Table 8. *L-Leucine metabolism of isolated hemidiaphragms, sciatic nerves and nerves undergoing Wallerian degeneration: comparison of ¹⁴CO₂ production and ¹⁴C incorporation into proteins*

Tissues were preincubated for 1 h and incubated for 2 h as described in Table 1 in media containing 16.5 mM-glucose and 0.1 mM-leucine. [1-¹⁴C]Leucine (0.02 μ Ci/ml) was present during incubation only. ¹⁴CO₂ production and ¹⁴C incorporation into protein was expressed in nmol of [¹⁴C]leucine; for calculation see Table 1. Means $\pm$ S.E.M. are tabulated. *, Sciatic nerves severed close to origin 8–14 days before the experiment.

	Hemidiaphragm	Sciatic nerve	'Cut'* nerve
¹⁴ CO ₂ produced (nmol/2h)			
(per g of tissue)	172 $\pm$ 18	365 $\pm$ 22	231 $\pm$ 22
(per 0.1 g of protein)	128 $\pm$ 14	906 $\pm$ 48	807 $\pm$ 76
¹⁴ C incorporated into protein (nmol/2h)			
(per g of tissue)	130 $\pm$ 12	107 $\pm$ 11	222 $\pm$ 28
(per 0.1 g of protein)	97 $\pm$ 9	271 $\pm$ 31	786 $\pm$ 76
¹⁴ C oxidized $\times$ 100			
¹⁴ C incorporated into protein	132 $\pm$ 17	335 $\pm$ 30	103 $\pm$ 22
Protein (mg/g of tissue)			
DNA (μ g/0.1 g of tissue)	134 $\pm$ 2.7	40 $\pm$ 1.4	29 $\pm$ 1.8
No. of observations	35 $\pm$ 1.5	109 $\pm$ 15	192 $\pm$ 30
	7	12	12

sciatic nerves the ratio $^{14}\text{CO}_2$ production/ ^{14}C incorporation into proteins is approx. 3:1. Table 8 also compares the leucine metabolism of intact sciatic nerves with that of their severed pairs, which were undergoing Wallerian degeneration. One sciatic nerve of each rat was cut at its origin 8–14 days before the experiment; its intact pair served as control. The weight of the dissected intact and severed nerves was not significantly different at the time of death (21.8 ± 1.5 mg for controls and 20.0 ± 1.8 mg for the severed nerves). However, the relative protein content of the severed nerves was approx. 30% less than that of the controls. When expressed on the basis of tissue weight, the severed nerves oxidized significantly less leucine than the controls ($P < 0.01$). When leucine oxidation was related to the protein content of the tissue, there was no significant difference in leucine oxidation between intact and severed sciatic nerves. A marked increase in leucine incorporation into proteins as well as a twofold increase in tissue DNA content was observed in the cut nerves. Proliferation of Schwann cells and stimulation of protein synthesis has been reported in nerves undergoing Wallerian degeneration (Takahashi *et al.*, 1961).

Discussion

The data presented demonstrate that rat peripheral nerve, spinal cord and the aorta are among the tissues that have a high capacity for oxidizing branched-chain amino acids. We have also shown that factors that affect the oxidation of the branched-chain amino acids generally also affect the oxidation of 4-methyl-2-oxopentanoate, the product of the deamination of leucine, in the same way. Thus octanoate stimulated oxidation of leucine and of 4-methyl-2-oxopentanoate by hemidiaphragms, whereas pyruvate inhibited the oxidation of both substrates. In peripheral nerves octanoate and pyruvate inhibited the oxidation of branched-chain amino acids and that of 4-methyl-2-oxopentanoate; the degree of inhibition was of the same magnitude with both substrates. This parallelism suggests that the agents tested regulated the oxidation of the branched-chain amino acids at a step beyond transamination. Since the oxidation of the three branched-chain amino acids was similarly affected, it appears reasonable to assume that one of the early reactions was involved, e.g. the oxidative decarboxylation to acyl-CoA thioesters or their subsequent dehydrogenation.

The first reaction appears to be a plausible regulatory site, since it is catalysed by an enzyme complex that is thought to be similar (Connelly *et al.*, 1968; Shinnick & Harper, 1972) to the pyruvate dehydrogenase system (Ishikawa *et al.*, 1966). Substrates of either system inhibit the oxidation of substrates of the other. Pyruvate inhibited the oxidation of branched-chain amino acids in kidney (Dawson & Hird, 1968),

heart, diaphragm (Buse *et al.*, 1972), peripheral nerves and spinal cords (the present study), as well as the oxidation of branched-chain oxo acids by liver mitochondria (Danner & Bowden, 1966), and by the tissues tested in the present study. Inhibition of pyruvate oxidation by branched-chain α -oxo acids has been reported in studies of rat liver (Bowden *et al.*, 1970), brain (Bowden *et al.*, 1971) and with purified pyruvate dehydrogenase (Kanzaki *et al.*, 1969).

The fact that 2-oxoglutarate stimulated the oxidation of the branched-chain amino acids and that of 4-methyl-2-oxopentanoate in muscles suggests that, in addition to being a substrate of the transaminase reaction, it may also stimulate the branched-chain α -oxo acid dehydrogenase system. As shown in Fig. 1, in muscle, at low concentrations of substrate, the branched-chain α -oxo acid dehydrogenase reaction appeared to be stimulated also by the substrate, 4-methyl-2-oxopentanoate. The transamination of the branched-chain amino acids is fully reversible in liver and skeletal muscles (Walser *et al.*, 1973). It is possible that 2-oxoglutarate stimulated 4-methyl-2-oxopentanoate oxidation by hemidiaphragms, because as an amino-group acceptor it prevented the formation of leucine from the branched-chain α -oxo acid, thus increasing the intracellular concentration of the substrate. On the other hand, it may have a more direct effect on the branched-chain α -oxo acid dehydrogenase system. Whichever the mechanism, 2-oxoglutarate may regulate branched-chain amino acid oxidation in muscle at two points: as an amino-group acceptor by accelerating transamination, and by accelerating the oxidation of the resulting branched-chain α -oxo acid. In nerves, 2-oxoglutarate stimulated the oxidation of leucine but not that of 4-methyl-2-oxopentanoate, suggesting that in this tissue 2-oxoglutarate accelerated only branched-chain aminotransferase.

At all substrate concentrations tested 4-methyl-2-oxopentanoate was oxidized faster by hemidiaphragms than leucine, and this difference was also observed at relatively high substrate concentrations (1 mM), which exceeded the physiological range of leucine concentration in rat plasma (Scharff & Wool, 1966; Meikle & Klain, 1972). One of two explanations appears likely: (1) the transport of 4-methyl-2-oxopentanoate into muscle cells, or into a specific compartment of muscle cells, is faster than the transport of leucine; (2) under our conditions *in vitro* transamination was limiting the rate of leucine oxidation by hemidiaphragms. It is noteworthy that in skeletal muscle branched-chain aminotransferase is present in very high activity relative to its activity in the liver (Ichihara & Koyama, 1966; Taylor & Jenkins, 1966).

As judged by the response to octanoate, during incubation with branched-chain amino acids at physiological concentrations the rat tissues tested fell

into two categories: heart and hemidiaphragm showed stimulation of branched-chain amino acid oxidation; peripheral nerves, the spinal cord and the aorta showed inhibition. The observed differences between muscles and nerves could be explained by assuming an active and an inactive form of the branched-chain α -oxo acid dehydrogenase complex in muscles, the latter being activated by the substrate as well as by 2-oxoglutarate and octanoate; if the enzyme was present mainly in the active form in nerves the above agents would cause no further stimulation. The inhibitory effect of octanoate in nerves and aorta may be attributed to an increase in the NADH/NAD⁺ ratio and/or an increase in the acetyl-CoA/CoA ratio; these factors are thought to account for the inhibition of the pyruvate dehydrogenase system during fatty acid oxidation by muscles (Randle *et al.*, 1966). It is noteworthy that in the presence of unphysiologically high substrate concentrations (e.g. 2mM-isoleucine) octanoate inhibits branched-chain amino acid oxidation also in rat hemidiaphragms (Buse *et al.*, 1972).

In recent years it has become increasingly evident that the delivery of certain amino acids, e.g. alanine and glutamine, from the peripheral tissues, primarily muscles, is an important factor in the regulation of gluconeogenesis (Felig *et al.*, 1969; Pozefsky & Tancredi, 1972). Stimulation of branched-chain amino acid oxidation by muscles in conditions of stress and carbohydrate deprivation may serve a dual role: the carbon skeleton is used as fuel for muscles, and the amino group becomes available for the formation of alanine and glutamine, substrates for gluconeogenesis by liver and kidney. Ryan *et al.* (1974) reported marked stimulation of leucine oxidation by isolated muscle fibres obtained from monkeys which had been traumatized 48 h before the experiment. The conditions that are characterized by accelerated branched-chain amino acid oxidation in muscles are also accompanied by accelerated catabolism of muscle proteins and negative nitrogen balance. It is tempting to speculate that stimulated oxidation of the branched-chain amino acids may result in a relative deficiency of a critical essential amino acid in a cellular compartment, which could act as a signal for the accelerated breakdown and/or decreased synthesis of muscle proteins. Preliminary experiments indeed suggest that in isolated hemidiaphragms, leucine may play a role in regulating the turnover of muscle proteins (Buse & Reid, 1974). Rannels *et al.* (1974) reported that in the isolated perfused heart the branched-chain amino acids alone were as effective as a mixture containing all of the plasma amino acids in promoting the incorporation of [¹⁴C]phenylalanine into muscle proteins.

The cells responsible for the brisk oxidation of branched-chain amino acids by peripheral nerves and the aorta need to be identified. The data shown in

Table 8 suggest that branched-chain amino acid oxidation by peripheral nerves occurs in the Schwann cells, although an intact axon appears to be required for optimum efficiency. Caston & Singer (1969) reported that approx. 20% of the [¹⁴C]leucine that was incorporated into the sciatic nerves of amphibians after an intraperitoneal injection was in the lipid fraction. Leucine is a significant precursor of lipid and cholesterol synthesis in several tissues (Rosenthal *et al.*, 1974; Stillway *et al.*, 1974). The possibility that some structural lipids derived from branched-chain amino acids may contain branched-chain fatty acids should be considered; their prevalence could depend on factors that regulate branched-chain amino acid oxidation.

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