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Normal 2-Aminobutyrate Oxidation and Increased Valine Oxidation in Fibroblasts Deficient in Pyruvate Dehydrogenase

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Human skin fibroblasts deficient in pyruvate dehydrogenase and five normal control strains were incubated with one of the following labelled substrates: DL-[1-^{14}C]-2-amino-n-butyric acid, DL-[3-^{14}C]-2-amino-n-butyric acid, L-[1-^{14}C]leucine, L-[1-^{14}C]valine, L-[1-^{14}C]alanine, and [1-^{14}C]pyruvate. The rate of $^{14}CO_2$-production in the deficient cells was normal from 2-aminobutyrate and leucine, increased from valine, and decreased from alanine and pyruvate. These results indicated that in human skin fibroblasts the decarboxylation of 2-oxobutyrate is catalysed by an enzyme system different from the pyruvate dehydrogenase complex.

In 1947 it was shown that pigeon breast muscle oxidized pyruvic acid and 2-oxoglutaric acid by two specific enzymes, pyruvic oxidase (pyruvate dehydrogenase complex (PDH)) and α-ketoglutaric oxidase (2-oxoglutarate dehydrogenase complex (OGDH)), with very similar properties (Stumpf *et al.*, 1947). Neither of the enzymes oxidized 2-oxobutyric acid. Stumpf *et al.* (1947) concluded that the two oxidases in their preparation were specific for pyruvic acid and 2-oxoglutaric acid, respectively. 20 years later similar findings supporting these conclusions were published (Connelly *et al.*, 1968). However, Kanzaki *et al.* (1969) concluded from purification studies on pig heart PDH that no enzyme specific for 2-oxobutyrate existed, and that the PDH itself performed the degradation of 2-oxobutyrate as well as pyruvate. Bremer (1969) also suggested that pyruvate and 2-oxobutyrate were oxidized by the same enzyme, the PDH, in rat and pig organs.

A patient suffering from lactic acidosis (McKusick 20880) due to a defect in the pyruvate dehydrogenase (EC 1.2.4.1) of the PDH has been described (Strømme *et al.*, 1976). In the present study skin fibroblasts from this patient (PDH-deficient cells) were used to test their capacity for 2-oxobutyrate oxidation. A normal degradation rate would indicate that in these cells the decarboxylation of 2-oxobutyrate is performed by an enzyme system different from PDH, whereas a decreased rate would indicate that PDH is responsible for the degradation of 2-oxobutyrate as well as pyruvate.

MATERIALS AND METHODS

Human fibroblasts were grown as previously described (Strømme *et al.*, 1976; Borud and Strømme, 1977). All experiments were done on cells between the 16th and 23rd trypsination. Mycoplasma infections were tested for by the radioactive uridine/uracil ratio (Schneider and Standbridge, 1975). No mycoplasma infections were detected in the cell cultures after the experiments. The ^{14}C-isotope experiments were performed as previously described (Borud and Strømme, 1977) except that the $^{14}CO_2$ formed was trapped during the experiments as well as for 4 hours after the addition of $NaHCO_3$/acetic acid.

Radioactive isotopes and chemicals of the highest available purity were used. L-[1-^{14}C]leucine, [1-^{14}C]pyruvic acid, L-[1-^{14}C]valine and L-[1-^{14}C]alanine were purchased from the Radiochemical Centre, Amersham, UK, DL-[3-^{14}C]-2-amino-n-butyric acid from Commisariat A L'energie Atomique, Gif-sur-Yvette, France, and DL-[1-^{14}C]-2-amino-n-butyric acid from ICN Pharmaceuticals, Cal., USA. The DL-[1-^{14}C]-2-amino-n-butyric acid contained about 10% volatile ^{14}C-labelled impurities which were removed in a stream of N_2 at 25°C after addition of $NaHCO_3$ and acetic acid (pH 4). In addition some of the isotope solution was further purified by DEAE-Sephadex column chromatography (Chalmers and Watts, 1972), and used for the experiments shown in Figure 1. The amino acids were used in preference to the keto acids because the latter are commonly contaminated with volatile radioactive compounds and are unstable under conditions of storage and incubation.

For the results shown in Table 1, untrypsinized cells (subculture number 16–23) were washed with Hank's salt solution and incubated in the same solution containing one of the following labelled substrates: [1-^{14}C]pyruvate, 2.3 μCi/flask; L-[1-^{14}C]alanine, 1.4 μCi/flask; L-[1-^{14}C]leucine, 1.0 μCi/flask; L-[1-^{14}C]valine, 1.0 μCi/flask (100 μmol/l) and 2.1 μCi/flask (400 μmol/l); DL-[1-^{14}C]-2-amino-n-butyric acid, 2.4 μCi/flask; DL-[3-^{14}C]-2-amino-n-butyric acid, 1.4 μCi/flask (25 μmol/l), 1.2 μCi/flask (100 μmol/l) and 4.6 μCi/flask (400 μmol/l). The flasks contained about 0.4 mg protein and were incubated with 2 ml medium at 37°C. The metabolism was stopped by acidification as previously described (Strømme *et al.*, 1975). Three parallel incubations of each strain were done; the mean values of these were used for the calculations.

Paired comparison was done statistically using Student's *t* test.

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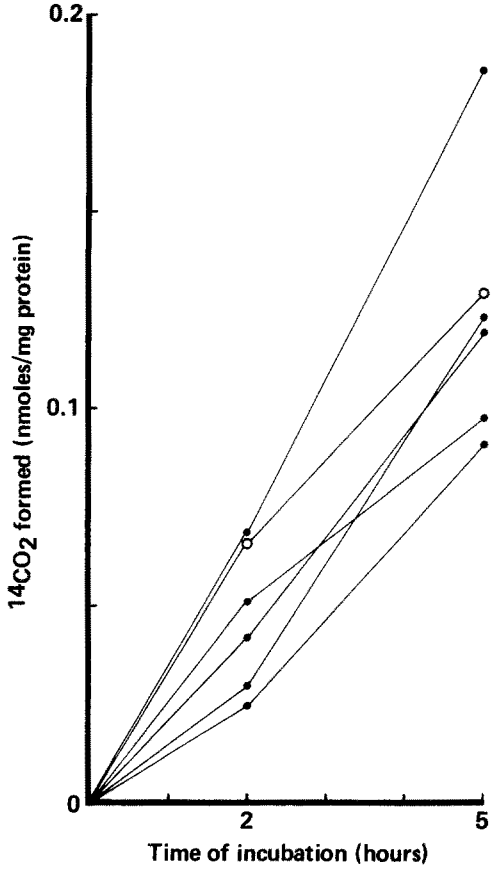

Figure 1. Oxidation of purified [1-^{14}C]-2-amino-n-butyric acid to $^{14}CO_2$ in five normal fibroblast strains (●) and in a pyruvate dehydrogenase deficient strain (○)

RESULTS

Table 1 shows the formation of $^{14}CO_2$ from ^{14}C-labelled pyruvate, alanine, valine, leucine, and 2-aminobutyrate in untrypsinized fibroblasts (five normal control cell strains and the PDH-deficient strain).

The rates of oxidation of alanine and pyruvate were 14% and 13%, respectively, in the PDH-deficient strain compared with the five normal cell strains in accordance with earlier findings (Borud and Strømme, 1977).

The experiments with [1-^{14}C]- and [3-^{14}C]-2-aminobutyrate as substrates showed a normal oxidation rate in the PDH-deficient cells. Figure 1 shows the results from another experiment using purified [1-^{14}C]-2-aminobutyric acid as substrate.

Table 1 shows that the oxidation of valine was significantly increased ($p < 0.001$) in the PDH-deficient cells (360% of the normal rate). Figure 2 shows the results from another experiment, with ^{14}C-valine as substrate.

DISCUSSION

The pyruvate dehydrogenase complex in mammalian cells is a large protein complex with a molecular weight of about 8×10^6 daltons (Guest, 1978). Other 2-oxoacid dehydrogenase complexes described are the OGDH and the branched-chain 2-oxoacid dehydrogenase complex(es), both large protein complexes with similar physical properties to the PDH. The different dehydrogenase complexes are difficult to separate from each other, and as stated above there are different opinions with regard to the existence of a 2-oxobutyrate dehydrogenase complex different from PDH and OGDH.

In the present study fibroblasts from a patient with a known enzyme defect in the PDH were used as a tool for testing if the PDH and a hypothetical 2-oxobutyrate dehydrogenase complex were identical or two separate enzyme systems. Tissue from patients with known specific enzyme defects have been used to solve similar problems (Rhead and Tanaka, 1980). Our results shown in Table 1 and in Figure 1 indicate that in human skin fibroblasts two separate enzyme systems exist for the decarboxylation of pyruvate and 2-oxobutyrate, respectively. This conclusion is in accordance with the findings

Table 1 Formation of $^{14}CO_2$ from ^{14}C-labelled substrates by cultured fibroblasts, five normal control strains and one PDH-deficient strain

^{14}C-labelled substrate	*μmol/l*	*Incubation time (hours)*	*$^{14}CO_2$ formed (nmol/mg protein)*	
			Five normal cell strains mean (range)	*PDH-deficient cell strain*
[1-^{14}C]pyruvate	100	2	9.0 (3.8–11.6)	1.2
[1-^{14}C]alanine	100	5	4.1 (3.0–5.2)	0.59
[1-^{14}C]leucine	100	2	4.6 (2.1–5.1)	4.6
	100	5	7.9 (4.5–10.9)	9.2
[1-^{14}C]valine	100	2	0.33 (0.11–0.43)	1.3
	100	5	0.87 (0.23–1.39)	3.45
	400	5	1.58 (0.66–2.60)	5.95
[1-^{14}C]-2-aminobutyrate	100	2	0.064 (0.053–0.083)	0.061
	100	5	0.18 (0.14–0.23)	0.19
[3-^{14}C]-2-aminobutyrate	25	5	0.013 (0.004–0.029)	0.011
	100	5	0.030 (0.013–0.067)	0.048
	400	5	0.13 (0.06–0.25)	0.16

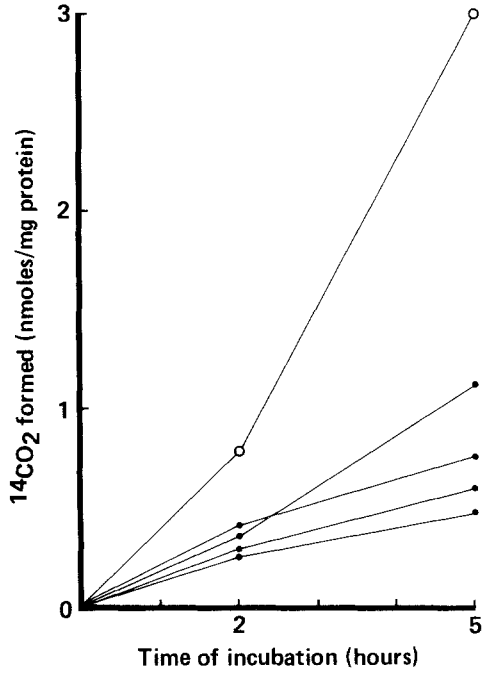

Figure 2. Oxidation of [1-^{14}C]valine to $^{14}CO_2$ in four normal fibroblast strains (●) and in a pyruvate dehydrogenase deficient strain (○)

of Stumpf *et al.* (1947) and Connelly *et al.* (1968), who studied the metabolism of 2-oxobutyrate in pigeon breast muscle and bovine liver, respectively.

In maple syrup urine disease (McKusick 24860) with deficiency of the branched-chain 2-oxoacid dehydrogenase complex(es) no increased excretion of 2-aminobutyrate or 2-oxobutyrate was found by Menkes (1959). His findings would be in accordance with the view that 2-oxobutyrate is not a substrate for the branched chain 2-oxoacid dehydrogenase complex. Further support for this suggestion might be obtained by fibroblast experiments, similar to those in the present study, using cells from patients with maple syrup urine disease.

The oxidation of alanine, leucine and valine in normal fibroblasts shown in Table 1 is in the nanomol/hour range, in accordance with earlier findings in normal human skin fibroblasts (Dancis *et al.*, 1972). The oxidation rate of 2-aminobutyrate was low compared with that of alanine and leucine.

The increased oxidation of valine in the PDH-deficient cells was an unexpected finding since the branched-chain 2-oxoacids derived from leucine, isoleucine and valine are probably oxidized by a single enzyme complex similar to the PDH (Guest, 1978). Increased transamination of valine caused by the increased pyruvate in the deficient cells and a secondarily increased oxidation of the 2-oxo-isovaleric acid may be an explanation for this finding since a specific valine transaminase has been demonstrated in man (Dancis *et al.*, 1967). In accordance with this, leucine was oxidized normally in the PDH-deficient cells. Valine has also been shown to stimulate its own oxidation more than the other branched-chain amino acids do (Roberts and Sokatch, 1978), and this regulatory system may also operate in the valine metabolism in our PDH-deficient cells.

Our results from experiments in human skin fibroblasts indicate that the decarboxylation of 2-oxobutyrate is catalysed by an enzyme system different from the pyruvate dehydrogenase complex. Further work has to be done to show if a specific 2-oxobutyrate dehydrogenase complex does exist.

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