

Positron emission tomography using pyruvate-1-¹¹C in two cases of mitochondrial encephalomyopathy

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Positron emission tomography (PET) using pyruvate-1-¹¹C was carried out to investigate the in vivo metabolism of pyruvate in the brains of patients with mitochondrial encephalomyopathy and Leigh's disease. Two epileptic patients were studied as control subjects. Radioactivity was eliminated from the brain tissue of the epileptic patients soon after injection of pyruvate-1-¹¹C. PET images of mitochondrial encephalomyopathy patients showed an increase in radioactivity in the cerebral cortex, basal ganglia and thalamus, with elimination of radioactivity being slower than that of epileptic patients. One patient with Leigh's disease showed similar PET images. PET using pyruvate-1-¹¹C is useful for the evaluation of mitochondrial energy metabolism in the brain.

Key words: positron emission tomography, mitochondrial encephalomyopathy, pyruvate-1-¹¹C

INTRODUCTION

MITOCHONDRIAL ENCEPHALOMYOPATHIES¹ are clinically heterogeneous disorders, characterized by morphological and biochemical abnormalities of the mitochondrial metabolism. In recent years it has been demonstrated that there are several different sites of biochemical defects in the mitochondrial metabolism, including pyruvate dehydrogenase, pyruvate carboxylase, respiratory chains, and carnitine.² There are several clinically distinctive syndromes, such as the Kearns-Sayre syndrome,³ MELAS (mitochondrial encephalopathy, myopathy, lactic acidosis and stroke-like episodes),⁴ MERRF (myoclonus epilepsy with ragged-red fibers),⁵ Leigh's disease,⁶ etc.⁷⁻¹⁰ Although the diagnosis of these diseases depends upon the identification of histochemical abnormalities and/or specific biochemical

defects in the skeletal muscle, in some cases, due to the lack of abnormal findings in muscle mitochondria, only clinical and/or autopsy examinations can be used for diagnosis. There may be isolated abnormalities of mitochondrial energy metabolism in brain tissues. For example, a pyruvate dehydrogenase deficiency restricted to the brain was reported.¹¹

Recently, positron emission tomography (PET) has been applied to a noninvasive investigation of "in vivo" brain metabolisms, such as oxygen metabolism¹² and glucose metabolism.¹³ Hara et al. (1985) reported the synthesis of pyruvate-1-¹¹C as a radiopharmaceutical tracer for tumor imaging.¹⁴ Our study focuses on "in vivo" brain mitochondrial metabolism by PET, using pyruvate-1-¹¹C, and has obtained significant findings about mitochondrial encephalomyopathies.

METHODS

¹¹CO₂ was produced by bombardment of a gas target of N₂+O₂ (trace) with a proton beam in a baby cyclotron (Japan Steel Works BC-105 cyclotron). After trapping ¹¹CO₂ in a small volume of water, pyruvate-1-¹¹C was prepared enzymatically by an ex-

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Table 1 Enzyme activities in biopsied muscle mitochondria

	NADH cytochrome c reductase*	Succinate cytochrome c reductase*	Cytochrome c oxidase*	Carnitine		
				Total	Free	Acyl
Patient (Case 1)	87.2	310.2	79.3	3.10**	1.07**	2.03**
Controls	226.9±119.6	232.9±96.6	270.7±133.0	2.34±1.00	1.02±0.43	1.33±0.65
Patient (Case 2)	0.23	51.39	80.96	11.94***	5.19***	6.75***
Controls	6.28	27.54	1,449.86	15.6±2.8	10.2±2.6	5.4±2.0
Patient (Case 3)	182.1	258.9	455.2			
Controls	226.9±119.6	232.9±96.6	270.7±133.0			

*: Activities expressed as nmoles/min/mg mitochondrial protein.

**: Activities expressed as micromoles/g-wet weight.

***: Activities expressed as nmoles/ml.

Control values±SD

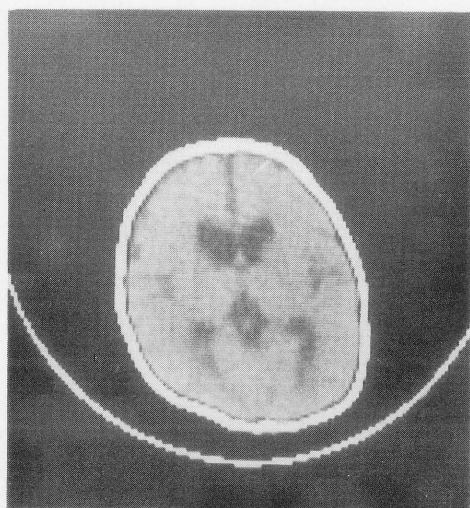


Fig. 1 Case 1. CT shows dilated bilateral anterior horns of the lateral ventricle, bilateral sylvian fissures and a longitudinal fissure which suggests atrophy of the caudate nucleus and cerebral cortex.

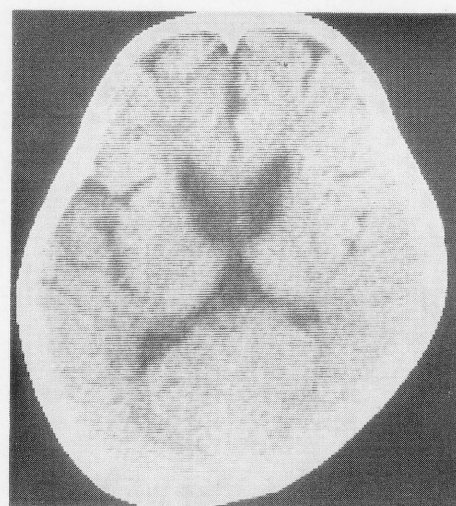


Fig. 3 Case 3. CT reveals dilated bilateral anterior horns of the lateral ventricle, cortical sulci and left sylvian fissure, indicating atrophy of the caudate nucleus and cerebral cortex.

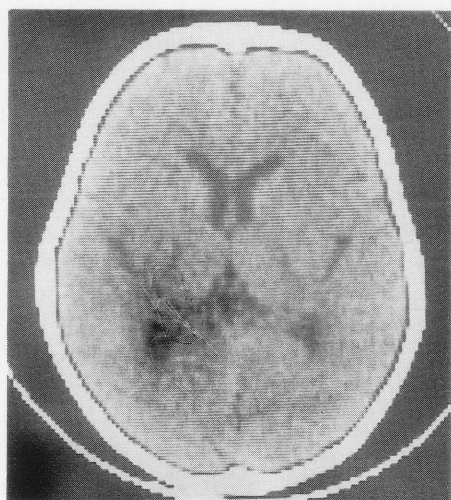


Fig. 2 Case 2. No remarkable abnormalities are seen except for mild dilatation of the bilateral sylvian fissures.

change reaction of $^{11}\text{CO}_2$ and the carboxyl group of pyruvic acid, using pyruvate-ferredoxin oxidoreductase of *Clostridium butyricum* (Seikagaku Kogyo Co. Tokyo).¹⁴ ^{11}C -labeled pyruvate was purified by sublimation in dry-sterilized glassware. At the end of the procedure, the evaporated pyruvic acid was soaked in 3 ml of water with 0.5 ml of 7% sodium bicarbonate added. As this amount of sodium bicarbonate was equivalent to the amount of pyruvic acid employed, the resulting solution was at the neutral pH level and immediately injectable. The injection solution thus prepared was found to be sterile and free from pyrogenic materials (Limulus and rabbit tests). Intravenous injection of the solution into human subjects produced no vascular pain or any other adverse effects. The radiochemical yield of pure pyruvate-1- ^{11}C was almost 100%, at 30 minutes after the end of bombardment.

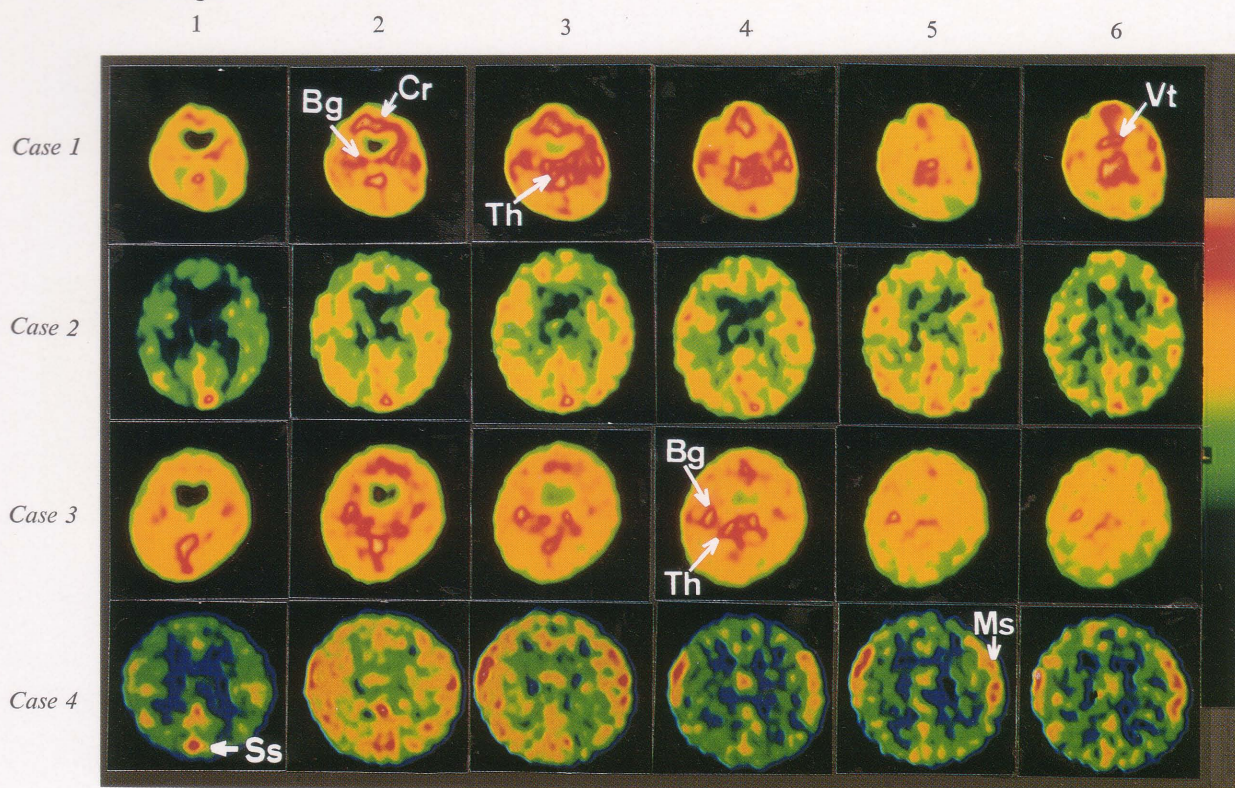


Fig. 4 PET images using pyruvate-1- ^{11}C .

Case 1. Early images (image 1-3) showing higher uptake of radioactivity in the cerebral cortex (Cr), basal ganglia (Bg) and thalamus (Th). Later images (image 4-6) demonstrating slow clearance of radioactivity in those areas and its remarkable accumulation in the ventricles (Vt).

Case 2. Early images showing the higher uptake mostly in the cerebral cortex. Later images demonstrating slow clearance in those areas.

Case 3. PET images showing basically the same as those of case 1.

Case 4. Early images show less uptake in brain tissues than in skull muscles (Ms). Later images reveal rapid clearance in brain tissues and ventricles. Ss; straight sinus.

A serial dynamic PET scan was performed using Headtome II (Simazu Co. Ltd., Kyoto, Japan). This camera has 2 detector rings with 68 BGO detectors in each ring, providing 3 tomographic images at 15 mm intervals simultaneously. After a transmission scan with a ^{68}Ga standard ring source for the correction of photon attenuation on the emission images, a serial PET scan was obtained every 6 minutes for a total period of 36 minutes soon after intravenous administration of pyruvate-1- ^{11}C (2-8 mCi). PET images were corrected for decay of ^{11}C . All subjects were fasted for at least 5 hours and received sedatives such as tricloryl (po) and diazepam (iv) in PET study.

CASE REPORTS AND RESULTS

Patients

Five patients were studied, including two patients

with mitochondrial encephalomyopathy and one with Leigh's disease. Two epileptic patients were studied as control subjects.

Case 1

A six-month-old girl with mitochondrial encephalomyopathy. She was born of a normal full-term pregnancy, weighing 2,540 g at birth. Family history was negative for metabolic disorders. The maternal grandparents were first cousins. Shortly after birth, the patient developed apnea, cyanosis, vomiting, generalized tonic clonic convulsion, and metabolic acidosis. She was successfully treated with intubation and intravenous administration of NaHCO_3 . However, she thereafter suffered from a feeding problem and poor gaining weight. At the age of 4 months, she was admitted to a hospital because fever, tachycardia, tachypnea and hyperlactic acidemia were noted. Two months later, she was transferred to our

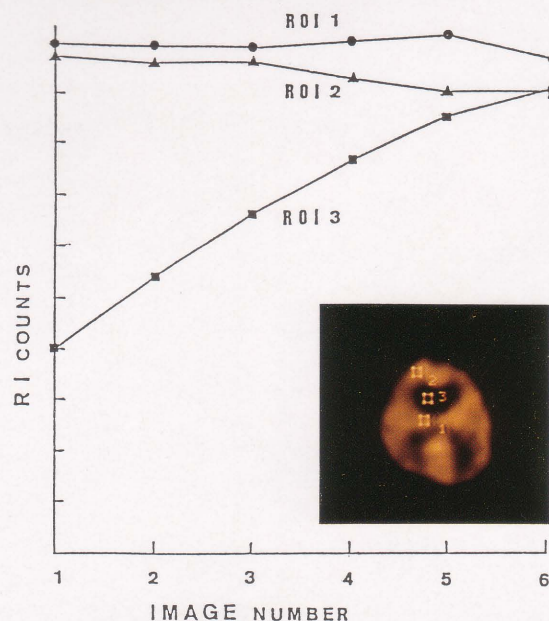


Fig. 5a Case 1. Time activity curves of pyruvate-1- ^{11}C in the regions of interest (ROI). Radioactivity is cleared slowly in the areas of the thalamus (closed circle, ROI #1) and cerebral cortex (closed triangle, ROI #2). Radioactivity accumulates in the ventricle (closed square, ROI #3).

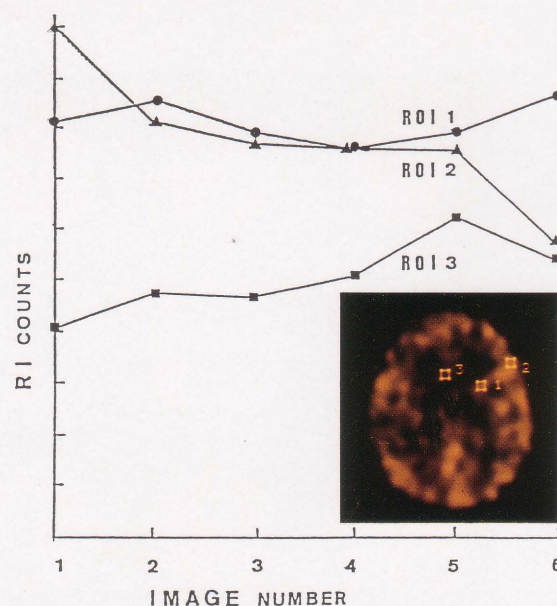


Fig. 5b Case 2. Time activity curves showing slow clearance of radioactivity in the basal ganglia (closed circle, ROI #1) and cerebral cortex (closed triangle, ROI #2). And radioactivity accumulates mildly in the ventricle (closed square, ROI #3).

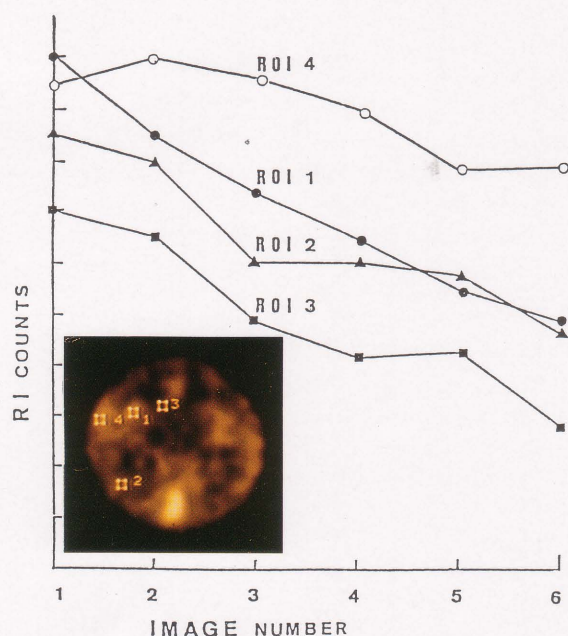


Fig. 5c Case 4. Time activity curves show basically eliminating patterns in the basal ganglia (closed circle, ROI #1), cerebral cortex (closed triangle, ROI #2) and ventricle (closed square, ROI #3). Radioactivity in the skull muscle (open circle, ROI #4) clears more slowly than in the other areas (ROI #1, 2, 3).

hospital for further evaluation. A physical examination on admission revealed small stature, poor activity, weak crying, no head control, tachycardia, tachypnea and hepatomegaly. A neurological examination showed that she was mentally retarded, having severe hypotonia mainly in the axial muscles and muscle atrophy of all extremities with decreased deep tendon reflexes. Serum GOT, GPT and aldolase were all increased. Serum pyruvate was 3.38 mg/dl and lactate 63.7 mg/dl, both being above the normal value. Pyruvate and lactate in the cerebrospinal fluid (CSF) were also increased to 2.82 mg/dl and 75.1 mg/dl, respectively. Enzyme studies using biopsied muscles revealed that NADH cytochrome c reductase and cytochrome c oxidase were decreased (Table 1). An EEG showed no abnormality. The CT revealed a diffuse atrophy of the cerebral cortex and caudate nucleus. Ventricular systems were also dilated (Fig. 1).

Case 2

A 8-year-old girl enzymatically diagnosed as having mitochondrial encephalomyopathy. She was born of a normal pregnancy and delivery. She developed generalized tonic convulsions twice, first at the age of 2 years and then at the age of 3 years. She was otherwise healthy until the age of 6 years, when it became difficult for her to climb stairs and she complained of being easily fatigued. At the age of 7 years, she was admitted to a hospital where a

muscle biopsy was carried out, indicating a decrease in NADH cytochrome c reductase, and total and free carnitine activities (Table 1). She thereafter developed myoclonus attacks and her school performance deteriorated. At the age of 8 years, she was admitted to our hospital for further evaluation. Serum pyruvate and lactate values were within normal limits. Pyruvate and lactate values of CSF were slightly increased (pyruvate, 1.46 mg/dl; lactate, 32.1 mg/dl). An EEG showed diffuse high voltage spike and wave bursts. The CT revealed no remarkable abnormalities but mild dilatation of the bilateral sylvian fissures (Fig. 2).

Case 3

A 3-year-old girl clinically diagnosed as having Leigh's disease. Psychomotor retardation was noted in her elder sister by the age of 2 years, when she developed neurological deterioration, followed by choreoathetosis and convulsions. The family history was not otherwise unusual and no consanguinity was indicated. The patient's birth was complicated by an urgent delivery during 2 to 3 months of gestational age and placenta dysfunction. Abnormal postural reflexes were first noted at two and a half months of age when she had started on a rehabilitation program. At 3 years, she was admitted to our hospital for further evaluation. Neurological examination on admission disclosed hypotonia, increased deep tendon reflexes, choreoathetosis, absent gag reflex and abnormal eye movements. Mild metabolic acidosis and a slight increase in pyruvate and lactate in serum were found in laboratory examinations. Muscle biopsy in histochemical and biochemical examinations (Table 1) revealed no abnormalities of mitochondria. An EEG showed no paroxysmal discharges. The CT showed atrophy of the cerebral cortex and caudate nucleus (Fig. 3).

Case 4

An 8-year-old boy with autonomic seizures. The family history was negative for neuromuscular diseases. Born to nonconsanguineous parents after a normal pregnancy and delivery, he experienced episodic headache attacks from the age of 7 years. An EEG revealed spike discharges in the right parietal and posterotemporal areas but no marked changes by hyperventilation. The neurological examination was normal. The CT revealed no abnormality.

Case 5

An 11-year-old girl with astatic seizures. Her family history was not remarkable. She developed astatic seizures about the age of 2 years. An EEG revealed diffuse spike and wave complexes. She was placed

on anticonvulsants, which had failed to stop seizures completely. At the age of 10, she was admitted to our hospital for evaluation and control of the seizures. A CT scan revealed low density on the occipital areas and calcification of the bilateral basal ganglia. Serum Ca, P, lactate and pyruvate were normal. Lactate and pyruvate in CSF were also normal. An EDTA loading test suggested the possibility of hypoparathyroidism.

Positron emission tomography with pyruvate-1-¹¹C (Fig. 4)

Mitochondrial encephalomyopathy patients (case 1 and 2) had abnormal PET images, demonstrating positive retention of ¹¹C in the areas of the basal ganglia and/or cerebral cortex. In addition, the ventricular system revealed a higher uptake of radioactivity, which increased with time. The patient with Leigh's disease (case 3) showed basically the same PET image patterns as those of case 1. The PET images of control subjects, on the other hand, showed a lower uptake of radioactivity in the brain and retention of ¹¹C only in the skull muscles.

Time activity curve of pyruvate-1-¹¹C in the brain

On this curve cases 1 and 2 (Fig. 5a, 5b) had a pattern of retaining radioactivity in the basal ganglia (region of interest, ROI, #1) and cerebral cortex (ROI #2). It is interesting to note that the ventricular system showed a pattern of accumulating radioactivity when ROI was taken in the anterior horn areas (ROI #3). In comparison, the control subject (Fig. 5c) showed a pattern of eliminating radioactivity in the areas of basal ganglia (ROI #1) and cerebral cortex (ROI #2). Skull muscles (ROI #4) showed somewhat slower clearance than in those areas (ROI #1, #2). The ventricular system (ROI #3) had quick clearance compared with cases 1 and 2.

Estimated blood clearance using blood and PET counts

No metabolic compartment model is available at the present time because of complicated pyruvate metabolism in the brain. Our study presents only the PET images and time activity curves of pyruvate-1-¹¹C in the brain, yet some complicated factors regarding the significance of estimation by these methods might still remain. A blood clearance curve obtained from venous blood counts from a control subject (case 5) showed three apparent stages (Fig. 6). Rapid clearance was observed in the first 3 minutes after the injection of pyruvate-1-¹¹C ($T_{1/2}=0.9$ min). The second phase, which had an intermediate biological half life ($T_{1/2}=3.5$ min), occurred between 3 to 10 minutes after the injection. The last phase was observed more than 10 minutes after the injection ($T_{1/2}=13$ min). Blood clearance in the brain was

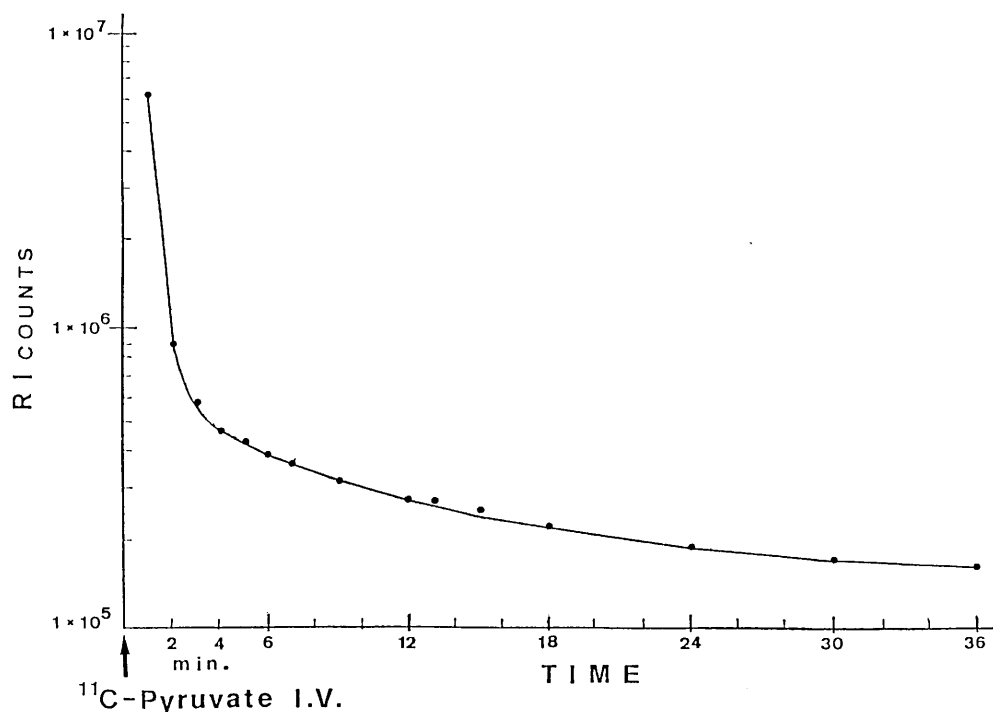


Fig. 6 Case 5. Venous blood clearance curve of case 5 shows three stages. The first phase is observed within 3 minutes after the administration of pyruvate-1-¹¹C, which was cleared quickly from the venous blood. The second phase is present between 3 to 10 minutes after injection when radioactivity is cleared slower than in the phase 1. The last phase is more than 10 minutes after injection.

estimated by PET counts in the straight sinus areas. Brain blood clearance followed a pattern basically similar to that of venous blood.

DISCUSSION

Mitochondrial encephalomyopathy is a symptom complex, showing various clinical, biochemical, and pathological heterogeneities. Some of them are characterized by ragged-red fibers in the biopsy muscle and can be diagnosed by enzymatic studies. However, it has been pointed out that the morphological characteristics of muscle are not necessarily representative of mitochondrial encephalomyopathy. Prick et al.¹¹ described an infant with a pyruvate dehydrogenase deficiency restricted to the brain. They found an increased lactate concentration in CSF with almost normal serum lactate content. This is the same as in case 2 of our series which was consistent with altered mitochondrial functions in the brain.

Pyruvate is transported into the mitochondria where it is metabolized into the TCA cycle through acetyl-CoA by several enzyme reactions. Abnormality of the mitochondrial electron transfer system causes possible abnormal pyruvate metabolism in the mitochondria.¹⁵ Therefore, a PET study using

pyruvate-1-¹¹C is very useful for the noninvasive study of in vivo pyruvate metabolism in the brain. In normal brain tissues, ¹¹C of pyruvate-1-¹¹C is rapidly eliminated from the brain tissues through the above metabolic pathway. Patients with epilepsy (case 4 and 5) showed rapid elimination of radioactivity from the brain after injection of pyruvate-1-¹¹C, indicating no abnormal pyruvate metabolism.

Patients with mitochondrial encephalomyopathy (case 1 and 2) showed abnormal PET images when pyruvate-1-¹¹C was used, and were characterized by a higher uptake of radioactivity in the cortex and/or basal ganglia. Interestingly enough, biochemical abnormality could be detected by this PET study even in the case (case 2) which did not have any abnormal findings in the CT. In addition, the fact that abnormal pyruvate metabolism in the brain was found in case 3, a patient suspected of having Leigh's disease clinically but demonstrating no evidence of abnormal mitochondrial electron transfer system biochemically or morphologically is very important. This indicates that PET studies using isotope labeled pyruvate make it possible to detect abnormal pyruvate metabolism localized only in the brain tissues.¹¹ Accumulation of radioactivity in the ventricular systems is also quite understandable in patients (cases 1 and 2) with increased pyruvate and

lactate in cerebral tissue fluids, because of diffusion equilibrium CSF and cerebral tissue fluids.¹⁶

Abnormal mitochondrial energy metabolism is also found in several conditions such as hypoxia,^{17,18} ischemia,^{19,20} convulsions,^{21,22} and tumors²³⁻²⁵ etc. PET with pyruvate-1-¹¹C must be useful in diagnosing cases with brain pathological conditions involving mitochondrial energy metabolism such as mitochondrial encephalomyopathies, because it is possible to investigate "in vivo" brain mitochondrial metabolism by this method.

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