D-Lactate Is Present in Much Larger Amount than L-Lactate in Cephalopods and Gastropods

Authors: Ohmori, Shinji, Ohsaki, Yuko, Akagi, Shinsuke, Kondoh, Chie, Kawase, Michi, et al.

Source: Zoological Science, 14(3) : 429-434

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.429

ZOOLOGICAL SCIENCE 14: 429–434 (1997)

D-Lactate Is Present in Much Larger Amount than L-Lactate in Cephalopods and Gastropods

Shinji Ohmori*, Yuko Ohsaki, Shinsuke Akagi,
Chie Kondoh, Michi Kawase and Toshie Nagai

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima Naka 1-1, Okayama 700, Japan

ABSTRACT—It has long been known that only L-lactic acid is found in animals and that D-lactic acid is produced in microbial organisms. During the course of study of D-lactate formation from methylglyoxal (the methylglyoxal bypass) in animals, we found that D-form is mainly present in *Octopus vulgaris* and very little L-lactate was found in octopus muscle. There is an inverse relationship between octopus and normal animals for concentrations of D- and L-lactate. The activities of D-lactate dehydrogenase (D-LDH) was predominantly found in octopus muscle, while L-LDH activity was scarcely detected. Methylglyoxal was the best substrate for D-lactate formation in octopus foot homogenate and pyruvate was the second best substrate. It was also found that D-lactate is present in much higher amounts than the L-form in some animals or plants.

INTRODUCTION

D-Lactate was thought to be an intermediate of glucose metabolism in organisms from 1913 to about 1933 (Dakin and Dudley, 1913; Neuberg, 1913; Neuberg and Kobel, 1928, 1930; Vogt, 1929). Ever since Embden, Meyerhof and others established the glycolytic pathway (Embden *et al.*, 1933; Meyerhof and Lohman, 1934), D-lactate has been forgotten. The presence of D-lactate in fermented products by *lactobacilli* is well known, but whether D-lactate is normally present in animals and plants has not been known and the physiological meaning of its absence or its presence in them has not been described in any textbook. In microbes, D-lactate is mainly formed via methylglyoxal (MG) from triosephosphates as shown in Fig. 1. In order to study this metabolic pathway in higher plants and animals we first developed quantification methods for D-lactate (Ohmori and Iwamoto, 1988; Ohmori *et al.*, 1991) and MG (Ohmori *et al.*, 1987a,b). Thereafter, we investigated the plasma level of D-lactate in human after exercise (Kondoh *et al.*, 1992b; Ohmori and Iwamoto, 1988). D-Lactate in blood plasma increased 3 to 3.6 times even after a short period of exercise such as 5 min-running; however, MG content in red blood cells decreased to 13% of the initial levels. Next we measured concentration of D-lactate and its related metabolites in liver, blood and muscle of diabetic and starved rat (Kondoh *et al.*, 1992a; Ohmori and Iwamoto, 1988; Ohmori *et al.*, 1987a,b). These rats had significantly higher levels of D-lactate in plasma, liver and skeletal muscle compared with the control group (Kondoh *et al.*, 1992a). Successively, we studied the age-related changes in the levels of D-lactate and its related compound in rat tissues. The hepatic levels of D- or L-lactate, MG, pyruvate and inorganic phosphate markedly decreased during aging process (Kawase *et al.*, 1995). Recently, rats were fed a diet containing 0.064% 3'-methyl-4-dimethylaminoazobenzene (a hepato-carcinogen) for 21 weeks. After the start of the diet hepatic contents of MG and D-lactate increased to 7 and 3 times that of the control, respectively (Kawase *et al.*, 1996). Up to present, we have studied the biochemistry of D-lactate in mammals, however, we wanted to study further the biochemistry of D-lactate in lower animals and plants. At first we studied the distribution of D-lactate in various organisms, which can be easily available. These results are reported in this paper.

MATERIALS AND METHODS

Chemicals

Hydrazine sulfate, o-phenylenediamine and rabbit muscle aldolase were purchased from Wako Pure Chemicals (Osaka, Japan). Fructose-6-phosphate and γ-glycerophosphate dehydrogenase (γ-GDH) from rabbit muscle, pig heart LDH, phosphoenolpyruvate, and NAD^+ were obtained from Oriental Yeast (Tokyo, Japan). Lithium L- and D-lactate, triosephosphate isomerase from rabbit muscle, fructose-1,6-diphosphate and S-lactoyl-glutathione (S-LacGS) were from Sigma Chemicals (St. Louis, Mo., USA). D,L-6,8-Thioctamide was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). MG was prepared just before use by hydrolysis of the dimethylacetal (Aldrich, Milwaukee, Isc., USA) (Kellum *et al.*, 1978). D-Lactate dehydrogenase (D-LDH) from *Staphylococcus* sp. and diaphorase from *Clostridium kluiveri* were kindly supplied by Amano Pharmaceutical (Nagoya, Japan). Reduced glutathione (GSH) was kindly supplied by Senju Pharmaceutical (Itami, Japan).

* Corresponding author: Tel. +81-86-251-7933;
FAX. +81-86-251-7933.

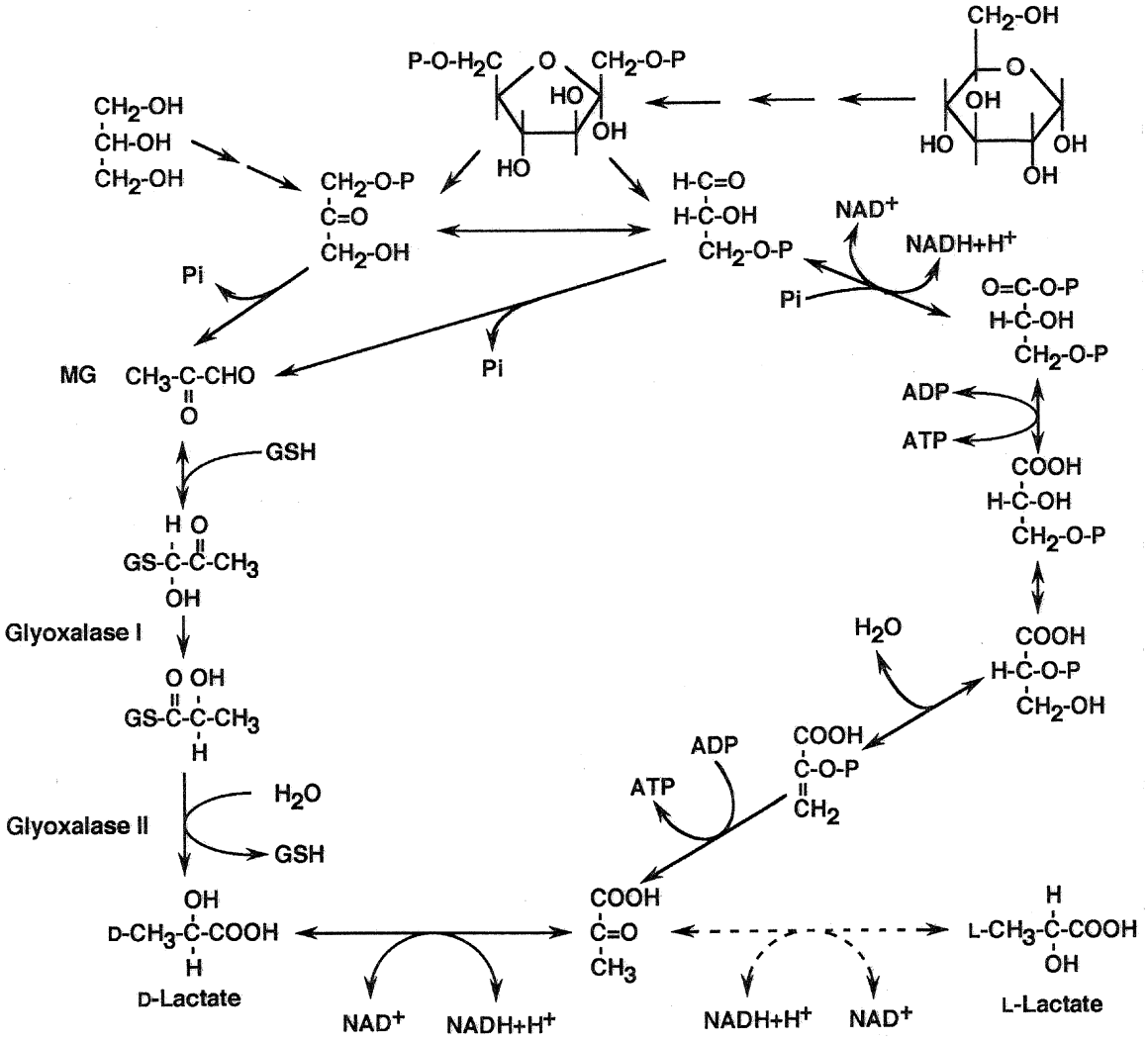

Fig. 1. Proposed pathway for the formation of D-lactate in octopus.

Animals and plants

Octopus, squid, turbo and all vegetables were purchased from appropriate local stores. Other marine animals were kindly provided by the Ushimado Marine Laboratory of Okayama University and African giant snail was kindly supplied by Prof. H. Takeuchi, the medical school of Gifu University. All animals were alive before the experiment.

Homogenization and extraction

Most biological samples were homogenized at 10,000 rpm for 5 min with a Waring blender in 5 volumes of the ice-cold 10 mM potassium phosphate (pH 7.0). Livers were homogenized at 4°C for 30 s in a glass Teflon homogenizer. Fresh baker's yeast was boiled in 100°C for 15 min and centrifuged at 700 × g for 15 min.

Determination of metabolite concentrations

D-Lactate and pyruvate were determined by our methods (Ohmori and Iwamoto, 1988). 3-Methyl-2-quinoxalinol generated from pyruvate which was also derived D-lactate was measured with a Gasukurokogyo liquid chromatograph, model 572 (Tokyo, Japan) with a Gasukurokogyo UV-detector, model 502s. L-Lactate concentration was measured by the UV method (F-kit, Boehringer, Mannheim, FRG), when a large enough quantity of sample had to be assayed. MG was measured as 6,7-dichloro-2-methylquinoxaline by our GLC-method with electron capture detection (Ohmori *et al.*, 1987a). A Shimadzu gas chromatograph, model GC-4CMPEE (Kyoto, Japan) equipped with a ^{63}Ni electron capture detector was used. Inorganic phosphate was determined by the colorimetric method of Lanzetta *et al.* (1979). Measurements of glycerol were performed using an UV detection kit (Boehringer). GSH and oxidized glutathione (GSSG) were measured by a modification of the method of Griffith (Matsumoto *et al.*, 1996). Glucose was determined at 505 nm using a kit (glucose CII-Testwako, Wako Pure Chemicals, Osaka, Japan). Protein concentrations were measured by the biuret method (Beisenherz *et al.*, 1953).

Enzyme assays

Activities of glycerokinase (GK), 6-phosphofructokinase (PFK), pyruvate kinase (PK), aldolase, glyoxalase I, and glyoxalase II were determined by methods previously described in the literatures (Bergmeyer, 1983; Gracy *et al.*, 1970; Kemp, 1975; Racker, 1955; Valentine and Tanaka, 1966), respectively. The L- and D-LDH activity were measured by the UV method of Vassault (1983).

Configuration of lactate in octopus foot

Octopus foot was boiled for 10 min and the boiled foot was cut into small portions by scissors. The cut tissues were homogenized by a mixer with the boiled water. The homogenate was further boiled for 5 min. The filtrate from the boiled homogenate was passed through a

Dowex 50 w x8 (H^+-form) column. The effluent and the washing by 50% methanol from the column were combined and concentrated under reduced pressure. The concentrate was extracted by n-hexane and the water layer was automatically extracted with ether for 40 hr. After the ether layer was evaporated under reduced pressure, the residue was analyzed by a chiral column, Sumichiral OA-5000 (150 × 4.6 mm) (Sumika Chemical Analysis Service, Ltd. Osaka, Japan). The column was eluted by 2 mM copper sulfate containing 5% acetonitrile at flow rate 1.0 ml/min and built in a Shimadzu liquid chromatography, Model LC-6A (Kyoto, Japan). All runs were performed at room temperature and the eluate was monitored at 254 nm.

D-Lactate formation from pyruvate in octopus foot homogenate

Octopus feet (8.0 g) were homogenized with Waring blender in 5 volumes 50 mM potassium phosphate (pH 7.0). The homogenate was centrifuged at 6,000 × g for 15 min. The supernatant was dialyzed at 4°C for 2 hr against 5 l of 50 mM potassium phosphate (pH 7.0). The composition of the reaction mixture was as follows: 150 to 600 μl of 1 mM sodium pyruvate, 100 μl of 1 mM NADH, 100 μl of 10 mM nicotinamide, 100 μl of the dialyzed supernatant, and 50 mM potassium phosphate (pH 7.0) for a final volume of 1.0 ml. After the mixture was incubated at 25°C for 30 min, 3 ml of methanol was added and the mixture was centrifuged at 3,000 × g for 15 min. The precipitate was extracted with 4 ml of 70% methanol (methanol : water, 7 : 3, v/v) and the suspension was centrifuged as above. One ml of the combined extract supernatants was evaporated using a Savant Speed Vac Concentrator (Model SUC-100H, New York). The residue was assayed for D-lactate by our method (Ohmori and Iwamoto, 1988; Ohmori *et al.*, 1991).

RESULTS AND DISCUSSION

The distribution of D-lactate and its related metabolites in some organisms

As can be seen in Table 1, the most striking finding is that lactic acid in octopus was almost all in the D-form. Other cephalopods and gastropods tested had relatively high amounts of D-lactate. However, since turbo is an exception to the general observation tested here, we are widely studying the D-lactate content in these kinds of animal. Surprisingly, some plants also have exceedingly high levels of the D-form. In Fig. 1, two routes for the D-lactate formation are shown, one is the route via MG from triosephosphates, the other is the Embden Meyerhof pathway via phosphoenolpyruvate. As can be seen in the figure, the former is less effective to produce energy and the latter is energy-productive. D-Lactate and its related metabolites shown in Fig. 1 were determined in octopus foot muscle and rat tissues and the determined values are listed in Table 2. As can be seen from this table, D-lactate concentration in octopus was much higher than those of rat tissues and also than the concentration of L-lactate in rat tissues. On the other hand, L-lactate level in octopus was very low. The configuration of the intrinsic lactate in octopus foot was confirmed by the chiral column. L- and D-Lactate were eluted at retention times of 12 and 15 min, respectively. The

Table 1. D-Lactate and L-lactate in various organisms

		D-lactate*	L-lactate*	D/L	L/D
Animal	Octopus (*Octopus vulgaris*)				
	foot muscle	22.9	0.24	95	
	mantle muscle	4.37	0.21	20	
	Squid (*Sepia madokai*)				
	foot muscle	3.47	0.19	18	
	mantle muscle	2.49	0.23	11	
	African giant snail (*Achatina fulica ferussac*)	7.31	0.44	17	
	Asari clum (*Tapes japonica*)	0.81	0.047	17	
	Turbo (*Turbo cornutus*)	0.089	0.58		6.5
	Crab (*Potamon dehaani*)	0.043	11.8		274
	Shrimp (*Natantia penaeus*)	0.085	9.78		115
	Starfish (*Astropecten polyacanthus*)	0.085	0.55		6.5
	Mackerel (*Pneumatophorus japonicus japonicus*)	1.43	78.2		55
	Ayu fish (*Plecoglossus altivelis*)	0.44	37.8		86
	Frog (*Rana nigromaculata*)				
	skeletal muscle	0.19	31.2		164
	liver	0.028	1.09		39
	Rat skeletal muscle	0.049	3.07		63
	liver	0.469	3.19		6.8
	Human plasma	0.046**	1.57**		34
Plant	Onion (*Allium cepa L.*)	780	76.6	10	
	Potato (*Solanum tuberosum L.*)	1.18	0.52	2.3	
	Asparagus (*Suaeda asparagoides Makino*)	14.6	8.13	1.8	
	Cabbage (*Brassica oleracea L. var. capitata L.*)	8.43	6.85	1.2	
	Broccoli (*Brassica oleracea L. var. botrys L.*)	0.7	4.81		6.9
	Spinach (*Spinacia oleracea L.*)	0.10	0.94		9.4
	Corn seeds (*Zea mays L.*)	0.30	0.80		2.7
	Baker's Yeast	not detectable	0.043		

*, μmol/g wet weight; **, μmol/ml.

Table 2. Concentrations of D-lactate and its related metabolites in octopus

	D-lactate*	L-lactate*	MG**	GSH* GSSG*	Inorganic phosphate*	Glycerol*	Glucose*
Octopus foot muscle	22.9 ± 2.2	0.238 ± 0.022	6.04 ± 0.51	0.264 ± 0.022 0.019 ± 0.003	2.82 ± 0.08	0.277 ± 0.018	3.07 ± 0.19
Rat skeletal muscle	0.060 ± 0.001	3.585 ± 0.069	4.25 ± 0.32	0.96 ± 0.10 0.11 ± 0.01	3.5 – 4.0	0.250 ± 0.008[(a)]	1.76 ± 0.11[(a)]
Rat liver	0.133 ± 0.005	0.820 ± 0.025	12.50 ± 1.14	5.86 ± 0.56 0.18 ± 0.02	3.50 ± 0.17[(b)]	0.029 ± 0.005[(b)]	5.42 ± 0.19[(b)]

Values are means ± S.E.M., n = 3; *, μmol/g wet weight; **, nmol/g wet weight.
[(a)] Edington DW, Ward GR, Saville WA (1973) Am J Physiol 224: 1375–1380
[(b)] Greenbaum AL, Gumaa KA, McLean P (1971) Arch Biochem Biophys 143: 617–663

Table 3. Enzyme activities related to the D-lactate formation

	L-LDH*	D-LDH**	Glyoxalase I*	Glyoxalase II**	PFK*	Aldolase*	PK*	GK*
Octopus foot muscle	0.17**	36.43	0.412 ± 0.067	9.06 ± 1.38	0.457 ± 0.052	1.21 ± 0.17	1.79 ± 0.61	0.046 ± 0.007
Rat skeletal muscle	13.39	not detectable	0.405 ± 0.055	2.79 ± 0.37	0.114 ± 0.014	0.251 ± 0.057	1.79 ± 0.38	not detectable
Rat liver	1.43	not detectable	0.48 ± 0.06	76.2 ± 7.7	0.007 ± 0.001	0.020 ± 0.005	0.35 ± 0.03	4.3 ± 0.5

Values are means ± S.E.M., n = 3; *, μmol/min/mg protein; **, nmol/min/mg protein.
For determination of L- and D-lactate, pyruvate and NADH were incubated at 25°C for 5 min in the concentrations of 1 and 0.2 mM, respectively, with the 12,000 × g supernatant of octopus foot homogenate in 1 ml of 100 mM potassium phosphate (pH 7.0). After addition of 3 ml of methanol, the mixture was centrifuged at 1,700 × g for 15 min. The precipitate was washed with 4 ml of 70% methanol. The combined supernatant was evaporated by Speed Vac Concentrator. The residue was determined for L- and D-lactate.

latter was found to be produced in much larger quantities. Glutathione level was lower in octopus muscle than in rat tissues. The present report describes for the first time the contents of D-lactate and the MG bypass-related compounds in octopus.

Some enzyme activities related to the D-lactate formation

In Table 3, enzyme activities related to the D-lactate formation are listed. PK and PFK are regulating enzymes in the glycolytic pathway in most organisms. The GK activity was also assayed, because it was found to play an important role for the D-lactate formation in rat liver (Kondoh *et al.*, 1994). Aldolase is the enzyme of the division point of the MG bypass and the glycolysis. These enzyme activities in octopus resembled those in rat skeletal muscle more than those in liver except for the D-LDH and L-LDH activity which was scarcely detected in octopus. From Tables 2 and 3 or Fig. 1, higher level of D-lactate in octopus muscle (in Tables 1 and 2) can be understandable, that is, D-lactate appears to be produced from both the MG bypass and the Embden Meyerhof pathway in octopus, while D-lactate must be formed only from the MG bypass in rat, because it possesses no D-LDH.

The D-lactate formation in the homogenate of octopus

As a next problem, we want to know which route is major for the D-lactate formation in octopus . As shown in Fig. 2,

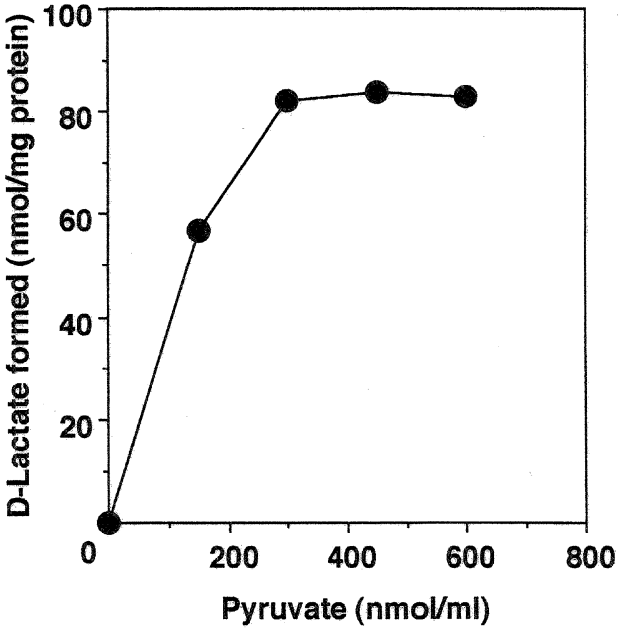

Fig. 2. D-Lactate formation from pyruvate in octopus foot homogenate. Octopus feet were homogenized and the homogenate was centrifuged. The supernatant was dialyzed. Various amounts of pyruvate were incubated with NADH in the supernatant and D-lactate formed was determined. Details of the method are written in Materials and Methods.

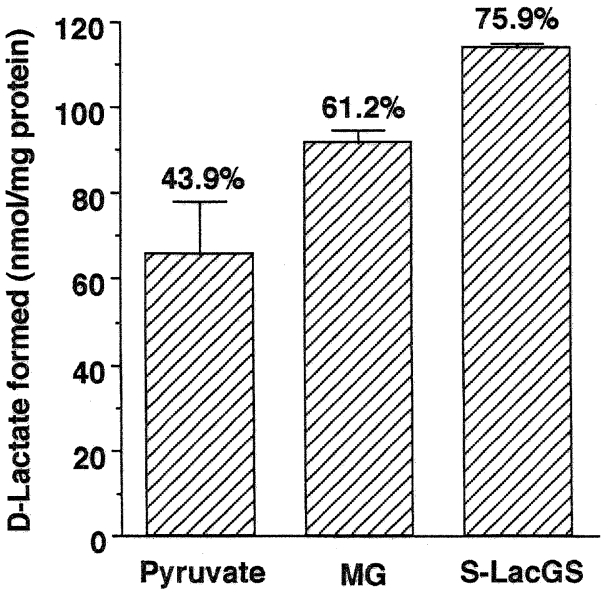

Fig. 3. D-Lactate formation from pyruvate, methylglyoxal and S-lactoylglutathione in octopus foot homogenate. The incubation condition was described in Materials and Methods with the exception that substrates used were 150 μl of 1 mM sodium pyruvate, methylglyoxal, or S-lactoylglutathione. GSH was added in concentration of 0.5 mM to the incubation mixture for methylglyoxal.

production of D-lactate from pyruvate was investigated using homogenate of octopus foot. D-Lactate was formed from 300 μM pyruvate with a 27% yield, which corresponded to an 81% yield on a NADH-added basis. In Fig. 3, the formation of D-lactate from pyruvate, MG and S-LacGS by the homogenate of octopus is shown. Yields were 43.9, 61.2 and 75.9% from pyruvate, MG and S-LacGS, respectively. From the results described here, it is thought that D-lactate is formed from both MG and pyruvate in octopus and the rate of the formation is more favorable from MG than from pyruvate at substrate concentrations of 150 μM.

The presence of high levels of MG in octopus may be due to low levels of GSH in the organism (Table 2).

In conclusion, many people have the common thought that L-lactate is the physiological lactate, however, as reported here, D-lactate is predominantly present in some organisms.

Recently, we isolated two forms of D-LDH from octopus foot muscle. They had the same molecular mass of 38 kDa on SDS-PAGE and were monomer. Sequencing of genes of two types of D-LDH revealed that the homology between this D-LDH and known L- and D-LDH was very low. These results will be soon published.

ACKNOWLEDGMENTS

We are grateful to Dr. J. Shiozawa of Max-Planck-Institute of Biochemistry (Munich) for improvement in English style.

We thank Prof. M. Yamamoto and Dr. Ohtsu (the Ushimado Marine Laboratory of Okayama University) for collection of marine animals.

REFERENCES

Beisenherz G, Boltze HJ, Bücher Th, Czod R, Carbade KH, Meyer-Arendt E, Pfleiderer G (1953) Diphosphofructose aldolase, phosphoglyceraldehyde dehydrogenase, lactic acid dehydrogenase, glycerophosphate dehydrogenase, and pyruvate kinase from rabbit muscle in one process. Z Naturforsch 8b: 555–577

Bergmeyer HU, Grassl M, Walter HE (1983) Glycerokinase, In "Methods of Enzymatic Analysis Vol 2" Ed by HU Bergmeyer, Verlag Chemie GmbH, Weinheim, pp 216–217

Embden G, Deuticke HJ, Kraft G (1933) Über die intermediären Vorgänge bei der Glykolyse in der Muskulatur. Klin Wochenschn 12: 213–215

Dakin HD, Dudley HW (1913) On glyoxalase. J Biol Chem 14: 423–431

Gracy RW, Lacko AG, Brox LW (1970) Structural relations in aldolases purified from rat liver and muscle and Novikoff hepatoma. Arch Biochem Biophys 136: 480–490

Kawase M, Kondoh C, Matsumoto S, Teshigahara M, Chisaka Y, Higashiura M, Nakata K, Ohmori S (1995) Contents of D-lactate and its related metabolites as well as enzyme activities in the liver, muscle and blood plasma of aging rats. Mech Ageing Dev 84: 55–63

Kawase M, Tada M, Akagi S, Ohmori S (1996) Changes in concentrations of methylglyoxal, D-lactate and glyoxalase activities in liver and plasma of rats fed a 3'-methyl-4-dimethylaminoazobenzene-rich diet. Res Exp Med 196: 251–259

Kellum MW, Oray B, Norton SJ (1978) A convenient quantitative synthesis of methylglyoxal for glyoxalase I assays. Anal Biochem 85: 586–590

Kemp RG (1975) Phosphofructokinase from rabbit skeletal muscle. In "Methods in Enzymology Vol 42" Ed by NO Kaplan, SP Colowick, Academic Press, New York, pp 71–77

Kondoh Y, Kawase M, Hirata M, Ohmori S (1994) Carbon sources for D-lactate formation in rat liver. J Biochem 115: 590–595

Kondoh Y, Kawase M, Kawakami Y, Ohmori S (1992a) Concentrations of D-lactate and its related metabolic intermediates in liver, blood, muscle of diabetic and starved rats. Res Exp Med 192: 407–414

Kondoh Y, Kawase M, Ohmori S (1992b) D-Lactate concentrations in blood, urine and sweat before and after exercise. Eur J Appl Physiol 65: 88–93

Lanzetta PA, Alvarez LJ, Reinach PS, Candia OA (1979) An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem 100: 95–97

Matsumoto S, Teshigahara M, Tsuboi S, Ohmori S (1996) Determination of glutathione and glutathione disulfide in biological samples using acrylonitrile as a thiol-blocking reagent. Anal Sci 12: 91–95

Meyerhof O, Lohmann K (1934) Über die enzymatische Gleichgewichtsreaktion zwischen Hexosediphosphorsäure und Dioxyacetonephosphorsäure. Biochem Z 271: 89–110

Neuberg C (1913) Weitere Untersuchungen über die biochemische Umwandlung von Methylglyoxal in Milchsäure nebst Bemerkungen über die Entstehung der verschiedenen Milchsäuren in der Natur. Biochem Z 51: 484–508

Neuberg C, Kobel M (1928) Die desmolytische Bildung von Methylglyoxal durch Hefenenzym. Biochem Z 203: 463–468

Neuberg C, Kobel M (1930) Über den Kohlenhydratstoffwechsel der höheren Pflanzen. Biochem Z 229: 433–442

Ohmori S, Iwamoto T (1988) Sensitive determination of D-lactic acid in biological samples by high-performance liquid chromatography. J Chromatogr 431: 239–247

Ohmori S, Kawase M, Mori M, Hirota T (1987a) Simple and sensitive determination of methylglyoxal in biological samples by gas chromatography with electron-capture detection. J Chromatogr 415: 221–229

Ohmori S, Mori M, Kawase M, Tsuboi S (1987b) Determination of methylglyoxal as 2-methylquinoxaline by high-performance liquid chromatography and its application to biological samples. J Chromatogr 414: 149–155

Ohmori S, Nose Y, Ogawa H, Tsuyama K, Hirota T (1991) Fluorimetric and high-performance liquid chromatographic determination of D-lactate in biological samples. J Chromatogr 566: 1–8

Racker E (1955) Glyoxalases. In "Methods in Enzymology Vol 1" Ed by SP Colowick, NO Kaplan, Academic Press, New York, pp 454–460

Valentine WN, Tanaka KR (1966) Pyruvate kinase, clinical aspects. In "Methods in Enzymology Vol 9" Ed by NO Kaplan, SP Colowick, Academic Press, New York, pp 468–473

Vassault A (1983) Lactate dehydrogenase. In "Methods of Enzymatic Analysis Vol 3" Ed by Bergmeyer HU, Verlag Chemie GmbH, Weinheim, pp 118–126

Vogt M (1929) Bildung und Isolierung von Methylglyoxal bei der Glykolyse durch tierisches Enzym. Biochem Z 211: 17–39

(Received February 18, 1997 / Accepted March 24, 1997)