

# *Acta Medica Okayama*

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*Volume 20, Issue 6*

1966

*Article 5*

DECEMBER 1966

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## Abstract

Recently, by histochemical observations the changed activities of the enzymes of heart muscle in experimentally induced ischemia have been reported by several investigators<sup>1-4</sup>. SHNITKA and NACHLAS<sup>4</sup> demonstrated that the ligation of coronal artery of dog heart induced an increase in the activities of succinic dehydrogenase and NAD-diaphorase four to six hours after the ligation. However, extracorporeal circulation induced no distinct changes in the activities of succinic dehydrogenase and cytochrome oxidase as has been revealed quite recently by BJORK and associates<sup>5</sup> from their histochemical studies of the specimen from left ventricular myocardium by a method of drill biopsy, but only the myocardial edema and fibrosis occurred. This report deals with the distribution and activities of oxidative enzymes of human myocardium of fortyone cases of congenital heart disease and four cases of mitral stenosis and two controls, the specimen of which were obtained at the surgical operation. The purpose is to confirm the damaging effect of occlusion of blood flow in surgical operation on muscle fiber.

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**BRIEF NOTE**

Acta Med. Okayama 20, 277—282 (1966)

**HISTOCHEMICAL OBSERVATION ON THE ENZYME  
ACTIVITIES OF HUMAN MYOCARDIUM**

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*Received for publication, November 4, 1966*

Recently, by histochemical observations the changed activities of the enzymes of heart muscle in experimentally induced ischemia have been reported by several investigators<sup>1-4</sup>. SHNITKA and NACHLAS<sup>4</sup> demonstrated that the ligation of coronal artery of dog heart induced an increase in the activities of succinic dehydrogenase and NAD-diaphorase four to six hours after the ligation.

However, extracorporeal circulation induced no distinct changes in the activities of succinic dehydrogenase and cytochrome oxidase as has been revealed quite recently by BJÖRK and associate<sup>5</sup> from their histochemical studies of the specimen from left ventricular myocardium by a method of drill biopsy, but only the myocardial edema and fibrosis occurred. This report deals with the distribution and activities of oxidative enzymes of human myocardium of forty-one cases of congenital heart disease and four cases of mitral stenosis and two controls, the specimen of which were obtained at the surgical operation. The purpose is to confirm the damaging effect of occlusion of blood flow in surgical operation on muscle fiber.

MATERIALS AND METHODS

The patients of congenital heart disease were those of twenty-four ventricular septal defect (VSD), ten atrial septal defect (ASD), three pulmonary stenosis (PS), one endocardial cushion defect (ECD), one Tetralogy of Fallot, one ruptured aneurysm of the sinus of Valsalva and one both great vessels from right ventricle.

In general, specimens were obtained from right atrial and ventricular myocardium. In the case of mitral stenosis only the specimens from left atrial appendage served as material. For the control, specimens were obtained from the patients of thoracic trauma. In these cases two specimens from right atrial appendage were observed, one in each heart.

All the specimens were obtained within two hours extracorporeal circulation

with 15% hemodilution of low molecular dextran under moderate hypothermia (30~32°C). Specimens were obtained before and after perfusion one in each. The histochemical observations were made on frozen sections unfixed. The

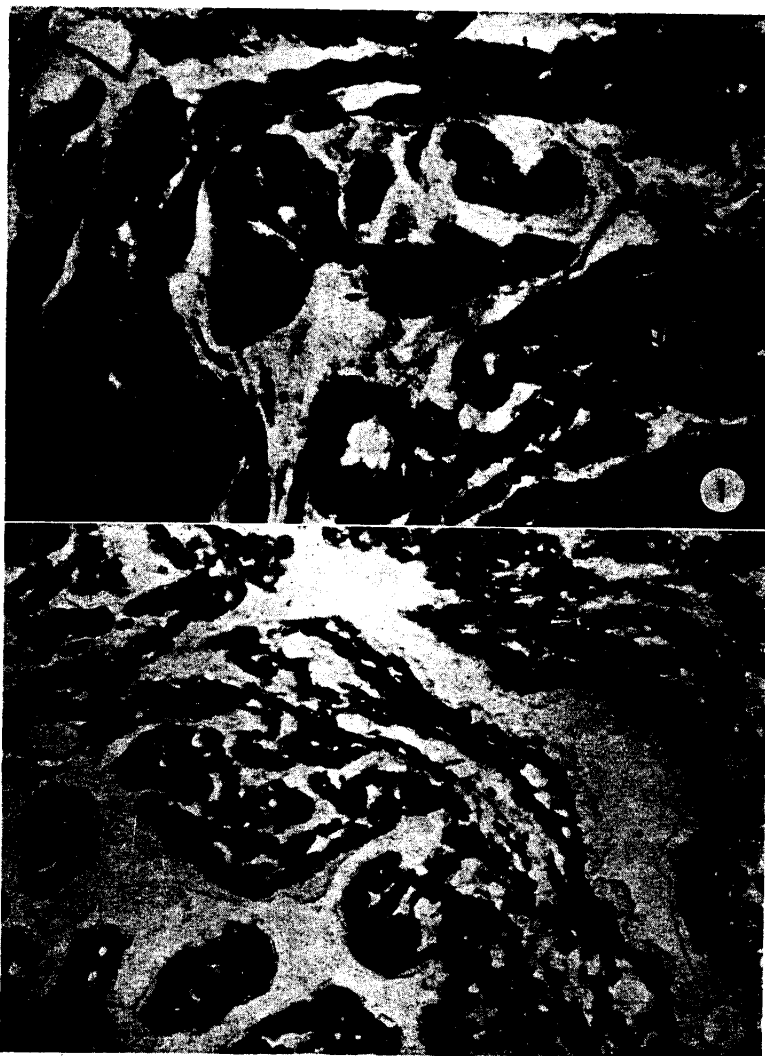


Fig. 1 Histochemical demonstration of lactic dehydrogenase activity of hypertrophic right ventricular myocardium of acyanotic Tetralogy of Fallot of 14 years old. Note strong positive activity of muscle fibres  $\times 350$

Fig. 2 Histochemical demonstration of succinic dehydrogenase activity of right ventricular myocardium of less severe VSD of 5 years old. Note the moderately positive activity of muscle fiber and negative reaction of endocardium, epicardium and interstitial tissues.

enzyme activities studied were those of succinic, malic, lactic, glutamic,  $\beta$ -hydroxybutyric dehydrogenases ( $\beta$ -HDH) and NAD, NADP-diaphorase, and the methods by PEARSE<sup>6</sup> were employed. In each sample a section was stained by hematoxylin and eosin staining. The intensities of enzyme reactions were observed dividing 6 grades in each, negative, undefined very slightly positive, slightly positive, moderately positive and strongly positive reactions.

## RESULTS

In all the myocardium examined the enzyme activities of dehydrogenase gave relatively high values, especially, the activities of succinic dehydrogenase (SDH), lactic dehydrogenase (LDH), NAD and NADP-diaphorase were strongly positive. The activities of malic dehydrogenase (MDH) and glutamic dehydrogenase (GDH) were moderate and that of  $\beta$ -HDH slightly positive. The histochemical pattern of the atrial and ventricular myocardium was completely identical. Blood vessels in interstitial tissues gave a moderate positive activity in every enzyme, except those of SDH and  $\beta$ -HDH which were negative or very slightly positive. The enzyme reactions appeared preferentially in tunica media and in endothelial cells of intima. The connective tissue gave a slightly to moderately positive reaction in LDH, MDH and NAD-diaphorase, very slight in GDH and  $\beta$ -HDH activities and negative in SDH. The epicardium and endocardium were slightly positive in LDH, very slightly or slightly positive in GDH, MDH and  $\beta$ -HDH and completely negative in SDH. The smooth muscle in endothelium gave a moderately positive activity in all the enzymes tested except SDH (Table 1). Concerning the enzyme activities of myocardium a positive correlation between the enzyme activities and the thickness of ventricular myocardium was found; a higher dehydrogenase activities in the hypertrophic muscle wall, for example, in pulmonary stenosis, Tetralogy of Fallot and Eisenmenger syndrome. Especially, a strong reactions appeared in LDH and MDH of hypertrophic heart muscles (Table 2).

By clamping the right atrial appendage during the open heart surgery, different enzyme activities of myocardium may be expected between the peripheral and central parts from the interceptive suture according to the time interval of occlusion, because the peripheral appendage from the suture is ischemic, but the central part is supplied with coronary flow except when the aortic clamp is undergone during complete bypass. Observations revealed the increased activity of LDH in most cases of the group in which the blood flow was occluded more than 90 minutes. Concerning the difference in enzyme activities of right ventricular myocardium resected before and after perfusion, observations on 20 patients of VSD revealed the increased activities in a few cases and the decreased

Table 1 Activities of Oxidative Enzymes in Human Myocardium

Disease	Enzyme	Myocardium	Vessel	Fibrocyte	Endocardium		Epicardium
					Endo-therium	Subendo-therium	
Control (2 cases)	GDH	+1	±	±	±	-	±
	LDH	+2	±	+1	+1	-	+1
	SDH	+3	±	-	-	-	-
	MDH	+2	+1	±	±	-	±
	β-HDH	+2	±	-	±	-	±
	NAD-D						
VSD (24 cases)	GDH	+1~+2	± ~+2	- ~±	± ~+1	- ~±	- ~±
	LDH	+3~+4	+2	± ~+1	± ~+2	- ~±	- ~+1
	SDH	+3	- ~±	-	-	-	-
	MDH	+2~+3	+1~+2	± ~+1	± ~+1	- ~±	± ~+1
	β-HDH	+1~+2	- ~±	-	- ~±	-	- ~±
	NAD-D	+4	+2~+3	± ~+1	± ~+1	- ~±	± ~+1
ASD (10 cases)	GDH	+1~+2	± ~+2	- ~±	± ~+1	- ~±	- ~+1
	LDH	+3~+4	+2	± ~+1	+1	±	± ~+1
	SDH	+3~+4	±	-	-	-	-
	MDH	+2~+3	+2	±	+1	±	± ~+1
	β-HDH	+1~+3	±	-	- ~+1	-	- ~±
	NAD-D	+2~+4	± ~+3	± ~+1	+1	- ~±	± ~+3
PS (3 cases)	GDH	+2	+1	±	±	-	±
	LDH	+3	+2	±	±	-	±
	SDH	+3	±	-	-	-	-
	MDH	+3	+2	+1	±	-	±
	β-HDH						
	NAD-D	+4	+3	±	+1	±	+1
MS (4 cases)	GDH	+2	+1	±	±	±	±
	LDH	+3	+2	±	+1	-	+1
	SDH	+3~+4	±	-	-	-	-
	MDH	+3	+1~+2	±	±	-	-
	β-HDH	+3	-	-	±	-	-
	NAD-D	+4	+3	±	+1	±	±

VSD: ventricular septal defect, ASD: atrial septal defect, PS: pulmonic stenosis, MS: mitral stenosis, GDH: glutamic dehydrogenase, LDH: lactic dehydrogenase, SDH: succinic dehydrogenase, MDH: malic dehydrogenase, β-HDH: β-hydroxybutyric dehydrogenase, NAD-D: nicotinamide adenin dinucleotide diaphorase. -: negative, ±: undefined, +1: very slightly positive, +2: slightly positive, +3: moderately positive, +4: strongly positive.

Table 2 Relationship Between the Enzyme Activities and Hypertrophy of the Right Ventricle

Thickness of muscle wall (mm) \ Enzyme Activities	Less than 5	6-10	11-20	21-30
GDH	+1(5), +2(2)	±(1), +1(4), +2(4)	+1(1), +2(2), +3(2)	±(1), +2(1)
LDH	+2(1), +3(1), +4(6)	+2(2), +3(1), +4(6)	+3(2), +4(3)	+4(2)
SDH	+3(5), +4(3)	+2(1), +3(6)+4(2)	+3(4), +4(1)	+4(2)
MDH	+1(1), +2(3), +3(4)	±(1), +1(1), +2(4), +3(3)	+2(1), +3(4)	+3(1), +4(1)
β-HDH	+1(1), +2(3), +3(3)	+1(2), +2(2), +3(3)	+1(1), +2(1)	+2(1)
NAD-D	+1(1), +3(3), +4(3)	+2(2), +3(4), +4(1)	+2(1), +3(1), +4(3)	+2(1), +4(1)

The number in parentheses indicates the number of cases observed. GDH, LDH, SDH, MDH, β-HDH, NAD-D, ±, +1, +2, +3, +4: See the explanation in Table 1.

Table 3 Relationship Between the Enzyme Activities and the Duration of Aortic Occlusion (20 cases of VSD)

Enzyme activities	Time (minutes)	Time (minutes)			
		0~10	11~20	21~30	31~40
GDH	A	8	2	1	3
	B	3	1		
	C	1			1
LDH	A	10	2	1	1
	B	1	1		
	C	1			3
SDH	A	8	1	1	3
	B	4	2		1
	C				
MDH	A	10	2		3
	B	1	1	1	
	C	1			1
β-HDH	A	9	1		1
	B	1		1	1
	C	2			1
NAD-D	A	5	1	1	1
	B	3	2		1
	C	4			

A: unchanged, B: increased, C: decreased.

enzyme reactions in some other cases according to the duration period of the aortic clamp, though in most cases no distinct changes in enzyme activities were found among these taken before and after perfusion, including a case of long term occlusion of aorta as long as 41 minutes (Table 3). The results seem to indicate that the duration time of the aortic occlusion in the course of operation gives actually no significant damaging effect on heart as far as the enzyme activities of muscle fiber are concerned.

#### ACKNOWLEDGEMENT

The authors wish to acknowledge the staff of thoracic and heart surgery, Sakakibara Juzen Hospital, Okayama, Japan for their kind supply of the materials.

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