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J Clin Invest. 1959;**38**(12):2111-2118. <https://doi.org/10.1172/JCI103989>.

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STUDIES ON ISOCITRIC AND LACTIC DEHYDROGENASES IN EXPERIMENTAL MYOCARDIAL INFARCTION * †

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(Submitted for publication June 2, 1959; accepted June 19, 1959)

It was the purpose of this investigation to attempt to explain the curious pattern of serum enzyme activity which has been seen clinically following myocardial infarction. This pattern, which consists of fairly uniform elevations of the activity of lactic dehydrogenase (LDH) (1-3), glutamic-oxaloacetic transaminase (GOT) (4-6) and aldolase (1), contrasts sharply with the failure to demonstrate increased activity of isocitric dehydrogenase (ICD) (6-8). Since myocardium has been reported rich in ICD activity (7, 9), and since it has been assumed that the increase in serum enzyme activity following myocardial infarction has been related to the release of the enzyme contents of the damaged tissue (10, 11), it appeared that ICD either failed to leave the damaged cells or, if it were liberated, that it had a fate in serum different from that of LDH or GOT. The elevation of serum ICD activity in liver disease has been well documented (7, 8), and therefore the failure to demonstrate increased ICD activity after myocardial infarction was even more striking.

The investigation to be reported was designed to provide answers to the following questions. 1) Is ICD released into the blood after myocardial infarction? 2) What is the fate of ICD and of LDH in blood following myocardial infarction? 3) At what rates are exogenously administered enzymes cleared from the blood of normal animals? 4) Is the rate of "clearance" altered following hepatectomy or nephrectomy? Experiments were divided into two parts: 1) the production of ex-

perimental myocardial infarctions in dogs, with the study of serum activity of ICD and LDH in coronary sinus and vena cava blood, and comparison of the activity of these enzymes in normal and infarcted myocardium; 2) a study of the rates of "clearance" of ICD and LDH after intravenous injection in normal and in hepatectomized and nephrectomized dogs.

METHODS

General procedures. Unselected mongrel dogs, weighing between 10 and 18 Kg., were used. All surgical procedures were performed under pentobarbital sodium anesthesia (30 mg. per Kg.) and dogs employed in experiments requiring thoracotomies received oxygen under positive pressure.

Blood samples for enzyme assay were drawn through No. 10 French siliconized, polyethylene catheters. Each sample was centrifuged and the serum was carefully separated and recentrifuged to insure that no cells remained as contaminants. Sera were frozen immediately following centrifugation and the enzyme activity was assayed within one week of the time of collection. ICD was assayed using the method of Wolfson and Williams-Ashman (12), and activity was expressed as millimicro-moles of triphosphopyridine nucleotide reduced per milliliter of serum per hour. LDH activity was determined with a modification of the method of Hill and Levi as described previously (1), and activity was expressed as the change in optical density per 0.01 ml. of serum per 30 minutes. The error of the determination of enzyme activity as based on duplicates of the same sample is about 5 per cent for ICD and about 2 per cent for LDH.

Open chest myocardial infarction procedure. Employing a left lateral thoracotomy incision, a catheter was guided into the coronary sinus via the right external jugular vein. The catheter was secured with 4-0 silk to the wall of the coronary sinus. A second catheter was guided into the superior vena cava through the left external jugular vein. The circumflex branch of the left coronary artery was exposed and dissected free. This branch was selected for ligation, because its occlusion produces a homogeneous infarct in the posterior papillary muscle (13). Homogeneous infarcts were desired to permit accurate appraisal of enzyme activity in infarcted myocardium.

Six dogs were subjected to coronary ligation at the time of thoracotomy. Ligatures were placed within 1

* Supported in part by Grant CY 2914 from the National Cancer Institute, National Institutes of Health, Bethesda, Md., and in part by a fellowship from the Josiah Macy, Jr. Foundation.

† This paper is the sixth in a series of investigations on serum enzymes.

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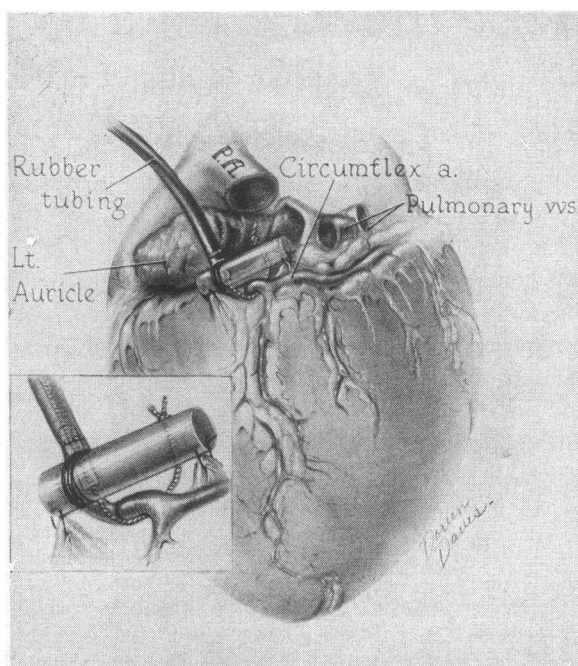


FIG. 1. APPARATUS EMPLOYED IN THE PRODUCTION OF CLOSED CHEST MYOCARDIAL INFARCTIONS

The apparatus consisted of a 2 cm. segment of polyethylene tubing attached at right angles to a rubber tube of sufficient length to extend from the heart to the chest wall. Tightening the nylon loop compressed the artery against the base of the polyethylene tube, producing coronary occlusion.

cm. of the coronary ostia. Five dogs developed ventricular fibrillation shortly after ligation; however, three of them were successfully defibrillated with electric shock. One dog, which developed acute heart failure and died four hours after ligation, was discarded. Dogs were followed continually with electrocardiograph recordings. Within an hour after ligation the chests were closed and the animals were permitted to recover. Samples were taken from the coronary sinus and vena cava following infarction at 2.5, 8, 16, 24, 36 and 48 hours. Thereafter, samples were drawn every 24 hours until the dogs died or were sacrificed.

Closed chest myocardial infarction procedure. Closed chest myocardial infarctions were performed on four dogs. After the circumflex artery was exposed, a loop of No. 2 braided nylon was placed around the artery about 3 cm. from the ostium and threaded into the infarction apparatus illustrated in Figure 1. The apparatus was secured to the myocardial fat pads with 4-0 silk, and the rubber tube containing the long end of the nylon loop was brought out through the chest wall and secured. This apparatus effectively produced occlusion in all of the animals studied and prevented the breaking of ligatures and tearing of lungs and pleural adhesions which have sometimes complicated closed chest myocardial infarctions produced by the overhand knot technique (11, 14). Coronary sinus and vena cava catheters

were filled twice daily with sodium heparin to prevent clotting. When the enzyme activity returned to pre-operative levels, usually within five days, the dogs were anesthetized as previously described and placed on positive pressure oxygen. Five minutes prior to tightening the nylon loop an intravenous infusion was started. This infusion, which contained papaverine hydrochloride, 8 to 10 mg. per Kg. diluted in 50 ml. of normal saline, was administered at a constant rate over a 10 minute period. According to the studies of McEachern, Smith and Manning, papaverine hydrochloride decreases significantly the mortality rate following circumflex artery occlusion in the dog (15). All dogs were followed with electrocardiograph tracings and serial blood samples were drawn as described above. Two dogs developed ventricular fibrillation and died. Atrial fibrillation was treated with lanatoside C; ventricular tachycardia was treated with procaine amide.

Myocardial enzyme assays. Assays of enzyme activity in myocardial tissue were performed on homogenates of tissue. Weighed samples of infarcted and normal myocardium were finely minced, and homogenized in a Potter-Elvehjem apparatus as described by Meister (16). After centrifugation at 5,000 G for 60 minutes, enzyme assays were performed on the supernatant. Activities were expressed in terms of protein content, and the protein content was determined by the quantitative turbidimetric method of Bossak, Rosenberg and Harris (17).

Hepatectomy procedure. Total hepatectomies were performed on five dogs, employing a modification of the procedure described by Firor and Stinson (18). A thoraco-abdominal approach was employed as it offered better exposure than the midline incision originally described. A siliconized glass "Y" tube was utilized to bypass the liver. Prior to dividing the portal vein, 5 ml. of 1 to 500,000 epinephrine was injected intraportally to minimize hepatic congestion, thereby decreasing the blood loss during hepatectomy. McMichael demonstrated that liver volume could be reduced by as much as 50 per cent following the intraportal injection of epinephrine (19).

Nephrectomy procedure. Bilateral nephrectomies were performed on two dogs. A midline abdominal incision was used to avoid cutting muscles and the consequent liberation of enzymes into the circulation. The kidneys were exposed and, after the pedicles were ligated and divided, the kidneys were removed.

Injection studies. With the exception of the portal vein studies, enzymes were injected through a polyethylene catheter into the vena cava. Portal vein injections were administered through a small catheter threaded into the portal vein through a mesenteric vein. In dogs, Sigma ¹ ICD, 0.4 to 0.7 mg. per Kg., and NBC ²

¹ The isocitric dehydrogenase used in these experiments was extracted from pig hearts by the Sigma Chemical Company of St. Louis, Mo., and was provided through the courtesy of Mr. Dan Broida.

² The lactic dehydrogenase was extracted from rabbit hearts by the Nutritional Biochemicals Corporation of Cleveland, Ohio.

TABLE I
Open chest myocardial infarction

Time	Dog No. 27 (15.5 Kg. male)				Dog No. 28 (12.6 Kg. female)				Dog No. 30 (13.2 Kg. male)			
	Coronary sinus		Vena cava		Coronary sinus		Vena cava		Coronary sinus		Vena cava	
	ICD*	LDH*	ICD	LDH	ICD	LDH	ICD	LDH	ICD	LDH	ICD	LDH
Preinfarction	160	45	160	46	160	68	165	71	135	50	135	48
Postinfarction:												
2.5 hrs.	240	48	215	47	260	171	220	168	335	140	255	143
8 hrs.	475	53	300	50	970	287	325	283	610	300	455	300
16 hrs.	650	126	430	127	Died				1,230	438	865	430
24 hrs.	680	148	650	141					1,360	504	1,220	452
48 hrs.	1,080	170	1,080	154	Died				Died			
76 hrs.	970	70	920	39								
96 hrs.	190	44	180	38								
124 hrs.	215	38	200	36								
150 hrs.	240	34	240	32								

* ICD = isocitric dehydrogenase; LDH = lactic dehydrogenase.

LDH, 0.1 to 0.2 ml. per Kg., were injected simultaneously over 10 minute intervals. Serial samples were drawn at 2, 10, 20, 40, 60, 90, 180, 240 and 420 minutes.

Several rabbits were studied to determine the normal "clearance" curves of ICD and LDH in a species other than the dog. The ICD employed was extracted from rabbit hearts and preserved by lyophilization according to the method of Ochoa (20).

RESULTS

Myocardial infarction studies

Table I and Figure 2 record the data obtained from the open chest experiments. That enzymes are released following surgical trauma has been well documented (14, 21, 22). It has been dem-

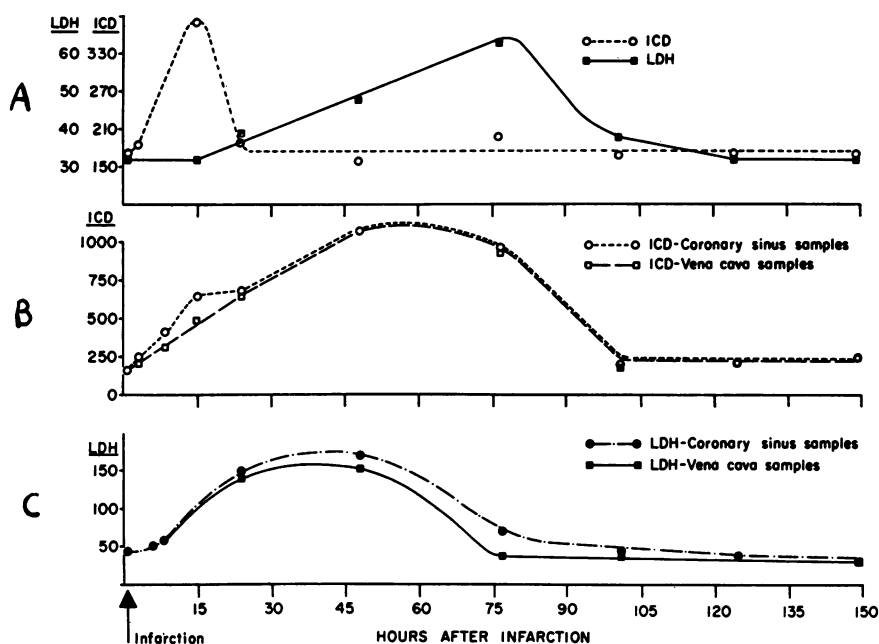


FIG. 2. ENZYME ACTIVITY AFTER OPEN CHEST MYOCARDIAL INFARCTION IN DOG No. 27

A. Differences between coronary sinus and vena cava enzyme activity after myocardial infarction. The graph is based on coronary sinus enzyme activity minus vena cava enzyme activity, using normal levels as a baseline. LDH = lactic dehydrogenase; ICD = isocitric dehydrogenase. B. ICD activity in vena cava and coronary sinus after myocardial infarction. C. LDH activity in vena cava and coronary sinus after myocardial infarction.

TABLE II
Closed chest myocardial infarction

Time	Dog. No. 42 (10.0 Kg. male)				Dog No. 44 (14.5 Kg. male)			
	Coronary sinus		Vena cava		Coronary sinus		Vena cava	
	ICD*	LDH*	ICD	LDH	ICD	LDH	ICD	LDH
Normal	120	94	115	92	115	80	110	74
Postinfarction:								
1 hr.	120	90	110	88				
3 hrs.	220	85	160	83	215	76	160	70
6 hrs.	310	228	215	142	325	135	190	125
10 hrs.	670	500	540	424	300	185	270	155
16 hrs.	500	420	325	340	215	250	110	220
24 hrs.	120	310	110	198	110	166	110	125
44 hrs.	115	100	110	88	120	80	115	78
52 hrs.					100	82	110	75
72 hrs.	110	92	100	90				
96 hrs.	120	88	110	84				

* See footnote, Table I.

onstrated that pentobarbital sodium anesthesia has no effect on serum enzyme activity (22). We observed increases in both ICD and LDH activity following thoracotomy; however, the increased activity of both these enzymes was similar in vena cava and coronary sinus samples. The elevations in enzyme activity illustrated in Figures 2B and 2C were the result of thoracotomy as well as of myocardial infarction. The period during which coronary sinus enzyme activity was greater than vena cava activity revealed the time during which the enzymes were being released from the injured

myocardium. Figure 2A demonstrates the periods during which ICD and LDH were being released as determined by differences between coronary sinus and vena cava enzyme activity.

To eliminate the confusing effect of the enzymes released as a result of surgery, closed chest myocardial infarctions were performed. The results of these experiments are illustrated in Figure 3 and Table II. Elevations of serum ICD activity occurred within three hours following coronary ligation and returned to normal limits within 24 hours. Increases in the activity of LDH were

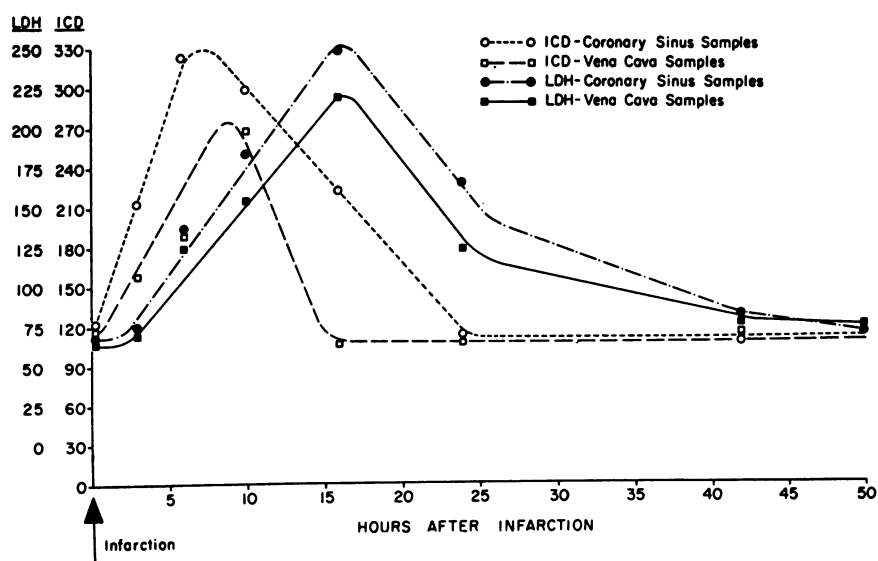


FIG. 3. ENZYME ACTIVITY AFTER CLOSED CHEST MYOCARDIAL INFARCTION IN DOG No. 44

LDH and ICD activity in vena cava and coronary sinus after myocardial infarction.

TABLE III
Enzyme activity in infarcted and normal
myocardium of five dogs

Tissue	ICD*	LDH†
Normal myocardium	20.3-27.0	23.1-32.3
Infarct. myocardium	1.8- 9.0	3.1-13.2

* Isocitric dehydrogenase expressed as millimicromoles triphosphopyridine nucleotide reduced per minute per milligram protein at 25° C.

† Lactic dehydrogenase expressed as micromoles diphosphopyridine nucleotide oxidized per minute per milligram protein at 38° C.

not observed until six hours after infarction; however, elevated activity was maintained for about 44 hours. These data are illustrated in Figure 3, which demonstrates the rapid rise and fall of ICD in comparison to LDH and confirms the open chest infarction profile illustrated in Figure 2A.

Myocardial enzyme assays

The enzyme activity in the homogenates prepared from normal and infarcted myocardium is recorded in Table III. The infarcted tissue contained less ICD and LDH activity than the normal myocardium.

Enzyme clearance studies

Figure 4 summarizes the findings of these studies. Injections of ICD and LDH into normal dogs and rabbits demonstrated the disappearance of exogenous ICD within 90 minutes following its injection; LDH activity was not observed to re-

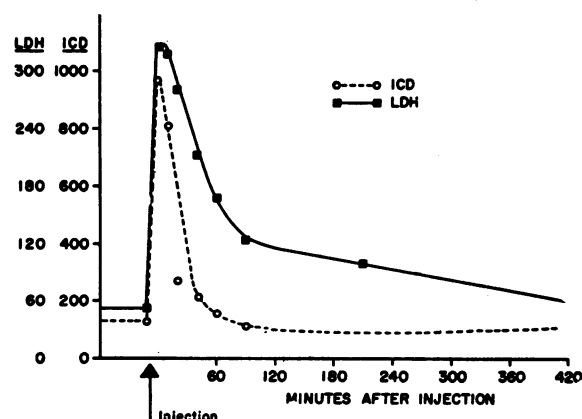


FIG. 4A. VENA CAVA ENZYME INJECTION

Rate of enzyme fall after the injection of 68.8 mg. ICD and 1 ml. LDH into the inferior vena cava of Dog No. 29.

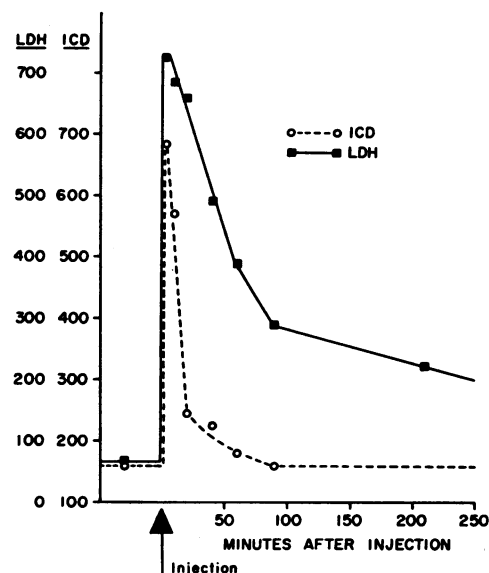


FIG. 4B. PORTAL VEIN ENZYME INJECTION

Rate of enzyme fall after the injection of 58 mg. ICD and 2.9 ml. LDH into the portal vein of Dog No. 27.

turn to normal levels until about seven hours after injection.

Injections into the portal vein did not produce "clearance" rates different from those observed

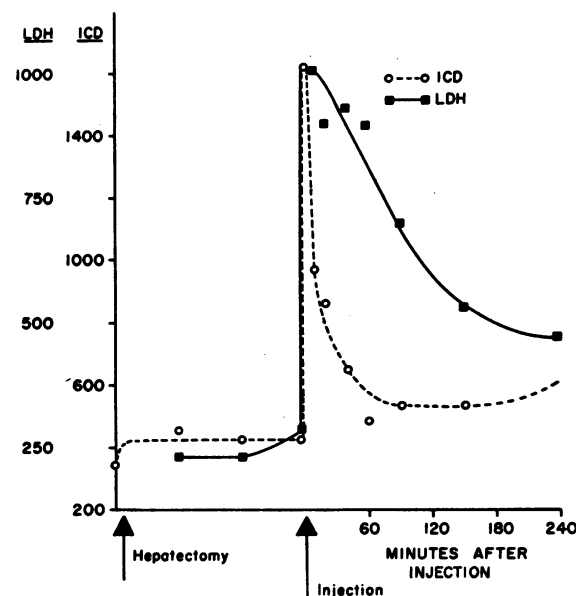


FIG. 4C. ENZYME INJECTION AFTER HEPATECTOMY

Rate of enzyme fall after the injection of 85.4 mg. ICD dehydrogenase and 2.4 ml. LDH into the inferior vena cava four hours after completion of total hepatectomy in Dog No. 31.

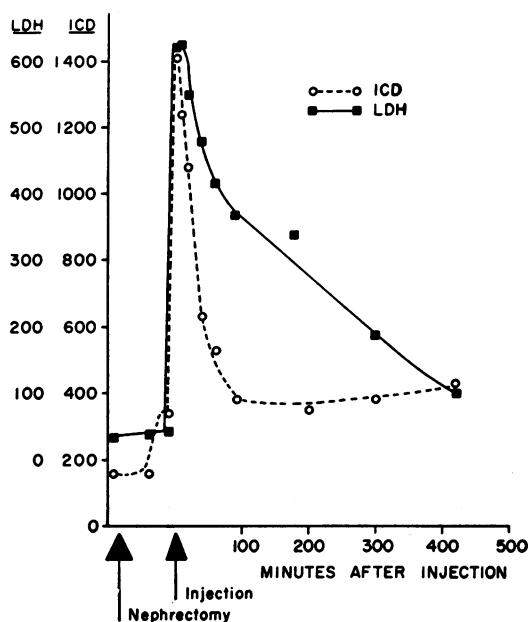


FIG. 4D. ENZYME INJECTION AFTER NEPHRECTOMY

Rate of enzyme fall after injection of 60 mg. ICD and 1.5 ml. LDH into the inferior vena cava two hours after completion of bilateral nephrectomy in Dog No. 34.

in the normal preparations. Injections following hepatectomy and nephrectomy also produced similar disappearance curves.

DISCUSSION

Myocardial infarction studies

These data document the release of ICD following myocardial infarction. ICD was released earlier than LDH, but ICD activity remained elevated for less than 24 hours; by contrast LDH activity remained elevated for at least 44 hours.

The coronary sinus enzyme levels are pertinent to previous discussions concerning the source of increased serum enzyme activity following myocardial infarction (11, 21). Following infarction, the activity of samples drawn from the coronary sinus was consistently greater than was the activity in samples drawn from the peripheral circulation. This difference corroborates the findings of Rueggsegger, Nydick, Freiman and LaDue (11) and indicates that the anoxic myocardium is responsible for a significant portion, if not all, of the liberated enzyme. It is important to note that, when the coronary sinus activity was no longer greater than the activity found in the vena cava,

the coronary sinus and vena cava enzyme activities had both returned to normal. This indicates that the observed elevations were primarily a direct result of enzyme release from the myocardium and not the result of a nonspecific response to trauma (21).

Elevation of serum LDH activity was not observed until six hours after coronary ligation. This delay prior to the release of LDH confirms the findings of Jennings, Kaltenbach and Smetters (23) and Rueggsegger and associates (11).

Myocardial enzyme assays

Several studies have previously demonstrated that homogenates prepared from infarcted myocardium contain less GOT, LDH, glutamic-pyruvic transaminase and succinic dehydrogenase activity than homogenates prepared from normal myocardium (11, 23). Our studies demonstrate that the activity of ICD in the infarcted tissue is similarly reduced. This observation along with the finding of increased ICD activity in coronary sinus blood after myocardial infarction is strong evidence that ICD is released from damaged myocardium.

Enzyme clearance studies

In these experiments sufficient amounts of enzyme were injected to produce elevations of activity which would be within the range observed in disease states. With ICD activity elevated to seven times normal, "clearance" was complete within 90 minutes. With similar elevations LDH was "cleared" in about seven to 10 hours.

The serum activity of LDH and GOT following experimental myocardial infarction has been found to correlate closely with the activity of these enzymes following myocardial infarctions in human beings (14, 24). If the activity of serum ICD after experimental infarction also parallels the clinical picture, the early and transient elevation of ICD explains the clinical failure to observe increased activity after infarction. The rapid disappearance of serum ICD activity following injection explains, at least in part, the transitory nature of the elevation which is observed after experimental myocardial infarction.

In view of the clinical finding that ICD has been reported elevated only in diseases involving

the hepatic parenchyma (7, 8), the liver was considered first as a possible site of ICD inactivation. However, the similar disappearance curves of ICD and LDH observed following vena cava injections, intraportal injections and injections in hepatectomized dogs led us to reject this hypothesis. One may thus infer that the elevation of serum ICD activity observed in liver disease is a result of the continuous liberation of ICD from damaged cells rather than a result of impaired hepatic inactivation of the enzyme.

The normal disappearance curves in nephrectomized and hepatectomized dogs in conjunction with the data obtained following intraportal injections indicate that the liver and kidney are not responsible for "clearing" ICD and LDH from the circulation.

Numerous investigators have commented on the speed of release of enzymes from damaged cells and on the rate of "clearance" of enzymes following injection into the blood stream (6, 10, 14). One of the variables considered in this respect has been enzyme molecular weight. Therefore a comparison of molecular weight with rate of "clearance" is of interest. The molecular weight of ICD has been reported as 61,000 (25), LDH as 135,000 (26) and GOT as 60,000 (27). Wroblewski injected homogenates of myocardium into dogs and observed similar disappearance curves for GOT and LDH over a period of four hours (10). His LDH "clearance" was comparable to that observed in our studies (10, 24). As noted above, ICD "clearance" was more rapid than LDH; however, comparison of the molecular weights of ICD, LDH and GOT with their rates of disappearance following injection demonstrates that although ICD and GOT have similar molecular weights, ICD is "cleared" more rapidly than GOT. On the other hand, LDH and GOT with similar rates of disappearance have different molecular weights. If the weights reported coincide with those of the enzymes used in these experiments, molecular weight cannot explain the differences observed in rate of "clearance."

SUMMARY

Isocitric dehydrogenase (ICD) is released from injured myocardium following myocardial infarction. ICD is released shortly after infarction

and for only a brief period of time; by contrast, lactic dehydrogenase (LDH) is released later than ICD and remains elevated longer.

ICD is rapidly "cleared" from the circulation following injection whereas LDH "clearance" is more protracted.

ICD and LDH are apparently not "cleared" by any single organ. The injection of these enzymes into the vena cava and portal vein of dogs and into the vena cava of hepatectomized and nephrectomized dogs demonstrates similar "clearance" rates.

ICD activity is transiently elevated during the first 24 hours following experimental myocardial infarction. The brief duration of this elevation is apparently due to the extremely rapid "clearance" of ICD and to the brief period of its liberation. If this brief period of elevation observed experimentally also occurs in patients with myocardial infarction, it presumably explains the clinical failure to demonstrate elevated levels of this enzyme after infarction.

ACKNOWLEDGMENT

We are indebted to Dr. Roy B. Cohn and Dr. Norman E. Shumway for their suggestions on surgical technique, to Dr. Raymond C. Stofer for his help and cooperation in the surgical laboratory, and to Mr. Charles J. Lewin and Mr. Donald R. Johnson for their suggestions and criticisms.

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