Changes in serum enzyme levels accompanying cardiac surgery with extracorporeal circulation

NORA WELBOURN¹, D. G. MELROSE, AND D. W. MOSS²

From the Nuffield Unit of Clinical Physiology, Departments of Surgery and Chemical Pathology, Postgraduate Medical School, London

SYNOPSIS Serum lactic dehydrogenase, alpha-hydroxy-butyrate dehydrogenase, iso-citric dehydrogenase, and glutamic oxaloacetic transaminase activities were measured daily for two weeks post-operatively in the serum of 22 patients undergoing cardiac surgery with extracorporeal circulation.

The length of perfusion was found to be a major factor affecting the extent of increased post-operative enzyme activities. Significantly higher levels were demonstrated in patients perfused for over one hour compared with those perfused for under one hour. Hepatocellular damage, age, and type of operation were not considered to be major factors in determining the extent of this increased activity.

A considerable increase in enzyme activity was found to occur during perfusion when the dilution introduced by mixing the patient's circulation with the priming fluid of the heart-lung machine was taken into account. This dilution, when accounted for, increased the observed enzyme activity by 30 to 50%.

The association of raised serum enzyme levels with damage to heart muscle, *e.g.*, myocardial infarction, is well documented. However, in patients undergoing cardiac surgery with extracorporeal circulation further study appeared to be necessary to elucidate the role extracorporeal circulation played in the release of these enzymes. Most workers have concentrated on changes in serum glutamic oxaloacetic transaminase (GOT), although some have included other enzymes such as lactic dehydrogenase (LDH) and glutamic pyruvic transaminase (GPT). It has been shown that GOT increases after most surgical operations (Nickell and Allbritten, 1957; Lawrence and Schulkins, 1956; Craver, Johnson, and Beal, 1957; Person and Judge, 1958; Ticktin, Ostrow, and Evans, 1956; Di Carlo and Parisi, 1958; Candura and Minardi, 1957) but that this rise seldom exceeds 30 international units (i.u.) per litre. The greatest increases of GOT occurred after heart operations, especially after cardiovascular surgery with extracorporeal circulation (Baer and Blount, 1960; Snyder, Barnard, Varco, and Lillehei, 1958; Werle, Trautschold, Gorris, and Zill, 1961; Quinn, Sirak,

Shabanah, and Frajola, 1960; Pyörälä, Gordt, Kontinnen, and Telivuo, 1963).

There is a wide divergence of opinion concerning the reasons for these post-operative increases. Fraser, Rossall, Black, and Dvorkin (1962) and Quinn *et al.* (1960) suggest that the release of enzyme is due to surgical procedures which produce direct heart trauma. They show significantly higher GOT levels in cases having ventriculotomy than those without. Walker and Morgan (1964) and Baer and Blount (1960) were unable to confirm this conclusion. Snyder *et al.* (1958) suggest that hepatic changes occurring during perfusion are the major sources of increased levels of GOT, but Walker and Morgan (1964), Baer and Blount (1960), and Norberg and Senning (1959) failed to verify this finding. It has generally been assumed that increased serum enzyme activity was due to the release of enzymes from necrotic cells and, in particular, organs, until Haus and Gerlach in 1958 advanced the concept of the acute syndrome. This proposed that GOT elevation was due to the complex response of the whole organism rather than a local factor.

These conflicting results have led us to re-examine the problem and to broaden the study to include LDH, GPT, hydroxybutyrate dehydrogenase (HBD), and isocitric dehydrogenase (ICD). Hydroxy-butyrate dehydrogenase activity is associated mainly

¹Correspondence to: Nora Welbourn, Nuffield Unit of Clinical Physiology, Dept. of Surgery, Postgraduate Medical School, Ducane Road, London, W.12.

²Present address: Department of Clinical Chemistry, Royal Infirmary, Edinburgh, 3.

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with the electrophoretically faster-moving fractions of LDH which occur predominantly in the myocardium (Rosalki and Wilkinson, 1960). This enzyme can be measured in the serum by a spectrophotometric method developed by Elliott and Wilkinson (1962). It has been shown to be a sensitive index of myocardial infarction (Elliott and Wilkinson, 1961; Elliott, Jepson, and Wilkinson, 1962; Hansson, Johansson, and Sievers, 1962; Konttinen, 1961; Konttinen and Halonen, 1963; Pagliaro and Notarbartolo, 1961) but at no time has it been found to rise in the serum without a concomitant increase in LDH (Wieme, 1962; Jontz, Bounous, Heimburger, Su, Teramoto, Shumacker, and Onnis, 1960; Rosalki and Wilkinson, 1960). Isocitric dehydrogenase is generally considered to reflect hepatic damage since a sharp increase in the serum activity follows liver cell damage, while normal values are obtained with myocardial infarction (White, 1958; Sterkel, Spencer, Wolfson, and Williams-Ashman, 1958; Sampson, 1958). However, Strandjord, Thomas, and White (1959) were able to demonstrate a transiently raised level of ICD during the first 24 hours after experimental myocardial infarction in dogs, thus showing that the damaged myocardium does release ICD. Its rapid disappearance from the circulation may account for failure to demonstrate increased levels following myocardial infarction.

METHODS

The patients studied were divided into two main groups. In the first group serum LDH, HBD, ICD, GOT, and GPT activities were measured in 22 patients pre-operatively, immediately post-operatively, and thereafter daily, when possible, for two weeks to ascertain the general enzyme pattern following surgery. The patients in this group were further subdivided and the results analysed with respect to the following factors: age, type of operation, and length of perfusion.

In group II the variations in serum LDH, HBD, ICD, and GOT were followed intensively throughout the perfusion and immediate post-perfusion period in 12 patients. In order to assess the actual amount of enzyme released from the patient during perfusion it was necessary to take into account the dilution which occurred when the patient's blood was mixed with the priming volume in the heart-lung machine. This dilution varies with the patient's blood volume as the volume necessary to prime the oxygenator remains fairly constant.

The observed enzyme activity was corrected so as to reflect more accurately the true enzyme activity of the patient using the equation

$$x = \frac{(\text{observed enzyme activity} - (\text{enzyme activity in pump} \times \text{total circulating volume}))}{\text{patient's blood volume} \times \text{pump volume}}$$

when x = the corrected enzyme activity.

All enzyme levels are expressed in i.u./litre and the blood volume is measured in litres.

This group was further subdivided in an attempt to see whether it was possible to detect increased serum enzyme activity during the perfusion period which could be attributed to myocardial damage. In order to do this, blood was simultaneously withdrawn from the coronary sinus, inferior vena cava, and oxygenator at the beginning and end of perfusion.

The operations were performed under mild hypothermia, and cardiopulmonary bypass was carried out using the Melrose-N.E.P. disc oxygenator (Melrose, 1961). The composition of the pump fluid generally consisted of 120 to 150 ml. of 4.2% sodium bicarbonate, 2.5 to 3 litres of whole blood, and 15 ml. per kilogram body weight of Rheomacrodex (Long, Sanchez, Varco, and Lillihei, 1961). The perfusion rate was maintained at 2.4 litres per sq. metre of body surface area per minute.

DETERMINATION OF ENZYME ACTIVITY Lactic dehydrogenase, HBD, ICD, GOT, and GPT were determined by the spectrophotometric methods described by Sigma using Sigma reagents. The results are expressed in international units (i.u.) per litre as recommended by the Joint Sub-Commission on Clinical Enzyme Units (King and Campbell, 1961) which defines 1 i.u. as the transformation of 1 μ mole of substrate per minute. All measurements were corrected to 25°C.

All the peak post-operative enzyme values expressed in this paper represent the highest level obtained within 72 hours after perfusion. This was necessary, even though the peak levels of GOT and ICD occurred within the first 24 to 48 hours, as the peak LDH and HBD activities often came within the 48- to 72-hour period.

COLLECTION OF BLOOD SPECIMENS All blood was withdrawn from the patient by venepuncture except during perfusion when samples were taken directly from the heart-lung machine. The serum was separated immediately and stored at 4°C. All the estimations (with the exception of ICD which was measured within 24 hours) were completed in one week, during which time the results were reproducible within $\pm 1\%$. Visibly haemolysed specimens were discarded since red blood cells contain appreciable amounts of LDH, HBD, ICD, and GOT, although Baer and Blount (1960) demonstrated that 80 to 100 mg. per 100 ml. of free haemoglobin did not significantly alter serum GOT levels. The special inferior vena cava and coronary sinus samples were taken by the surgeon at the operating table. The coronary sinus blood was taken by direct sampling from the right atrium immediately after the inferior and superior venae cavae had been snared and the systemic blood diverted, then just before the snares were released at the end of perfusion.

In one particular patient the hepatic veins did not join the inferior vena cava but entered the right atrium as a separate vessel. The hepatic vein was cannulated and samples withdrawn from it were compared with samples which had been drawn simultaneously from the coronary sinus.

TABLE I

RELEVANT DATA AND PEAK POST-OPERATIVE ENZYME LEVELS OF THE 22 PATIENTS STUDIED IN GROUP I

Diagnosis	Sex	Age	Length of Perfusion (min.)	Lowest Temperature (°C.) on Bypass	LDH (75-240)	HBD (60-120)	ICD (0.8-4.4)	GOT (4-20)	GPT (2.5-18)
Obstructive cardiomyopathy	Male	43	76	25	178	131	4.6	66	23
Subaortic stenosis	Male	44	61	27	433	294	7.1	36	6
Infundibular stenosis	Male	57	82	27	420	502	7.2	80	17
Mitral incompetence	Male	23	133	27	523	322	4.9	58	—
Mitral incompetence	Female	18	164	27	617	508	5.9	133	—
Aortic incompetence	Male	48	176	24	292	371	6.1	53	—
Mitral incompetence	Male	45	354	27	525	345	7.5	67	—
Ventricular septal defect	Male	7½	61	19	565	313	12.3	109	33
Ventricular septal defect	Female	5	62	24	630	565	8.2	116	22
Tetralogy of Fallot	Female	10	64	21	275	266	6.2	64	—
Atrioventricular canal	Male	5½	71	23	414	268	6.7	79	12
Tetralogy of Fallot	Female	10	77	20	517	537	5.4	78	15
Tetralogy of Fallot	Male	12	79	23	689	490	5.2	110	26
Ventricular septal defect	Female	6	89	22	505	178	6.9	107	16
Atrial septal defect	Male	15	39	31	351	245	3.6	72	29
Atrial septal defect and pulmonary valvotomy	Male	15	30	34	250	132	2.4	44	—
Ventricular septal defect	Female	10½	37	24	229	248	3.7	40	8
Ventricular septal defect	Male	6	40	26	—	246	5.0	61	11
Atrial septal defect	Male	15	41	34	263	234	3.5	55	—
Traumatic ventricular septal defect and aneurysm of left ventricle	Male	10	44	24	250	164	4.3	42	9
Atrial septal defect	Male	39	53	31	262	199	3.1	55	14
Ventricular septal defect	Male	17	55	26	262	183	4.6	39	10

RESULTS

GROUP I The distribution of the patients in this group according to age, sex, type of operation, length of perfusion, and peak post-operative enzyme activity is shown in Table I.

The GPT results showed no consistent variations and as no significant conclusions could be drawn from them they are only shown in Table I and not discussed further.

Effect of age Patients of 14 years of age and above were considered as adults. The scatter and mean of peak post-operative enzyme levels for both adults and children are shown in Figure 1. There is considerable overlapping in the individual peak post-operative levels of LDH, HBD, ICD, and GOT between children and adults but the main peak levels of all four enzymes are slightly higher in children.

Type of operation In considering the effects of

FIG. 1. Peak post-operative enzyme levels. Mean values shown by horizontal lines; normal range shaded.

FIG. 2. Peak post-operative enzyme levels in patients undergoing cardiac surgery with ventriculotomy (●) and without ventriculotomy (○). Mean values shown by horizontal lines; normal ranges shaded.

surgical trauma the patients were subdivided according to those having ventriculotomy (10 patients) and those not having ventriculotomy (12 patients). The patients having ventriculotomy included the following cases: ventricular septal defect (7 cases), tetralogy of Fallot (3), subaortic stenosis (1), obstructive cardiomyopathy (1), and those without ventriculotomy included atrial septal defect (4), mitral incompetence (3), aortic incompetence (1), infundibular stenosis (1), and atrioventricular canal (1). In Fig. 2 the scatter and mean peak post-operative levels between the two groups are indicated. These results indicate that incision of the right ventricle alone cannot account for the main

increases observed following perfusion as there are no significant differences between the two groups. *Length of perfusion* The patients were then subdivided according to the length of perfusion with the division arbitrarily chosen as one hour. Group A consists of the 14 cases perfused for over one hour and group B of the eight perfused for under one hour. In Fig. 3 each patient's daily enzyme level is indicated as well as the mean daily levels for groups A and B. From examining these results it can be seen that although the pre-operative levels are equal for both A and B, there are significant differences between their post-operative levels. The average peak levels of LDH, HBD, and GOT are approximately twice

TABLE II
MEAN PEAK POST-OPERATIVE ENZYME LEVELS BETWEEN CHILDREN AND ADULTS PERFUSED FOR UNDER AND OVER ONE HOUR

Group	Division by Age (adults over 14 yr. and children under 14 yr.)	No. of Cases	Average Peak Serum Enzyme Levels			
			LDH (75-240)	HBD (60-120)	ICD (0.8-4.4)	GOT (4-20)
A (perfused for over one hour)	Children	7	514	373	6.6	95
	Adults	8	427	335	5.3	65
B (perfused for under one hour)	Children	3	256	202	4.0	49
	Adults	5	258	189	3.4	53

TABLE III
MEAN PEAK POST-OPERATIVE ENZYME LEVELS BETWEEN PATIENTS WITH AND WITHOUT VENTRICULOTOMY PERFUSED FOR UNDER AND OVER ONE HOUR

Group	Type of Operation	No. of Cases	Average Peak Serum Enzyme Levels			
			LDH (75-240)	HBD (60-120)	ICD (0.8-4.4)	GOT (4-20)
A (perfused for over one hour)	Ventriculotomy	6	547	389	5.9	97
	No ventriculotomy	8	425	348	5.8	65
B (perfused for under one hour)	Ventriculotomy	4	225	196	4.1	45
	No ventriculotomy	4	251	212	4.1	46

as high in A, and, with the exception of two cases, only those patients perfused for over one hour have peak ICD levels outside the normal limit.

Since perfusion time is clearly an important factor affecting post-operative enzyme levels, groups A and B were examined separately to consider whether age or ventriculotomy affected the results when this source of variation was eliminated. From the results (Tables II and III) it is obvious that neither factor plays a determining role in affecting the mean peak enzyme levels in the group perfused for under one hour, but in the group perfused for over one hour the peak levels of LDH, HBD, and GOT are slightly higher in children and patients having ventriculotomy.

GROUP II In this part of the study an attempt was made to determine the actual amount of enzyme released from the patient during perfusion. This assessment was necessary to determine the dilution

which occurred when the patient's blood was mixed with the priming fluid of the heart-lung machine.

As care was taken to choose patients having little or no visible haemolysis, the increased enzyme activity in the oxygenator after perfusion (Table IV) cannot be attributed to haemolysis and therefore must represent cellular enzymes which have been released from the patient during perfusion.

By substituting the volumes given in Table V in the formula (described under methods) corrections were calculated for 12 patients. The observed and corrected values for five patients are shown in Figures 4-8. From examining these results it can be seen that without correction the enzyme activities determined in the first sample taken after just going on perfusion are actually lower than the pre-perfusion levels in most cases, whereas if corrected there is an immediate increase. It is also apparent that without correction the serum enzyme activity determined immediately after perfusion is often only

TABLE IV

AVERAGE SERUM ENZYME LEVELS PRESENT IN THE HEART-LUNG MACHINE BEFORE AND AFTER PERFUSION

No. of Cases	Serum Enzyme Levels in the Heart-lung Machine	Average Oxygenator Serum Enzyme Levels			
		LDH (75-240)	HBD (60-120)	ICD (0.8-4.4)	GOT (4-20)
15	Before perfusion	157	80	0.3	10
	After perfusion	276	153	2.5	29

TABLE V

RELEVANT DATA FOR PATIENTS IN GROUP II FROM WHICH THE DILUTION FACTOR WAS CALCULATED FOR CORRECTED AND UNCORRECTED ENZYME LEVELS SHOWN IN FIGURES 4 TO 8

Case No.	Age and Sex	Diagnosis	Length of Perfusion (min.)	Patient's Blood Volume (l.)	Oxygenator Volume (l.)
1	44 Male	Subaortic stenosis	60	3.6	4.85
2	58 Male	Aortic valve replacement	132	4.54	4.5
3	39 Male	Aortic valve replacement	80	5.06	4.4
4	53 Male	Aortic valve replacement	93	5.12	4.75
5	13 Female	Fallot's tetralogy	83	3.3	3.45

TABLE VI

Case No.	Age and Sex	Diagnosis	Perfusion Time (min.)	Blood Specimen	LDH		HBD		ICD		GOT	
					1	2	1	2	1	2	1	2
5	13 Female	Fallot's tetralogy	83	Coronary sinus	305	382	66	169	1.1	2.2	—	84
				Inferior vena cava	309	369	84	138	0.65	2.2	33	89
				Oxygenator	300	320	68	103	0.60	1.7	30	70
				Coronary sinus	230	290	136	151	1.3	1.1	28	34
6	29 Male	Ventricular septal defect	83	Inferior vena cava	175	247	105	142	1.2	2.1	22	33
				Oxygenator	180	190	110	115	1.1	1.1	20	28
				Coronary sinus	319	279	123	178	1.1	1.5	16	18
				Inferior vena cava	329	309	175	161	1.1	1.9	15	16
7	15 Female	Ventricular septal defect	39	Oxygenator	300	280	140	140	1.0	1.2	14	15
				Coronary sinus	320	369	86	124	1.1	2.1	20	30
				Inferior vena cava	278	369	82	107	1.3	1.9	15	23
				Oxygenator	260	310	75	90	1.0	1.5	15	20
8	14 Female	Ventricular septal defect	47	Coronary sinus	285	310	115	147	1.9	2.7	25	41
				Inferior vena cava	295	275	110	130	1.4	3.5	21	27
				Oxygenator	280	285	100	118	1.3	2.4	20	24
9	10 Male	Ventricular septal defect	55									

L D H

FIG. 3A.

H B D

FIG. 3B.

CASE 1

FIG. 4A.

CASE 1

FIG. 4B.

FIGS. 4-8. Enzyme levels during perfusion, with and without correction for priming volume of oxygenator. Normal ranges hatched.

slightly increased above the pre-operative level, whereas when the dilution is corrected for, these results become significantly raised by 30 to 50%.

In Table VI, the results are shown of the study which attempted to see if myocardial damage could be reflected in the serum enzyme activity of blood taken from the coronary sinus as opposed to either the inferior vena cava or the oxygenator. Only with HBD are there any consistent differences between the coronary sinus and inferior vena cava samples. All five patients have higher HBD activity in the coronary sinus sample at the end of perfusion.

In the one case in which the hepatic vein entered the right atrium directly, the post-operative ICD levels exceeded normal, and therefore if the liver were the main source of ICD one would expect to observe a higher enzyme activity in the specimen

from the hepatic vein. In actual fact the ICD activity was higher in the coronary sinus samples.

One significant point to emerge from this particular study is that in most cases the enzyme activities at the end of perfusion were higher in the samples from the patient than from the oxygenator. This indicates that the main increase in enzyme activity is due to the release of cellular enzymes from the patient's tissues and thus emphasizes the importance of considering the dilution factor when assessing damage to the patient during perfusion.

DISCUSSION

The results in group I show that the length of perfusion plays a major role in determining the amount of increased enzyme activity following

CASE 3

FIG. 6.

CASE 4

FIG. 7.

cardiac surgery with perfusion, the mean post-operative peak levels of LDH, HBD, ICD, and GOT being significantly higher in the group perfused for over one hour. An effect due to age or ventriculotomy could be established only in the group perfused for over one hour. The mean peak levels of LDH, HBD, and GOT were then seen to be slightly higher in cases having ventriculotomy, and although both adults and children had higher enzyme activities when perfused for over an hour, the levels for children were increased above the normal limits by approximately 30% more than those for adults.

Hepatocellular damage was not considered to be a major source of increased enzyme activity, and the

increased ICD activity could not be attributed solely to liver damage, as was shown by the one case in which there was abnormality of the hepatic veins. Campbell and Moss (1962) have shown that in the heart the predominating fraction of ICD is extremely labile whereas that of liver ICD is more stable; this could account for the difficulty in demonstrating increased ICD activity following myocardial infarction. In all our cases perfusion was carried out under mild hypothermia, so that ICD being released from the myocardium during perfusion would be more likely to be detected.

The results in group I demonstrate the importance of the duration of the perfusion period in influencing

CASE 5

FIG. 8.

the post-perfusion serum enzyme levels; therefore, in group II this period was studied in greater detail. The activities of all the enzymes studied increased during perfusion, and when corrected for dilution the majority of them exceeded the normal limits by the end of perfusion. Dilution by the oxygenator priming volume conceals this rapid rise in enzyme levels taking place during perfusion; the corrected levels are some 30 to 50% higher than the measured values, and emphasize the importance of perfusion as the factor which causes enzyme release from the tissues.

When the enzyme activity remaining in the oxygenator after perfusion was compared with the

pre-perfusion level, a marked increase was observed. Undoubtedly some of this came from haemolysis produced by the pump, but by choosing cases to follow with little or no haemolysis at the end of perfusion, this factor could be ignored, and the increased enzyme activity in the oxygenator at the end of perfusion could be attributed mainly to release of enzymes from the patient's tissues. When bleeding extensive in the immediate post-perfusion period it is extremely difficult to assess the actual increases in enzyme activity in the patient as the blood volume kept constant by replacement of losses with whole blood or plasma; thus, blood with elevated enzyme activity is being replaced by blood with a low

enzyme activity. The amount of enzyme activity lost cannot be accurately accounted for because the blood lost is removed by high-pressure suction which haemolyses it severely.

The results of other workers in this field are extremely diverse. Quinn *et al.* (1960), Snyder *et al.* (1958), and Fraser *et al.* (1962) demonstrated higher peak GOT levels in cases with ventriculotomy. Norberg and Senning (1959) noted increases of HBD as well as of GOT following ventriculotomy. In group I of this study, although the highest single HBD, LDH, and GOT levels occurred in patients with a ventricular incision, no correlation could be drawn in general between the mean peak enzyme levels and the degree of surgical trauma. This is corroborated by Baer and Blount (1960), who analysed their results statistically and could show no significant differences between Fallot operations, ventricular septal defects approached via the ventricle, and ventricular septal defects with an atrio-ventricularis communis defect approached via the auricle. Walker and Morgan (1964) also were unable to demonstrate significant differences in the peak GOT levels between cases with and without ventriculotomy. If an increase in GOT were related to the extent of surgical trauma then one would expect to demonstrate higher GOT levels with thoracotomies than with laparotomies. Neither our results nor those of Pyörälä *et al.* (1963), Quinn *et al.* (1960), and Werle *et al.* (1961) showed a difference between them.

Nyhus (1958) states that in his experiments the isolated liver tolerated perfusion poorly, developing severe oedema. Snyder *et al.* (1958) suggest that extracorporeal circulation might subject the liver to reduced oxygenation, or cause sufficient congestion to produce permeability changes in the cell membrane and allow enzymes to leak out. If this were a major source of increased enzymes, GPT would be expected to increase. We could not demonstrate significant changes in GPT, in agreement with the experience of Walker and Morgan (1964), Baer and Blount (1960), Pyörälä *et al.* (1963), and Norberg and Senning (1959). The last authors also measured serum ornithine carbamyl transferase, which is a more sensitive index of liver damage than the transaminases, and were unable to demonstrate significant changes in its activity post-operatively. Bang, Iversen, Jagt, and Tobiassen (1959) showed that GOT increases could come from centrilobular necrosis following acute heart failure superimposed upon chronic heart failure, but Baer and Blount (1960) were unable to show histological evidence of centrilobular necrosis in the livers of patients who died in the immediate post-operative period. They suggest that the chemical changes may precede the

anatomical changes and agree with the concept of 'acute syndrome' as put forward by Hauss and Gerlach (1958), which regards the release of enzyme as due to an overall response of the organism rather than to a single factor. Pyörälä *et al.* (1963) and Werle *et al.* (1961) also support this idea.

Baer and Blount (1960) observed a positive correlation between the post-operative enzyme levels and the duration of cardiopulmonary bypass. They found that the GOT levels were significantly higher in cases perfused for over 40 minutes. Fraser *et al.* (1962) and Pyörälä *et al.* (1963) were able to correlate post-operative enzyme activity with the length of perfusion only in cases having ventriculotomy. Snyder *et al.* (1958), Quinn *et al.* (1960), and Werle *et al.* (1961) suggest that no correlation exists between the post-operative enzyme levels and the duration of cardiopulmonary bypass. The failure of other workers to demonstrate such a correlation could be accounted for by the fact that the few investigators who studied the perfusion period did not consider the dilution from the priming fluid of the heart-lung machine to be relevant.

Other factors have been considered as possible causes for increasing enzyme activities. Lawrence and Schulkins (1956) found that the type of anaesthetic did not influence the post-operative GOT levels following surgical operations; March, Greenberg, and Rinehart (1954) demonstrated that haemolysis increased the enzyme levels; Crafoord, Norberg, and Senning (1957) found that, with seven to 59 minutes on bypass, the haemolysis values did not exceed 15 to 55 mg. haemoglobin/100 ml. of plasma, while Baer and Blount (1960) showed that 80 to 100 mg. per 100 ml. of free haemoglobin in haemolysed samples did not significantly alter GOT levels. Fraser *et al.* (1962) found that the post-operative GOT levels in children were considerably higher than in adults. Werle *et al.* (1961) were unable to correlate the increased enzyme activity with any of the following factors: type of operation, length of perfusion, hypothermia, type of anaesthetic, or age.

When these results are considered together it appears that the increased enzyme activities following cardiac surgery with extracorporeal circulation reflect the response of the whole organism to the perfusion. Increased activities do occur when cardiac surgery is performed without perfusion but to a lesser extent. The difference is even more impressive if corrections are applied for the dilution from the priming fluid of the heart-lung machine. The raised enzyme activity probably results from an increased permeability of cellular membranes, thus allowing the intracellular contents to enter the circulation. These changes could result from any of the following: variation in pH, lowered substrate levels, decreased

oxidative phosphorylation, anoxia occurring from decreased peripheral circulation and exclusion of certain vessels due to the reversed blood flow during perfusion, and collapse of small vessels from hypothermia.

In assessing our results we feel that although the release of cellular enzymes cannot be attributed to a single source a large proportion of them are coming from myocardial tissue. This can be seen from examining the HBD results for the patients perfused for over one hour in group I (Fig. 3). The mean level of HBD was still raised above normal after two weeks whereas the other three enzymes studied were generally within normal limits after one week. As increased HBD activity is indicative of myocardial damage, this can be taken as a sign that the heart is not fully recovered and is still releasing enzymes into the circulation from damaged cells.

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