Changes in Peripheral Serum Creatine Phosphokinase (CPK) and Lactic Dehydrogenase (LDH) in Acute Experimental Colonic Infarction

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No satisfactory laboratory test for the early diagnosis of bowel infarction exists at this time. We have delineated changes in serum CPK levels after acute superior mesenteric artery infarction; whether or not comparable changes occur with inferior mesenteric artery infarction has not yet been determined. Furthermore, the changes in LDH associated with acute bowel infarction have not been documented. To determine the changes in serum CPK and LDH in acute colonic infarction, laparotomies were performed on dogs after peripheral baseline blood samples were drawn and each subject was randomly placed in one of three groups: laparotomy alone, acute colonic obstruction, and acute colonic infarction by ligation of the inferior mesenteric artery. The marginal artery of the colon was ligated at the peritoneal reflection and at the cecum to interrupt arterial collaterals. Blood samples were taken from each subject at intervals of three hours for 48 hours after injury. Serum from each sample was analyzed for total CPK and LDH by automated spectrophotometry. Isoenzymes were determined by agarose gel electrophoresis. Necropsies were conducted on all the dogs to confirm that the intended condition had been produced and that no intercurrent disease was present. The data support the conclusion that total CPK, total LDH and their isoenzymes become elevated in the peripheral serum after colonic infarction. The maximal elevations were all seen within the first 12 hours after acute colonic infarction. Total LDH and LDH_3, the most prevalent isoenzyme of LDH in bowel, do not become elevated in the serum to as high a level as CPK, but the combination of serum elevations in both enzyme systems may prove to be of diagnostic significance.

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ALTHOUGH ACUTE COLONIC INFARCTION occurs in only 1-2% of major aortic reconstructions, the mortality rate remains very high.[1] One of the factors which contributes to the high mortality rate is the fact that there is no satisfactory laboratory test for the early diagnosis of colon infarction. Serial measurement of serum enzyme systems could offer clues to the extent and severity of colonic injury. Two enzymes which could fulfill this role are creatine phosphokinase (CPK) and lactic dehydrogenase (LDH). It has been demonstrated in previous experiments that serum CPK and its isoenzymes change with experimental superior mesenteric arterial infarction.[2,3] Based on two clinical case reports, analogous changes appear to occur with isolated colonic infarction.[4,5] Little has been written concerning the possible changes in serum LDH and its isoenzymes subsequent to large bowel infarction, but this serum enzyme system should be studied for two reasons: cellular destruction (*e.g.*, myocardial infarction) can cause shifts in the LDH serum isoenzyme pattern[6] and LDH_3, the isoenzyme of LDH reported to be most common in bowel, is only the third most common LDH isoenzyme in normal peripheral human serum.[6]

The following experiments were conducted to delineate the changes in the two serum enzyme systems noticed secondary to experimental arterial colonic infarction in the dog. In the first experiment, the dog was compared with man to ascertain the distribution of LDH and its isoenzymes in small and large bowel, thereby establishing the dog as an appropriate model in which to study serum LDH changes associated with colonic infarction. Similar studies have been conducted

In conducting the research decribed in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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previously for CPK in our laboratory.[3] Data presented from the subsequent studies reported here further expand the similarity between the distribution of CPK in the bowel of the dog and the bowel of man. In the second experiment, the changes found in peripheral serum CPK and LDH and their respective isoenzymes in dogs experiencing acute arterial colonic infarction have been compared with the changes seen in these enzyme systems in the peripheral sera of a group of dogs which had only laparotomies and to a group of dogs which had received large bowel obstructions.

Methods

In both experiments total enzyme activity for CPK and LDH was determined by automated spectrophotometry using a Centrifichem autoanalyzer (Union Carbide, Rye, NY).[7,8] Reagents and controls specific for this system were used throughout all analyses. Isoenzymes of CPK and LDH were determined by agarose gel electrophoresis using the Corning ACI® system equipped with a Corning 720 densitometer and a 722 data terminal.[9-13] Corning reagents and controls specific for this system were used for all electrophoretic analyses.

In the first experiment, full thickness samples of canine and human bowel wall were assayed for total activity of each of the enzymes. The isoenzyme distributions were determined for both CPK and LDH in the samples of bowel taken from both man and dog. Comparisons of LDH in the bowel wall and in the serum were conducted only for LDH, since a similar analysis for CPK has been reported previously.[3] Bowel samples were obtained by resecting full thickness specimens at the following anatomic sites: the second portion of the duodenum, the jejunum 20 cm distal to the ligament of Treitz, the ileum 20 cm proximal to the ileocecal valve, the middle portion of the ascending colon, and the middle region of the descending colon. All samples were cleaned mechanically of fecal material and washed in three successive separate baths of Ringer's lactate. Each one was placed individually in a fresh vial filled with Ringer's lactate and tranported to the laboratory. Small blocks of bowel, which contained all layers of bowel wall but no mesentery, were then weighed (±0.01 g) on a Sartorius type 2662 balance (Sartorius Werks, GMBM, Gottingen, Germany). Each sample was placed in a solution of 10 cc of Ringer's lactate per gram of sample and homogenized for one minute in a Virtis Model 23 homogenizer (Virtis Research Equipment, Gardener, NY). The homogenate was centrifuged for five minutes at 3000 RPM in a standard centrifuge. After the supernatants had been extracted, total enzyme activity and isoenzyme distribution for both enzyme systems were determined by the methods described previously.[3]

Canine bowel and serum samples were obtained from healthy animals (N = 12) placed under general endotracheal anesthesia induced by intravenous sodium pentobarbital 18 mg/kg accompanied by 0.4 mg/kg of atropine. After induction, satisfactory anesthesia was maintained with 1% Halothane®, 57% oxygen, and 42% nitrous oxide. After samples had been taken the animals were killed with overdoses of intravenous barbiturate. Complete necropsies were conducted to confirm the health of the animals.

Samples from human bowel were obtained at autopsy examination from patients (N = 9) who had died of disease processes which did not involve the gastrointestinal tract. Specimens were obtained only from fresh cadavers wherein death had preceeded the sample collection by less than 24 hours. Samples were also obtained from fresh surgical specimens, as soon as they had been resected. In surgical cases, samples were taken only from areas of the resected bowel which were not involved with the disease process by gross examination conducted by the responsible pathologist.

Serum samples were obtained only from healthy dogs who had no known disease. Human samples were obtained from sera collected as part of the routine evaluation of patients being prepared for elective surgery. All blood samples were separated by centrifugation at 3000 RPM for ten minutes. Sera were extracted by standard pipettes and were analyzed by the methods described previously.

Each parameter was tabulated for dog and man. Each parameter measured was evaluated for significant differences between dog and man by using Student's unpaired t-test.

In the second experiment, 13 healthy canines were selected and placed under general endotracheal anesthesia using the same techniques described in the initial experiment. Using sterile technique, laparotomies were performed after baseline blood samples had been obtained from a central venous catheter placed through an external jugular cutdown. The dogs were then assigned randomly to one of three groups. The laparotomy group (N = 4) was closed after exploration. In the obstruction group (N = 5), an acute mechanical obstruction was achieved by tying an umbilical tape around the colon just above the peritoneal reflection. The dogs were then closed in the same manner as those in the laparotomy group. The third group of dogs had complete arterial infarction of the colon from the peritoneal reflection to the cecum. In these dogs the inferior mesenteric artery was divided and ligated, and the marginal artery of the colon was divided and ligated at the peritoneal reflection and at the ileocecal valve. This inter-

TABLE 1. *The Distribution of CPK in Bowel*

		Total CPK (IU/g* ± SEM)	CPK Isoenzymes (Per Cent ± SEM†) MM	MB	BB
Small Bowel					
duodenum	(H)‡	90.2 ± 15.0	32 ± 3	36 ± 1	32 ± 3
	(D)§	57.4 ± 18.3	28 ± 3	30 ± 3	42 ± 3
jejunum	(H)	67.6 ± 14.6	27 ± 3	36 ± 3	37 ± 3
	(D)	47.1 ± 10.1	21 ± 3	32 ± 3	46 ± 5
ileum	(H)	81.0 ± 14.3	23 ± 4	31 ± 4	46 ± 7
	(D)	59.6 ± 10.7	24 ± 2	34 ± 2	42 ± 3
Colon					
ascending	(H)	93.1 ± 19.8	30 ± 3	33 ± 3	37 ± 4
proximal	(D)	54.4 ± 8.7	24 ± 2	35 ± 2	39 ± 2
descending	(H)	77.1 ± 8.9	24 ± 4	35 ± 3	41 ± 7
distal	(D)	52.9 ± 8.4	24 ± 3	37 ± 2	37 ± 3

* IU/g: International Units/gram.
† SEM: Standard error of the mean.
‡ H: Human.
§ D: Dog.

rupted the arterial blood supply of the colon from the ileocecal valve to the peritoneal reflection. In all four dogs in the infarction group no arterial pulses were present in the affected mesentery. The abdomens of these dogs were then closed in the same manner as the other two groups.

After surgery the dogs were allowed to recover before intravenous therapy was terminated. Blood samples were obtained at three, six, nine, 12, 24, 27, 30, 33, and 48 hours after the surgical procedure in each animal. The blood samples were separated by centrifugation at 3000 RPM for ten minutes. The serum samples obtained were assayed for the enzymes by the methods described previously. Each parameter was tested for statistically significant differences between the three groups by the Wilcoxon rank sum test.[14]

After the last blood sample was drawn, the dogs were killed by overdoses of intravenous barbiturates. Complete necropsies were performed by an independent pathologist to confirm that only the desired injury had

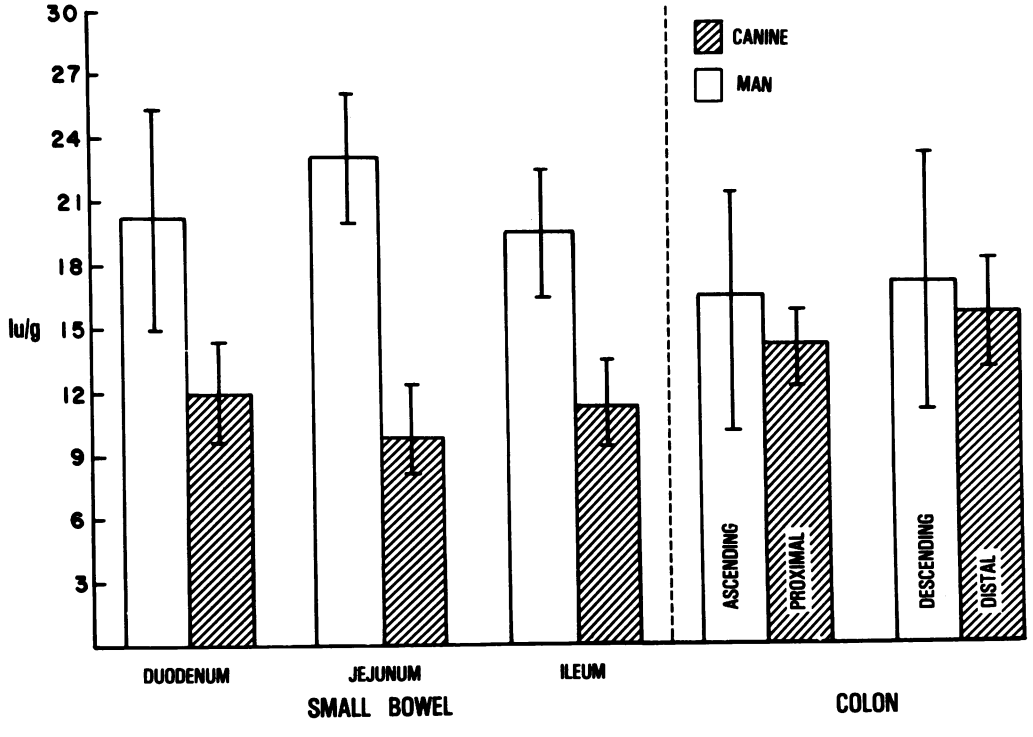

FIG. 1. Total LDH in the bowel of humans and dogs. Bars represent standard errors of the mean. Although the values recorded for the small bowel appear to be different, no statistically significant difference was found.

TABLE 2. *The Distribution of LDH in Bowel*

		Total LDH (IU/g* ± SEM)	LDH Isoenzymes (Per Cent ± SEM†)				
			1	2	3	4	5
Small Bowel							
duodenum	(H)‡	20.4 ± 5.3	16 ± 2	23 ± 1	24 ± 2	19 ± 2	19 ± 1
	(D)§	12.0 ± 2.5	18 ± 2	24 ± 2	22 ± 1	18 ± 2	18 ± 2
jejunum	(H)	22.9 ± 2.8	17 ± 4	20 ± 2	23 ± 1	18 ± 2	22 ± 2
	(D)	10.6 ± 1.9	18 ± 3	21 ± 1	20 ± 2	19 ± 1	21 ± 1
ileum	(H)	19.7 ± 3.8	14 ± 2	21 ± 4	24 ± 1	20 ± 3	21 ± 2
	(D)	11.4 ± 1.9	16 ± 1	23 ± 1	21 ± 1	19 ± 1	20 ± 1
Colon							
ascending	(H)	16.3 ± 5.1	18 ± 4	25 ± 2	23 ± 4	16 ± 2	18 ± 4
proximal	(D)	14.0 ± 1.7	18 ± 1	24 ± 1	21 ± 1	18 ± 1	19 ± 2
descending	(H)	17.5 ± 5.9	16 ± 2	24 ± 1	23 ± 3	18 ± 2	19 ± 4
distal	(D)	15.9 ± 2.6	19 ± 1	23 ± 1	21 ± 1	17 ± 1	20 ± 1

* IU/g: International Units/gram.
† SEM: Standard error of the mean.
‡H: Human.
§D: Dog.

occurred and that no significant intercurrent disease was present.

Results

In the first experiment, all three isoenzymes of CPK were found throughout the small and large bowel. No significant difference was found in the total or isoenzyme distributions of CPK between man and dog (Table 1). CPK-BB was the most prevalent isoenzymes while CPK-MM was least prevalent.

Analysis of total LDH and its isoenzyme distribution in the bowel samples showed that the dog and man were comparable (Fig. 1 and Table 2). Although total LDH appeared to be greater in human small bowel, calculations revealed that statistically significant differences did not exist. All five isoenzymes of LDH were present in all small and large bowel samples assayed for both man and dog. LDH_2 and LDH_3 were the most prevalent isoenzymes. Analysis of serum LDH for both man and dog showed that total LDH activity was comparable (Table 3). The isoenzyme distribution, however, was different. LDH_2 was most common in human serum and was followed in descending order by LDH_1, LDH_3, LDH_4 and LDH_5. In the dog, serum LDH_5 was the most common isoenzyme, while LDH_1 and LDH_3 were the next most common isoenzymes with essentially equal concentrations. LDH_2 and LDH_4 were the least prevalent isoenzymes in canine serum. The concentrations of LDH_3 found in normal serum and in bowel wall were quite similar for both man and dog.

In the second experiment, total serum CPK started at the same level for each of the three groups of dogs (Fig. 2) but those who received inferior mesenteric infarction experienced significantly greater increases by six hours after infarction than did the dogs having laparotomies or obstructions ($p < 0.001$). This elevation in total CPK was greatest at 12 hours and declined throughout the rest of the 48-hour study period.

The isoenzyme with the highest concentration in this elevation was CPK-MM (Fig. 3). When considering CPK-MM for all three groups of dogs in the second experiment, the levels for the initial analysis for each group were quite analogous to those for the other two groups. In the dogs who had acute colonic infarctions, serum CPK-MM rose quickly and reached its peak at 12 hours after injury. The laparotomy and the obstruction groups reached their maximal elevations by 12 hours after surgery as well and declined thereafter, but the elevations seen in the sera of the dogs having re-

TABLE 3. *Normal Serum Values of LDH in Man and Dog*

	Total LDH (IU/L* ± SEM)	LDH Isoenzymes (Per Cent ± SEM†)				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Man (n = 20)	77.8 ± 3.7	24.3 ± 0.6	31.5 ± 0.6	21.9 ± 0.3	12.2 ± 0.4	11.5 ± 0.7
Dog (n = 15)	65.7 ± 15.1	21.2 ± 2.6	15.7 ± 1.7	20.8 ± 1.6	15.5 ± 1.0	27.2 ± 4.4

* IU/L: International Units/L.
† SEM: Standard error of the mean.

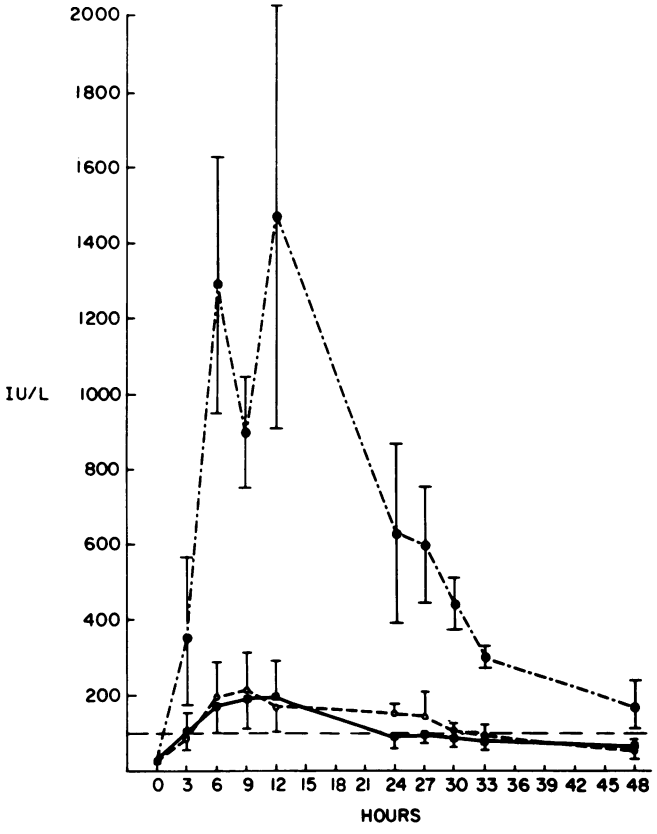

FIG. 2. Serum levels of total CPK in each of the three groups of dogs in the second experiment. Bars represent standard errors of the mean. ●: Infarction (N = 4). ■: Obstruction (N = 5). O: Laparotomy (N = 4).

ceived colonic infarctions were significantly greater ($p < 0.001$) than those recorded for the other two groups.

Serum CPK-MB rose most in the group of dogs with the experimental colonic infarctions (Fig. 4). The difference between the experimental infarction group and the other two groups is significant ($p < 0.001$), and is qualitatively different in that a second elevation appears between 24 and 33 hours. The maximal serum elevation of CPK-MB was at 12 hours.

CPK-BB became elevated in the peripheral serum by three hours after colonic infarction and reached its maximal value by six hours (Fig. 5). It then declined until it reached preoperative levels at the 30 hour samples. Comparison of this graph for the animals with infarctions with the graphs for the dogs having obstructions or laparotomies once again shows a significant difference ($p < 0.001$).

Figure 6 shows the changes in serum total LDH seen in all three groups of dogs. Total LDH increases most in the infarction group between six and nine hours after infarction but the values recorded for this group are only slightly above the upper limits of normal (110 IU/L). The difference between the infarction group and the laparotomy and obstruction group was significant ($p < 0.001$) for the first 24 hours after injury.

Figure 7 shows that serum LDH_3 had the same type of rise as serum total LDH. The values recored for the infarction group were significantly different from those for the obstruction or laparotomy groups ($p < 0.001$) for the first 24 hours after surgery. Thereafter, the groups were indistinguishable.

Figure 8 shows the percentage of the total LDH which was LDH_3 for all three groups. The percent LDH_3 was highest in the colonic infarction group in general, but the difference was not statistically significant.

Necropsies on all the dogs showed that no intercurrent disease existed. The only infarcted bowel was found in the dogs who had received colonic infarctions. Each dog in the infarction group had complete full thickness necrosis of the colon from the peritoneal reflection to the cecum. No evidence of myocardial injury was found in any of the subjects receiving colonic infarctions.

Discussion

Analysis of CPK in bowel and serum showed that the levels and distribution in dog and man are similar. The dog, therefore, is an adequate model in which to study any changes in these enzymes in the peripheral serum caused by acute colonic infarction. Previous experi-

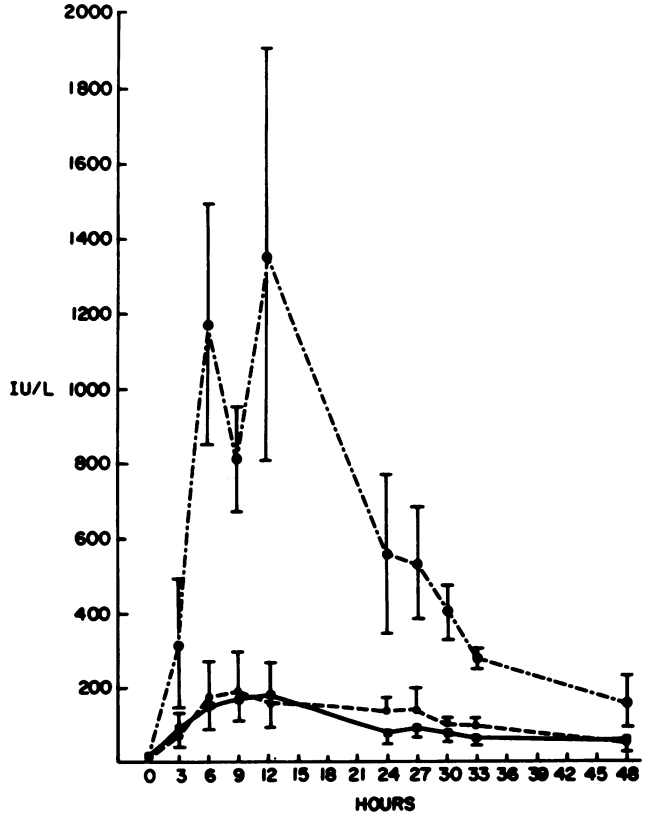

FIG. 3. Serum levels of CPK-MM in each of the three groups in the second experiment. Bars represent standard errors of the mean. ●: Infarction. ■: Obstruction. O: Laparotomy.

ments reported have shown that CPK-MB and CPK-BB are not present in normal human serum in quantities detectable by electrophoresis.[3] The presence of equal concentrations of all three CPK isoenzymes in bowel wall allows detection of infarction by identification of CPK-MB, and particularly, CPK-BB in the peripheral serum.[2,3] The material presented here on CPK in the colon suggests that CPK-MB and CPK-BB may have the same potential for being serum markers of early colonic infarction.

The analysis of LDH and its isoenzymes in bowel wall in both man and dog show that this enzyme system does not have the same potential as CPK for identifying mesenteric infarction. The data confirm that the concentrations of LDH and its isoenzymes in bowel wall of dogs and humans are comparable. LDH_3 and LDH_2 are the most prevalent enzymes in bowel in both man and dog but there is no difference between the concentration of these enzymes in bowel wall and the concentrations found in normal serum. All of the isoenzymes of LDH are detected in bowel wall and serum by agarose gel electrophoresis in both man and dog. Hence, a situation which is analogous to the situation found for CPK-MB and CPK-BB does not exist for LDH.

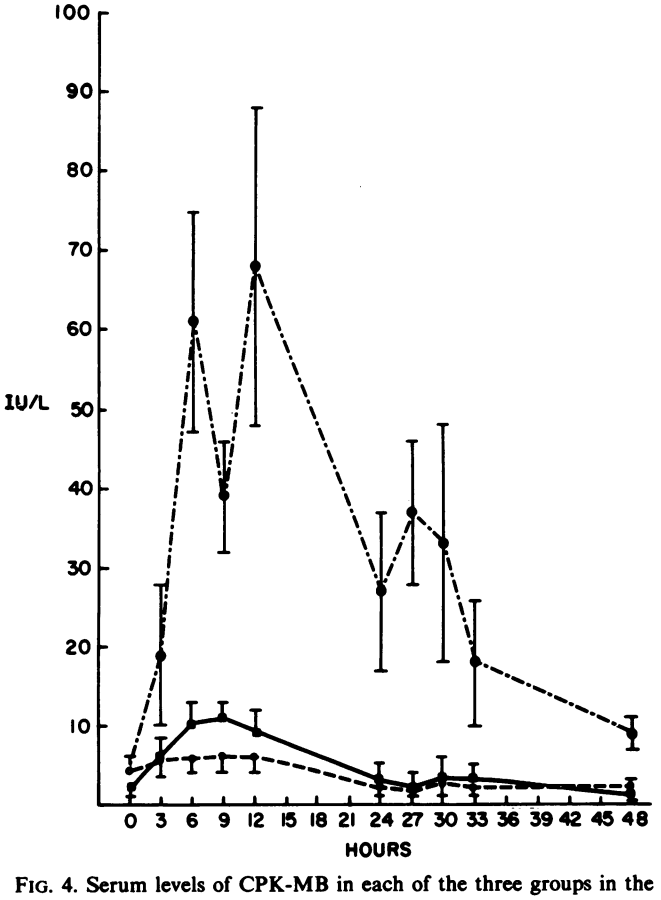

FIG. 4. Serum levels of CPK-MB in each of the three groups in the second experiment. Bars represent standard errors of the mean. Note that the vertical scale is expanded when compared with the scale in the preceeding two graphs. ●: Infarction. ■: Obstruction. O: Laparotomy.

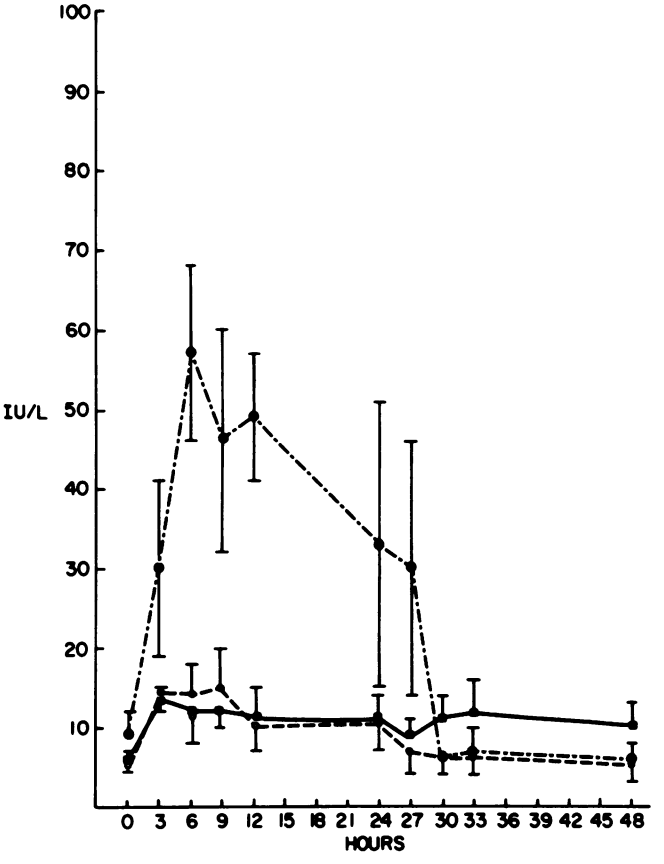

FIG. 5. Levels of serum CPK-BB in each of the three groups of dogs in the second study. Bars represent standard errors of the mean. The vertical scale is the same as for the previous graph. ●: Infarction. ■: Obstruction. O: Laparotomy.

The changes seen in peripheral serum CPK for experimental colonic infarction show both similarities and some differences with the changes reported previously to be associated with experimental infarction of the superior mesenteric system.[3] In both, total serum CPK becomes elevated and reaches its maximum value within 12 hours after the infarction.[3] The major component of the elevation is due to increases in CPK-MM. In both superior mesenteric infarction and inferior mesenteric (colonic) infarction, CPK-MM accounts for approximately 90% of the elevation in CPK seen in peripheral serum (Figs. 2-5).[3] CPK-BB has the earliest significant elevation in peripheral serum of the three isoenzymes of CPK. This elevation remains significant throughout the first 24 hours after infarction (Fig. 5).[3] Slight differences exist between the changes seen with peripheral serum CPK-MB when comparison is made between the results for the superior mesenteric infarction and those for inferior mesenteric infarction[3]. With superior mesenteric infarction, there were two distinct subgroups; those that suffered lethal infarction and those that survived throughout the 30-hour study period.[3] The dogs which died between 24 and 27 hours continued to have increasing serum CPK-MB values; those which survived for 30 hours had elevations only

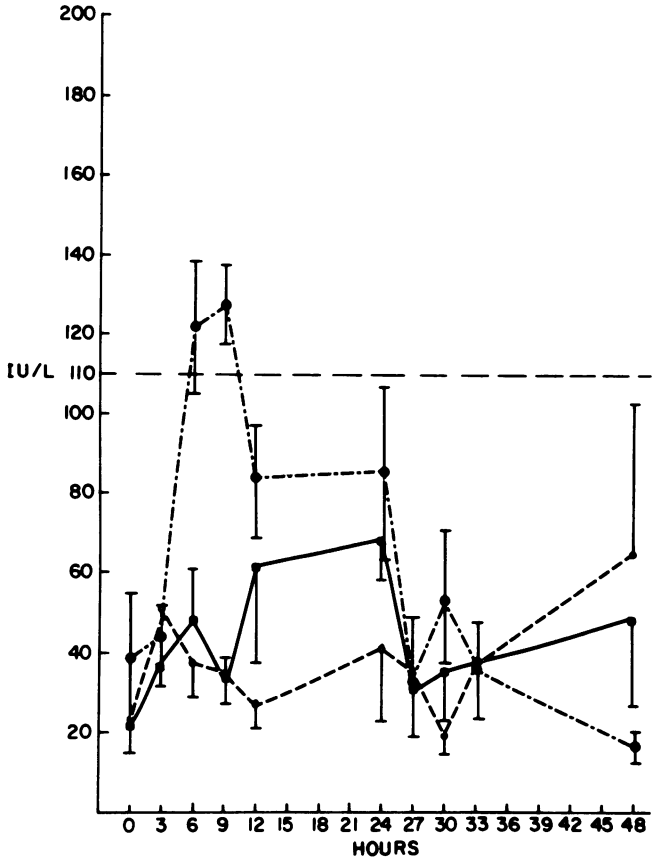

FIG. 6. This graph presents the values for total serum LDH in each of the groups of dogs in the second experiment. Bars represent standard errors of the mean. The broken line at 110 IU/L defines the upper limits of normal for our laboratory. ●: Infarction. ■: Obstruction. O: Laparotomy.

for the first 24 hours.[3] The findings in (colonic) inferior mesenteric infarction were different in that there was a second elevation of serum CPK-MB between 24 and 33 hours after the infarction. All of the subjects survived for 48 hours after inferior mesenteric infarction. The secondary rise in CPK-MB in this group was not due to cardiac disease, since none was found at necropsy in any of the dogs with colonic infarctions. Hence, the secondary elevation was probably due to the colonic infarction.

The significant changes recorded in total serum LDH in the colonic infarction group were not as great as the changes recorded for CPK. This finding was in accord with the results obtained from the analysis of bowel and serum in the first experiment. Review of Tables 1 and 2 and Figure 1 shows that there was approximately four and five times as much CPK as LDH per gram of bowel wall. Normal values for total serum CPK and LDH as defined for the systems used in the anslyses are comparable (CPK = 100 IU/L and LDH = 110 IU/L—Figs. 2 and 6).

LDH_3 was selected as the isoenzyme of LDH which might prove most helpful in detecting colonic infarction because previous work by another laboratory had noticed that this isoenzyme was the most common isoenzyme of LDH in bowel.[6] The results from the first experiment suggest that LDH_3 and LDH_2 are the most common isoenzymes in human and dog bowel, but neither one of them achieves a clear dominance. Analysis of LDH_3 in the peripheral serum of the dogs in each of the three groups in the second experiment showed that LDH_3 did have a significant increase in the serum of the dogs with experimental colonic infarctions but the elevation of this isoenzyme was not striking or as sustained as the changes in the CPK isoenzyme system (Fig. 7). In looking at LDH_3 as a per cent of total LDH (Fig. 8), per cent LDH_3 was highest in the group of dogs with infarctions but it did not achieve a significant difference when compared with the values recorded for the other two groups of dogs.

In summary, the material presented shows that peripheral serum total CPK changes in experimental (colonic) inferior mesenteric infarction. The changes are analogous to those recorded after experimental superior mesenteric artery infarction.[3] Concomitant analysis of

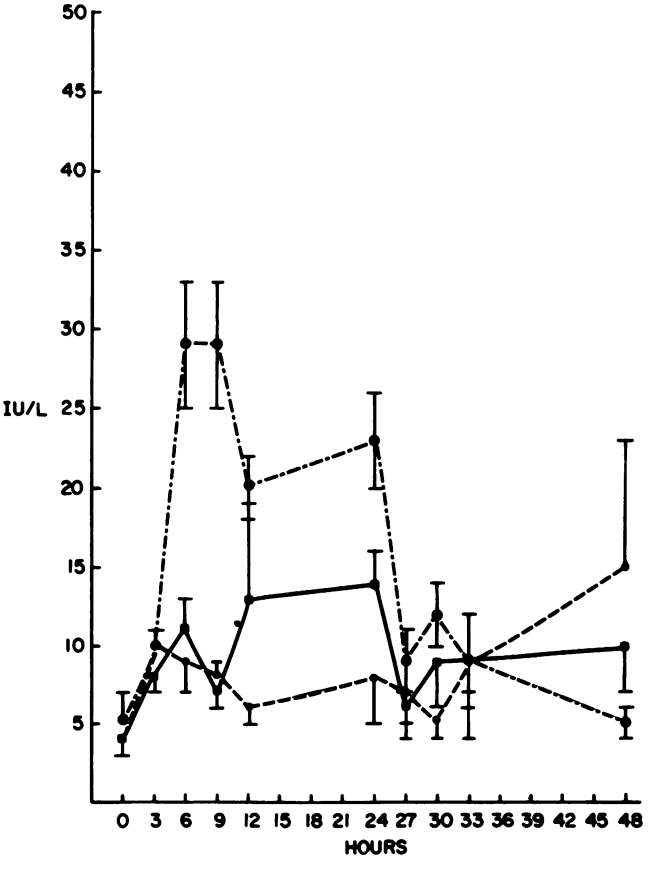

FIG. 7. This graph presents the values found for LDH_3 in each of the three groups of dogs in the second experiment. Bars represent standard errors of the mean. ●: Infarction. ■: Obstruction. O: Laparotomy.

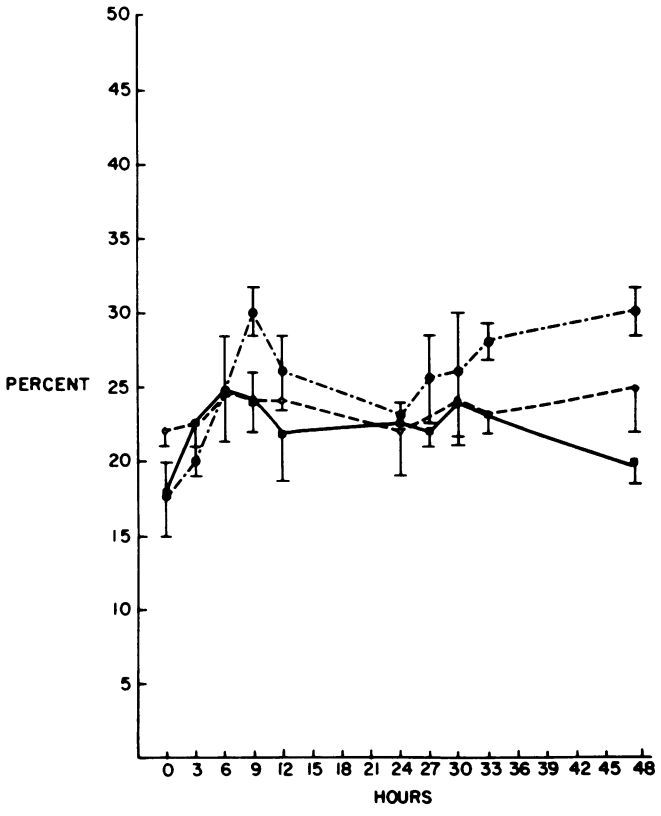

FIG. 8. This figure presents the percentage of total LDH which was represented by LDH_3 in each of the three groups of dogs in the second experiment. Bars represent standard errors of the mean. ●: Infarction. ■: Obstruction. ○: Laparotomy.

peripheral serum total LDH demonstrates that total LDH and LDH_3, one of the most common isoenzymes of LDH in bowel, become significantly elevated in peripheral serum after colonic infarction, however, the elevations seen are not as great or as sustained as those recorded for CPK. These data suggest that CPK will be more helpful in evaluating superior mesenteric infarction or infarction of the colon after major arterial reconstruction. The major benefit of knowing the changes in serum LDH associated with bowel injury is that it will further enable the clinician, theoretically, to confirm that the infarcted tissue is in the gastrointestinal tract. Myocardial infarction causes increases in total CPK, CPK-MM, and CPK-MB in the peripheral serum similar to those seen in mesenteric infarction.[6] Myocardial infarction also causes marked elevations in the peripheral serum of total LDH with a predominance of the LDH_1 isoenzyme.[6] Evaluation of peripheral serum in patients with mesenteric infarction should show elevations of total CPK with the presence of CPK-BB bands within 24 hours of the infarction. Concomitant elevations in LDH_3 rather than LDH_1, should further suggest the diagnosis of mesenteric infarction rather than myocardial infarction. Studies to elucidate these changes in the serum of patients suffering both mesenteric infarction and myocardial infarction are currently underway.

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