Changes in Serum Total Creatine Phosphokinase (CPK) and its Isoenzymes Caused by Experimental Ligation of the Superior Mesenteric Artery

GEOFFREY M. GRAEBER, M.D., MAJ., M.C., PATRICK J. CAFFERTY, SP4, USA, MICHAEL J. REARDON, PH.D., MAJ., V.C., CLAUDIA P. CURLEY, SP4, USA, NORMAN B. ACKERMAN, M.D., PH.D., F.A.C.S., JOHN W. HARMON, M.D., F.A.C.S., LT.C., M.C.

From the Divisions of Surgery and Veterinary Pathology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.; the Department of Surgery, the Uniformed Services University of the Health Sciences, Bethesda, Maryland; and the Department of Surgery, SUNY Upstate Medical Center, Syracuse, New York

The changes in serum total CPK and its isoenzymes have not been delineated in acute mesenteric infarction. As measurement of serum CPK levels could conceivably be a useful diagnostic test for bowel infarction, this experiment was performed to assess changes in serum CPK levels in bowel infarction in dogs, using sham operation and talc peritonitis as controls. Laparotomies were performed in 20 dogs, and each was assigned randomly to one of three groups: those having laparotomy (LAP), talc peritonitis (PER), and superior mesenteric artery infarction (MAI). Mixed venous blood samples were obtained from all subjects for 30 hours after surgery. All animals were killed, and complete autopsies were performed. Confirmation of infarction and determination of its extent were obtained through both gross and microscopic examination of the gut in canines subjected to arterial infarction. Total serum CPK levels were determined by spectrophotometric analysis. Agarose gel electrophoresis was used to determine the levels of each of the isoenzymes. Significant elevations of CPK and CPK-MM occurred nine hours after injury. CPK-BB reached maximum elevation by six hours, while CPK-MB did not reach its maximum until 24 hours after injury. From data in the study we conclude that total CPK and its isoenzymes become elevated in the serum of canines subjected to experimental superior mesenteric artery infarction. That CPK-BB elevations peak in the first 12 hours after injury and CPK-MB in the second 12 hours after injury may be of particular diagnostic significance.

THE MORTALITY RATE in acute mesenteric infarction due to arterial interruption has been reported at 85%.[1] One of the principal reasons for this very high mortality rate is that few diagnostic tests are specific for mesenteric infarction in its early stages.[1] A review of the literature revealed isolated case reports suggesting that serum CPK-BB might become elevated with intestinal necrosis.[2-4] Firm experimental data delineating the changes in serum CPK and its isoenzymes in relation to acute mesenteric infarction were not available. Hence, the following experiment was conducted to delineate these changes.

Reprint requests and correspondence: Geoffrey M. Graeber, M.D., MAJ., M.C., Division of Surgery, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Submitted for publication: October 7, 1980.

Methods

The first phase of the experiment was designed to evaluate the dog as a model. Samples were obtained from five segments of the bowel from human and canine subjects. Human samples were obtained at autopsy in conjunction with normal pathologic examination. In order to be included, the autopsy had to be conducted within 12 hours of death to assure relatively fresh samples for analysis. All autopsy subjects died of disease processes which did not affect the gastrointestinal tract. Samples were obtained from the second portion of the duodenum, the jejunum at 20 cm distal to the ligament of Treitz, the ileum 20 cm proximal to the ileocecal valve, the middle of the ascending colon, and the middle of the descending colon. All samples included full thickness of bowel wall. Each specimen was mechanically cleaned of fecal material and washed in three successive baths of Ringer's lactate. Each was transported to the laboratory immediately in a separate container filled with Ringer's lactate.

Canine samples were obtained from normal animals. Animals were excluded from the study if necropsy

0003-4932/81/0400/0499 $00.85

showed any acute disease process. Full-thickness samples of bowel wall were obtained from each of the three parts of the small bowel in a manner analogous to that conducted in the human subjects. Samples were taken from the proximal and descending colon in the dog to provide samples analogous to those from man. All specimens were washed in three successive baths of Ringer's lactate after being mechanically cleaned of fecal material and transported to the laboratory in individual containers filled with Ringer's lactate.

Upon arrival in the laboratory, 1 g samples of full-thickness bowel wall were excised. No mesentery was included in the sample. Weight of the sample for analysis was determined to ±0.01 g on a Sartorious type 2662 balance (Sartorius Werks, GMBM, Gottingen, Germany). Each 1 g sample was placed in 10 cc of fresh Ringer's lactate solution and homogenized for one minute in a Virtis model 23 homogenizer (Virtis Research Equipment, Gardener, New York 12525). The homogenate was centrifuged for five minutes at 3000 RPM in a standard centrifuge. Total enzyme activity was determined by spectrophotometric analysis using a Centrifichem® autoanalyzer (Union Carbide, Rye, NY) and standard Union Carbide reagents and controls.[5,6] Isoenzyme analysis was conducted with agarose gel electrophoresis using the Corning ACI® (Corning Medical, Medfield, MA) system equipped with standard reagents and controls specific for the Corning System.[7–10] Results were tabulated and plotted in graphic form. Levels of significance were evaluated for individual parameters using the Student's t-test.

Serum samples obtained from dogs and from man on a routine basis were used to determine normal levels of the enzymes and the isoenzymes. The human samples, obtained by standard venipuncture, were selected from the sera of healthy patients submitted for routine screening. Canine blood samples were drawn from healthy animals using standard venipuncture techniques. All blood samples were centrifuged at 3000 RPM for ten minutes. Serum was extracted using standard pipettes. All samples were analyzed for total CPK activity by spectrophotometric analysis using the techniques already described. Isoenzymes were determined using the same method of agarose gel electrophoresis described previously.[5–10]

In the second phase of the experiment, 20 mongrel dogs weighing between 15 and 30 kg were selected as subjects. General anesthesia was induced with intravenous sodium pentothal (19 mg/kg) accompanied by atropine (0.4 mg/kg). After induction, general endotracheal anesthesia was maintained with 1% Halothane®, 57% oxygen, and 42% nitrous oxide. Midline laparotomies were performed in each animal after a central venous line had been established via a cutdown into the external jugular vein and after baseline blood samples had been obtained. The mesentery was dissected, and the superior mesenteric artery was identified. The animals were then selected randomly and placed in one of three groups: those having laparotomy (LAP), peritonitis (PER), and mesenteric artery infarction (MAI). In the dogs who had laparotomy only, the abdomen was closed. Those in the peritonitis group had 10 g of talc in 100 cc of sterile water placed in the peritoneal cavity prior to closure. The dogs who were selected to have the mesenteric artery infarction had the superior mesenteric artery ligated and divided within 2 cm of the vessel's origin prior to closure.

After surgery, intravenous therapy was terminated, and the animals were allowed to recover. Blood samples were obtained at three, six, nine, 12, 24, 27, and 30 hours after surgery. The dogs were then killed, and complete autopsies were conducted to confirm that the desired experimental injury had occurred and that no significant intercurrent disease existed.

The blood samples were centrifuged at 3000 RPM for ten minutes immediately after collection, and the serum was removed for analysis of CPK. Total enzymatic activity was determined by spectrophotometric analysis using Union Carbide (Centrifichem® Series 400) autoanalyzer (Union Carbide, Rye, NY). Union Carbide reagents and control samples were used in all analyses.[5,6] The isoenzyme fractions were determined by agarose gel electrophoresis using the Corning ACI system equipped with a Corning 720 densitometer and 722 data terminal.[5–10] Reagents specific for this system were used throughout all analyses. The results were tabulated and expressed in graphic form to identify trends. The Wilcoxon rank sum test[11] was used to test whether the distribution of a given variable (*e.g.*, total CPK) was the same between two comparison groups. Observed significance levels (p values) were obtained using the normal approximation and are reported as one sided values.

Results

In the first phase, total mean CPK activity in the bowel ranged between 50 and 100 IU per gram of tissue in man (Fig. 1). Total CPK activity in the canine was between 40 and 60 IU per gram of tissue. Although the activity in the canine was consistently lower than in the human, statistically significant differences did not exist in the population presented. In all specimens assayed (humans: N = 9; dogs: N = 12), all three isoenzymes of CPK were present. CPK-BB tended to have the highest concentration and CPK-MM the lowest. At all levels, the dog and man were comparable as to the distribution of the isoenzymes.

The normal serum values for CPK in both man and dog are presented in Table 1. The standard

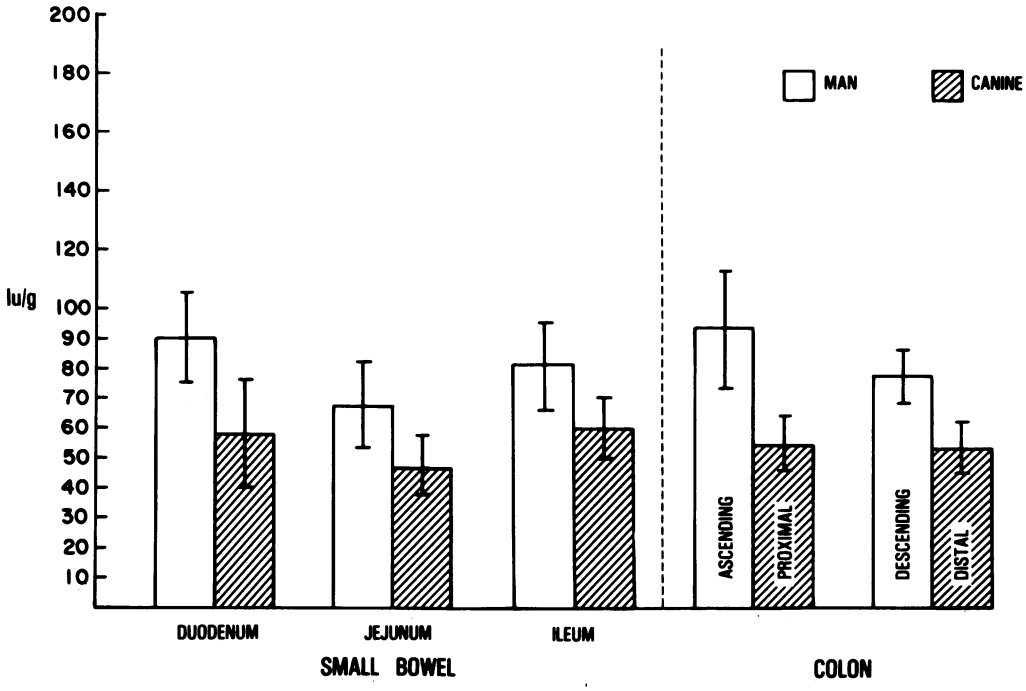

FIG. 1. Total CPK as expressed in international units per gram of bowel tissue is presented for both man and canine. Bars represent standard errors of the mean. Humans: N = 9; Canines: N = 12.

clinical normal range for serum CPK is 0 to 100 IU/L (Standards for Union Carbide Reagents as stated by manufacturer: Union Carbide, Rye, NY). The normal values for the dogs were comparable but were slightly lower than those recorded for the humans. The most notable finding was the difference in isoenzyme patterns. In man, only CPK-MM was present. In the dog we were able to detect all three isoenzymes of CPK in all serum samples. Both CPK-MM and CPK-BB were present in relatively large quantities. CPK-MB was present but it had the smallest concentration of the three isoenzymes.

In presenting the results from the surgical procedures conducted in the second phase of the study, the necropsies added confirmation that the desired effects had occurred. They showed that all seven dogs which had laparotomies had only very mild peritoneal injection secondary to surgery. Only one had a clot associated with the central venous cannula; none had any inherent cardiac pathology. All four dogs receiving intraperitoneal talc had severe fibropurulent peritonitis. One had a clot associated with the tip of the central venous cannula; none had cardiac pathology. All of the dogs having mesenteric infarction had necrosis of the jejunum and ileum. Two also had necrosis of the ascending colon, while one other had necrosis of part of the duodenum and distal stomach as well as the ascending colon. Two had clots associated with venous cannulae, while one had a small hemorrhage into the septal leaflet of the tricuspid valve. None of the subjects had intrinsic cardiac pathology.

The elevations of serum total CPK in each of the three groups are presented in Figure 2. All three groups entered the experiment with nearly identical mean serum total CPK values. Note that the serum total CPK activity remained significantly elevated in the canines that had mesenteric artery infarctions throughout the 30 hours after surgery. Significant elevations in serum total CPK appeared at three hours after injury and reached maximal values at nine hours after injury in the dogs undergoing mesenteric artery infarctions. Although the serum total CPK levels rose to twice the upper limits of normal (100 IU/L) in the dogs that were in the LAP and PER groups, they could not be distinguished from each other. The group undergoing MAI, however, was significantly different ($p < 0.01$) from either the PER or LAP groups.

Serum levels of CPK-MM, the isoenzyme found in the largest concentrations in skeletal muscle, rose significantly in the MAI group by three hours after injury, peaked at nine hours after injury, and remained elevated throughout the 30-hour study period (Fig. 3). The levels of serum CPK-MM in the LAP and PER groups rose but were significantly different ($p < 0.01$) from the values obtained for the canines in the MAI group. Once again, the LAP and PER groups were indistinguishable.

The serum values of CPK-MB (the cardiac isoen-

TABLE 1. *Normal Serum Values of CPK in Man and Dog*

	Total CPK (IU/L ± SEM)	CPK Isoenzymes (% ± SEM)		
		MM	MB	BB
Man (N = 20)	50.5 ± 6.3	100	0	0
Dog (N = 15)	33.2 ± 2.9	34.7 ± 3.1	18.2 ± 4.7	45.1 ± 3.6

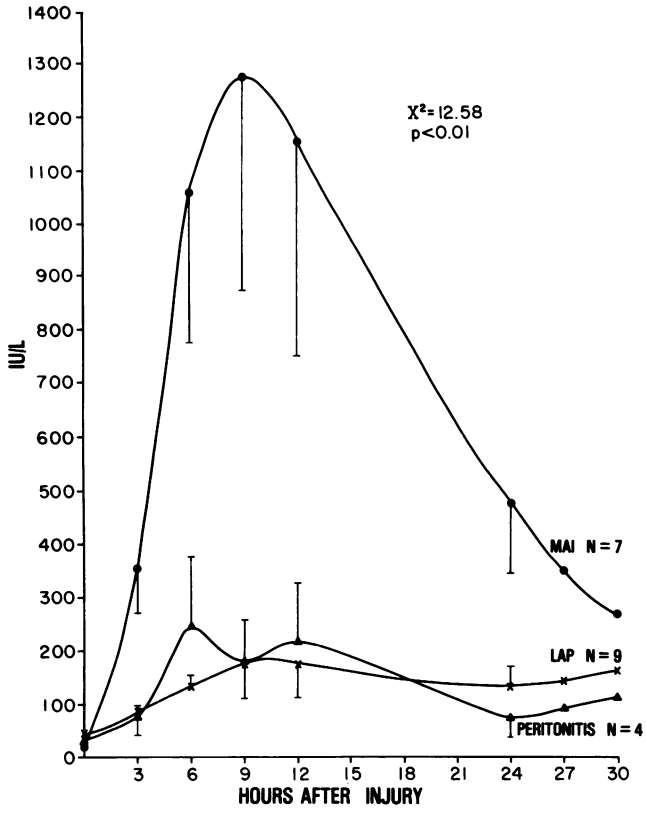

FIG. 2. The total activity of CPK in the dogs experiencing mesenteric infarctions (MAI) was at maximum elevation at nine hours after injury. When compared with the dogs who had laparotomies (LAP) or peritonitis the difference is significant ($p < 0.01$). Bars represent standard errors of the mean.

zyme) rose in the MAI group, but the values were not significantly different until nine hours after injury (Fig. 4a). The values fell from their maximal determinations after the 24-hour sample but remained elevated up to the 30-hour sample. When compared with the LAP and PER groups, the values of CPK-MB in the sera of the MAI animals were significantly elevated ($p < 0.01$). The serum values for the LAP and PER groups were virtually identical for the MB isoenzymes.

The group of MAI dogs can be divided into two subgroups: those that died between 24 and 27 hours after arterial ligation (mortalities: $n = 3$) and those that survived to complete the experiment (survivors: $n = 4$). The mean values of serum CPK-MB for each subgroup are represented in Figure 4b. Note that the survivors tended to have lower serum CPK-MB values than did the subjects that died. Statistical differences between the subgroups were not demonstrable, as is evident from the bars which represent the standard errors of the mean for each value.

Serum values for CPK-BB (the isoenzyme generally found in brain and smooth muscle) became significantly elevated in the sera of the dogs having MAI's by three hours after injury (Fig. 5). Maximal values in the sera of the MAI group were obtained six hours after injury. Serum values of this isoenzyme declined after six hours even though they remained elevated throughout the 30-hour study period. The values in the MAI group were significantly different ($p < 0.01$) when compared with the LAP and PER groups. Serum values for this isoenzyme were virtually the same in the LAP and PER group.

Figure 6 shows a composite of the serum levels of CPK and each of its three isoenzymes in the canines experiencing mesenteric artery infarctions. The skeletal muscle isoenzyme (CPK-MM) always is the major component present. CPK-BB rises early and then falls but does not return to preoperative levels. CPK-MB has a slow and steady rise and peaks at 24 hours after the procedure.

Discussion

The levels of total CPK activity we found in full-thickness, human bowel wall are similar to those in previous reports. Roberts and associates[12] found levels of 180 IU/g of tissue in the surgically resected bowel specimens which they investigated. The amount present was of a much smaller order of magnitude than skeletal muscle (3,000 IU/g) and ventricular myocar-

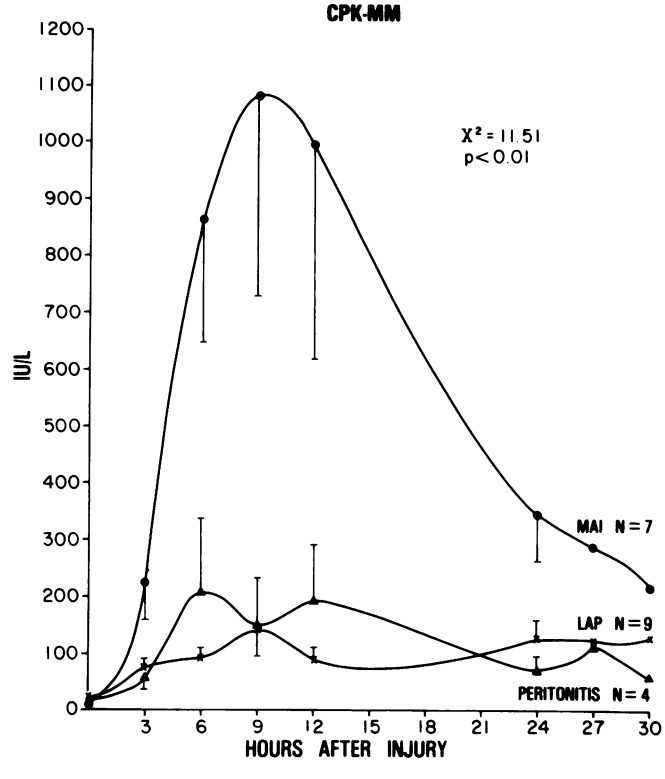

FIG. 3. Activity of CPK-MM in the serum of dogs subjected to laparotomy (LAP), peritonitis, and mesenteric artery infarction (MAI). The values in the MAI group are significantly different from the LAP and peritonitis groups at the $p < 0.01$ level. Bars represent standard errors of the mean.

dium (1200 IU/g).[12] Other studies conducted on surgical specimens yielded results of the same order of magnitude.[13]

In our studies, we found all three isoenzymes of CPK in every homogenized sample of full-thickness bowel we processed. Other authors have reported finding only CPK-BB.[12,13] Several possible explanations for the variance may be offered. The most plausible concerns dilution of the supernatant for electrophoresis. We found that by sequentially diluting the supernatant to achieve high resolution of each band, one could reach dilutional levels wherein the electrophoretic apparatus would detect only the CPK-BB and would not register the other two. A further source of error could have been in extraction and storage of the specimens in the other methods. In our samples, the assays were completed as soon as possible after the supernatants were removed from the homogenate. All samples were processed within 24 hours to prevent any bacterial digestion of the enzymes. CPK samples that could not be processed as soon as they were obtained were frozen to preserve activity even though the time until processing would be only a few hours.

The levels of total CPK in human bowel samples were consistently higher than the canine values. Statistical analysis, however, did not reveal any significant difference between man and dog at any of the levels of the gastrointestinal tract that were studied. Inspection of the levels of isoenzymes of CPK in the gastrointestinal tract shows that the dog and man are quite comparable. All three isoenzymes of CPK were present in all samples. CPK-BB tended to have the highest concentration at all levels, and CPK-MM had the lowest. In comparing these findings with serum values, it is clear that CPK-MB and CPK-BB have the greatest potential prognostic value in evaluating mesenteric infarction in man since they are virtually nonexistent in normal human serum. Sophisticated techniques are capable of detecting CPK-MB in the sera of healthy patients at levels between 0 and 6 IU/L.[12] Such levels have been considered to reflect the normal values for this isoenzyme in serum. Canine serum, on the other hand, had levels of all three isoenzymes in the normal state. Hence, the increased presence of CPK-MB or CPK-BB in canine serum cannot be construed as ab-

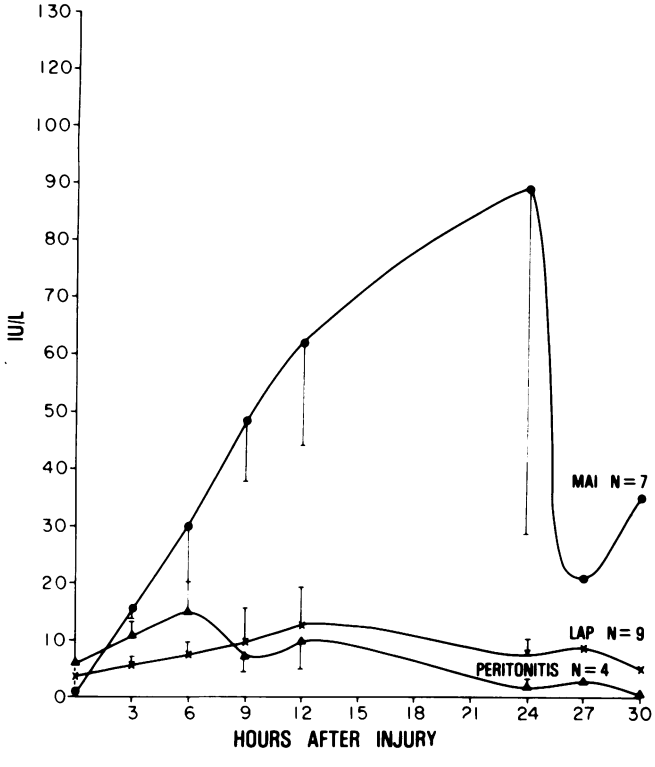

FIG. 4a. The levels of CPK-MB (cardiac isoenzyme) in the serum of the three experimental groups. Bars represent standard errors of the mean. The scale on the vertical axis (international units/L) is expanded more than in Figures 1 and 2. $\chi^2 = 10.81$. $p < 0.01$.

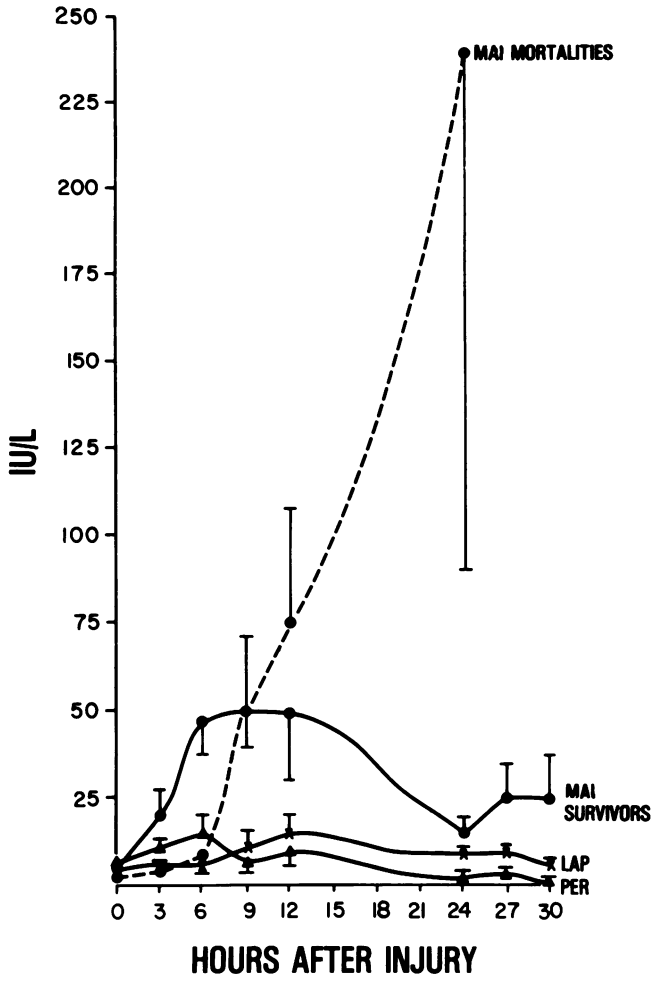

FIG. 4b. In this graph, the group of MAI dogs has been divided into two subgroups: three dogs dying between 24 and 27 hours and the four survivors who completed the entire experiment. The continuing increase in CPK-MB in the sera of the dogs suffering mortalities explains the large standard error of the mean in the 24-hour samples for the entire group.

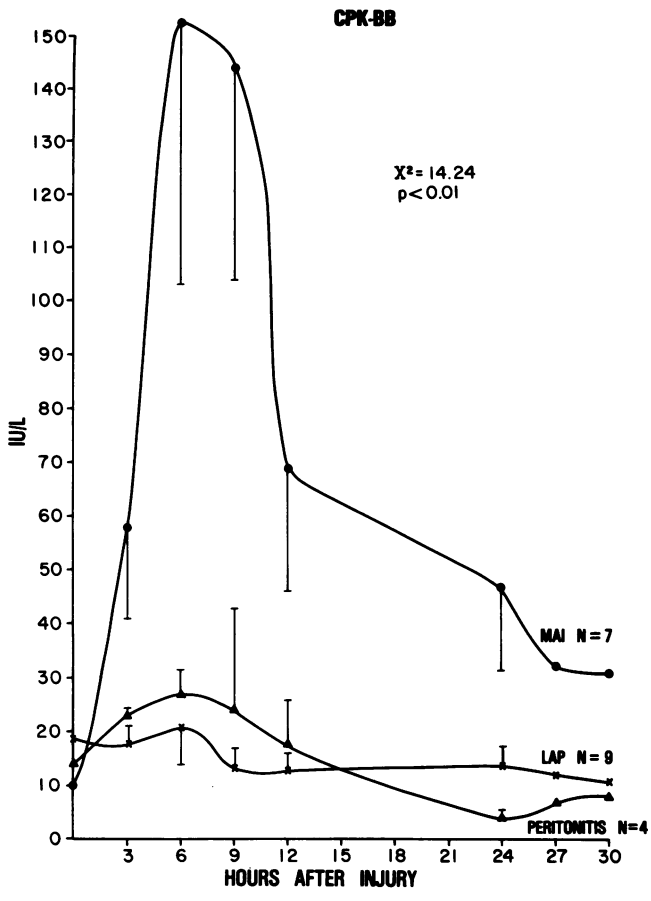

FIG. 5. Changes in CPK-BB (smooth muscle isoenzyme) with time after procedure. Note that CPK-BB elevates significantly ($p < 0.01$) within three hours after infarctions. The canines who had laparotomies are indistinguishable from those who had peritonitis. Note that the scale on the vertical axis is expanded. Bars represent standard errors of the mean. $\chi^2 = 14.24$. $p < 0.01$.

normal. When these isoenzymes are used to evaluate mesenteric infarction in the dog, the baseline level has to be determined for each subject and the absolute increase in each of the two has to be plotted.

The results from the experimental infarctions need to be interpreted with respect to the data generated in the analysis of the bowel samples. Elevations of total CPK seen in the serum of these dogs consisted primarily of skeletal muscle CPK (MM isoenzyme), despite the fact that our tissue investigations have shown that all three isoenzymes are apparent in nearly equal concentrations throughout all levels of the small and large bowel. Several plausible explanations for this phenomenon exist. CPK-BB, the isoenzyme of CPK generally accepted to be predominant in smooth muscle, has a very short half-life in serum of approximately 40 minutes.[14] Whether it is metabolized to an inert product or to the MM isoenzyme is not clear. Local tissue metabolism of both the MB and BB isoenzymes could certainly decrease the amount that is transported to the serum. Some investigators, for example, have postulated that only 15% of the relatively large quantities of CPK-MB liberated in myocardial infarction ever reaches the serum because much is metabolized in the myocardium and during lymphatic transport.[15] It could be postulated that the same metabolic transformations and/or degradation could be operating on both CPK-MB and BB liberated during mesenteric infarction. Substantiation for this theory is not available at present.

The large standard error of the mean apparent in the 24-hour determinations of serum CPK-MB levels in the dogs receiving mesenteric artery ligations can best be understood by dividing the group into two subgroups (Fig. 4b). In the first subgroup, the collateral circulation in the mesentery was not sufficient to sustain life beyond 24 hours after injury. In these three dogs, the infarction led to a progressive downhill course which resulted in their deaths between 24 and 27 hours. These dogs, which had very high levels of total CPK in their serum, died before the 27-hour samples were obtained. The remaining four dogs had sufficient collaterals to allow them to survive through the 30-hour

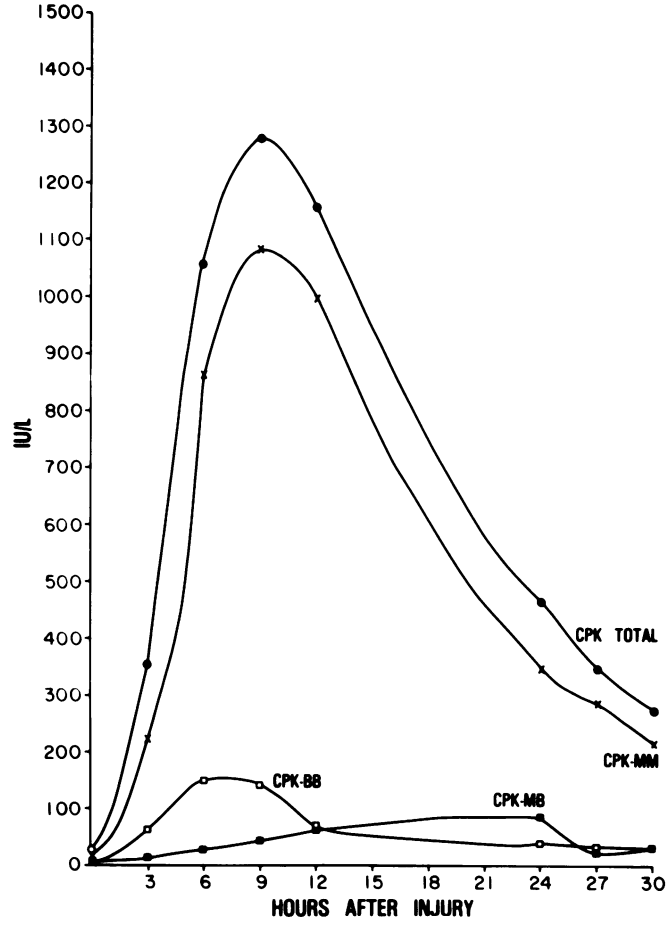

FIG. 6. This summarizes the changes in CPK and its isoenzymes seen in the sera of canines who have had mesenteric artery infarctions.

period. Their total serum CPK values were generally lower than in the three other canines.

The variability of response to superior mesenteric ligation in dogs is large. Our pilot study showed that some dogs have sufficient collateral circulation to withstand complete interruption of the superior mesenteric artery without bowel necrosis. For this reason, each dog in the study received a complete necropsy by a veterinary pathologist to confirm that the desired injury had occurred. A further benefit of the necropsy was that any intercurrent disease which could have affected the serum isoenzyme levels could be ruled out.

As we have shown, the dog is a good model in which to study CPK in acute mesenteric infarction because dog and man have comparable levels of total and isoenzyme fractions of CPK in each segment of the bowel. Correlation with human case reports can be conducted with reasonable certainty because of the marked similarities between dog and man. To our knowledge, only three reports discussing CPK in mesenteric infarction have been published.[2-4] In two of these, no detailed information concerning the patients with mesenteric infarction was rendered.[3,4] Lamar and his associates noted that five of their 41 patients who had been found to have CPK-BB in their sera were proven to have bowel necrosis.[4] In a review that focused on the potential of CPK-BB for diagnosing central nervous system injury, Itano found one case of membranous enterocolitis which was associated with the presence of CPK-BB in the serum.[3] In the most detailed report to date, Doran presented a case of acute infarction of a 12 cm segment of the sigmoid colon in a 33-year-old man who had associated serum changes in total CPK and its isoenzymes.[2] The total CPK and each of the isoenzymes were elevated in the patient's serum until the necrotic bowel was resected. After resection, the total CPK and the isoenzymes returned to normal. The patient died subsequently in renal failure. Autopsy examination confirmed that no myocardial damage existed. Electrophoresis of supernatants taken by that laboratory from human transverse colon showed that all three isoenzymes were present. These findings are in agreement with our studies on bowel specimens.

The temporal course of the elevations of each isoenzyme has not been addressed in any of the published reports cited above. As can be noted from the experimental data in this paper, the serum CPK-BB isoenzyme rises in the first 12 hours of a mesenteric infarction, while the CPK-MB isoenzyme rises during the second 12 hours. Two of the previously published articles refer only to the BB isoenzyme and do not discuss any temporal aspects of its elevation.[3,4] The one complete case report noted that all three isoenzymes of CPK were present in the patient's serum but there was no mention of their temporal relationship. We feel that the temporal appearance of each of the isoenzymes in the serum should be stated in any future reports because this may have diagnostic significance.

In summary, we have shown in the animal model that total serum CPK is elevated in the presence of ischemic bowel necrosis. By measuring the separate isoenzymes over time, we have further documented that the MM isoenzyme is predominant and that the BB isoenzyme peaks at six hours after superior mesenteric artery ligation. The MB isoenzyme is elevated also and peaks at 24 hours after arterial ligation. This study, plus isolated case reports of elevated serum CPK levels in humans with bowel necrosis, provides the impetus for a rigorous clinical study to assess the usefulness of serum CPK levels in diagnosing bowel ischemia and necrosis.

Acknowledgments

The authors wish to express their gratitude to Sp.5 Colleene E. Davidson for outstanding technical assistance and to Miss June Dubois and Miss Cheryl A. Currie for typing the manuscript.

References

1. Ottinger LW. The surgical management of acute occlusion of the superior mesenteric artery. Ann Surg 1978; 188:721-731.
2. Doran GR. Appearance of creatine kinase BB isoenzyme in the serum of a patient suffering from infarction of the colon. Clin Chem Acta 1979; 92:415-419.
3. Itano M. The detection of a CPK_1 (BB) in serum: A summary of sixteen cases. Am J Clin Pathol 1976; 65:351-355.
4. Lamar W, Woodward L, Statland BE. Clinical implications of creatine kinase-BB isoenzyme. N Engl J Med 1978; 299: 234-235.
5. Kornberg A. Reversible enzymatic synthesis of diphosphopyrodine nucleotide in inorganic pyrophosphate. J Biol Chem 1950; 182:779-784.
6. Oliver IT. Spectrophotometric method for the determination of creatine phosphokinase and myokinase. Biochem J 1955; 61: 116-122.
7. Hess JW, MacDonald RP, Nathow GJW, et al. Serum creatine phosphokinase: Evaluation of a commercial spectrophotometric method. Clin Chem 1967; 13:994-1005.
8. Nielson L, Ludvigsen BJ. Improved method for determination of creatine kinase. J Lab Clin Med 1963; 62:159-168.
9. Rosalki SB. An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med 1967; 69:696-705.
10. Wilkinson J, Steciw B. Evaluation of a new procedure for measuring serum creatine kinase activity. Clin Chem 1970; 16: 370-374.
11. Dixon WJ, Massey FJ. Introduction to Statistical Analysis, New York, McGraw-Hill, 1969, p 344.
12. Roberts R, Gowda KS, Ludbrook PA, et al. Specificity of elevated serum MB creatine phosphokinase activity in the diagnosis of acute myocardial infarction. Am J Cardiol 1975; 36:433-437.
13. Tseung SM. Creatine kinase isoenzyme patterns in human tissue obtained at surgery. Clin Chem 1976; 22:173-175.
14. Roberts R, Henry PD, Willeven SAGJ, et al. Quantification of serum creatine phosphokinase isoenzyme activity. Am J Cardiol 1974; 33:650-654.
15. Roberts R, Henry PD, Sobel BE. An improved basis for enzymatic estimation of infarct size. Circulation 1975; 52: 743-754.