<0.3 INR at a value of ~3 INR, so we can accept that there is no clinical significance in using samples that have been stored for 6 h in glass tubes. The EDTA sample tubes were not siliconized, and siliconization of tubes in fact improves sample stability.

In the coagulation factor tube, the blood is diluted (1:10) with citrate [0.109 mol/L (3.2%) or 0.129 mol/L (3.8%)], and this changes the sample in an nonphysiologic direction. In the EDTA sample, the blood is in a condition that most resembles physiologic conditions, and this should improve the stability of the sample.

Because the EDTA sample is suitable for many hematologic measurements, its use for coagulation tests affords the possibility of using the same sample for different purposes. This reduces costs, waste, and consumption. The use of the same tube for different purposes is also environmentally sound. After hematologic measurements are made, the sample can be centrifuged to obtain plasma for PT testing. This order of proceeding does not alter the relationship between plasma and cells. In my opinion, this method is suitable for laboratories of different sizes and capabilities for rapid response. The use of the same sample for different kinds of analyses opens up prospects of technical development with analyzers in the future.

I thank the staff of the Laboratory at Valkeakoski District Hospital for their cooperation.

References

In patients with acute myocardial infarction (AMI), the activity of creatine kinase isoenzyme MB (CKMB) in plasma consistently accounts for ~15% of the total CK activity (1, 2). By contrast, the CKMB content of cardiac tissue, although sometimes reported to be consistent with the 15% plasma activity of CKMB (2, 3), has also been reported to be negligible in healthy myocardium (4, 5). In these studies, the higher CKMB found in diseased hearts was thought to reflect cellular adaptation to disease. An alternative explanation for low CKMB is its limited thermostability and susceptibility to pH (6).

The objective of the present study was to examine whether inactivation of CKMB, either postmortem or during perimortal tissue acidosis, could explain the absence of CKMB in cardiac tissue at autopsy. The influence of tissue acidosis was studied by exposing heart tissue to pH values of 5.0–7.5. Finally, because myocardial ischemia and tissue acidosis attributable to AMI are located predominantly in the endocardium (7, 8), transmural differences in CKMB content were studied in the hearts of patients who died after AMI.

Slices (1 cm), midway between apex and base, were obtained from 20 hearts of patients (11 males and 9 females) who died from noncardiac causes and without history of cardiac complaints. Mean values for age, autopsy delay, and heart length and weight (± SD) were 71 ± 15 years, 29 ± 22 h, 167 ± 12 cm, and 467 ± 83 g, respectively. Similar heart slices were obtained from 6 patients who died within 6 h after AMI.

Tissue samples (133 ± 34 mg wet weight) were homogenized as described previously (9), and dry weight (dw) was determined by freeze drying the homogenate in a Leybold Heraeus GT2. CK, CKMBmass, CKMBact, and α-hydroxybutyrate dehydrogenase (HBD) in the supernatant were measured and expressed per milligram of dw of tissue. CK and CKMBact were determined at 37 °C, CK with a N-acetyl-cysteine-activated test from Merck Diagnostics and CKMBact with the Isomune assay of Roche Diagnostics. HBD was measured at 25 °C with the optimized HBD test from Roche Diagnostics. CKMBmass was
measured with the Immulite Automated Analyzer (detection limit, 0.42 μg/L; Diagnostic Products Corporation). The first nine non-AMI hearts were used for a detailed analysis of regional tissue protein content. The left ventricle was cut circumferentially into eight samples, each divided into epicardial and endocardial parts (9). Results were analyzed with variance component analysis (SPSS, Ver. 10.0; SPSS Inc.). Neither circumferential nor transmural location influenced protein content; only differences between hearts were significant (\( P, 0.05 \)). Therefore, single tissue samples were randomly taken from the remaining 11 non-AMI hearts. The six hearts from AMI patients were analyzed again in detail.

Linear regression analysis of the 20 non-AMI hearts showed no influence of sex, heart weight, or age on tissue protein content. A statistically insignificant tendency toward higher \( \text{CKMB}_{\text{mass}} \) in hearts with longer autopsy delays was noted, with mean values of 0.18 (\( n = 4 \)), 0.53 (\( n = 7 \)), and 0.88 (\( n = 9 \)) μg/mg in hearts with autopsy delays of <10 h, 10–25 h, and >25 h, respectively.

The high variability in tissue CKMB content, shown in the Table 1, was explained by very low CKMB values in 10 of the 20 non-AMI hearts (\( \text{CKMB}_{\text{mass}} < 0.1 \) μg/mg). Independently from these tissue data, total CK activity and \( \text{CKMB}_{\text{mass}} \) were also measured in plasma samples obtained 6 h after onset of symptoms from 164 patients with AMI. As shown in Table 1, the \( \text{CKMB}_{\text{mass}}/\text{CK} \) ratio for this in vivo protein release is much higher and less variable than in tissue. These data suggest that the loss of tissue CKMB is caused by a variable perimortal effect, which is already completed in the first few hours after death. A variable degree of tissue acidosis, depending on the agonal phase, could be such an effect and could also explain its all-or-none character.

Susceptibility of CKMB to tissue acidosis was studied in tissue samples (60–150 mg wet weight) incubated at 37 °C.

![Fig. 1. Influence of pH changes on the enzymes in heart tissue. Data are expressed as percentages of the tissue content obtained at time zero. ⊙, pH 5; ⊚, pH 5.5; △, pH 6.5; +, pH 7.5.](image-url)
in buffered phosphate–citrate with 20 g/L bovine serum albumin. After incubation, samples were stored at -70 °C. Tissue samples were homogenized, and CK, CKMB\textsubscript{act}, CKMB\textsubscript{mass}, and HBD were assayed in the homogenates as well as in the incubation buffers. CKMB\textsubscript{mass} completely disappeared within 2 h at pH 5.0 and 5.5 (Fig. 1). In contrast, HBD remained stable at all pH values, and CK remained stable for pH values down to 5.5, but was inactivated at pH 5.0. Because of the stability of total CK (predominantly CKMM) at pH 5.5, total inactivation of CKMB\textsubscript{act} at this pH may seem surprising. The Isomune assay, however, measures CK activity before and after blocking of M subunits and then subtracts the CK activity measured after the removal of all M units, including CKMB, from the sample. A CKMB molecule with an inactivated B subunit will not contribute to the activity in either fraction and thus will not be detected. We do not know if the M subunit activity remains.

The transmural ratios (endocardial/epicardial) of HBD and CKMB\textsubscript{mass} were computed in only four of the first nine non-AMI hearts and in four of the six AMI hearts because of the very low CKMB\textsubscript{mass} content (<0.1 μg/mg) in the remaining hearts. Because of the short time (<6 h) between onset of symptoms and death, infarcted areas in the AMI hearts had physiologic HBD content (>0.4 U/mg dw) and endocardial/epicardial ratios of HBD were equal in non-AMI and AMI hearts (1.08 ± 0.04 and 0.98 ± 0.05, respectively; mean ± 95% confidence interval). For CKMB\textsubscript{mass}, however, these values were 0.92 ± 0.12 and 0.53 ± 0.16, respectively (P <0.005; Student t-test). Apparently, endocardial CKMB\textsubscript{mass} disappears from AMI hearts even before significant leakage of proteins from the infarcted tissue has occurred.

In conclusion, our study shows that the low and highly variable CKMB content of myocardial autopsies is not related to the influence of sample location or autopsy delay, but may be caused by perimortal tissue acidosis. The narrow range of pH values at which inactivation occurs, between 5.5 and 6.0, could explain the noted all-or-none aspect. Myocardial pH after AMI may indeed reach values below 5.5, could explain the noted all-or-none aspect. Myocardial pH after AMI may indeed reach values below 5.5, and, after death, will increase again because of termination of lactate production. The tendency toward higher CKMB for longer autopsy delays may then indicate that this CKMB inactivation is partly reversible. Lack of CKMB in apparently healthy hearts from traffic accident victims (4, 5) could be explained by serious tissue acidosis in the prolonged agonal phase of these victims who survived long enough to die after admission to hospital.

References


Between-Assay Differences in Serum Growth Hormone (GH) Measurements: Importance in the Diagnosis of GH Deficiency in Childhood, Eduardo Chaler,* Alicia Belgorosky, Mercedes Maceiras, Mariano Mendioroz, and Marco A. Riverola (Laboratorio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires, Argentina 1245; * address correspondence to this author at: Laboratorio de Endocrinología, Hospital de Pediatría Garrahan, Buenos Aires, Argentina, Combate de los Pozos 1881 (1245); fax 54-11-4308-5325, e-mail echaler@yahoo.com)

The diagnosis of childhood growth hormone (GH) deficiency is controversial. The usefulness of provocative tests of GH secretion has been questioned for several reasons, one of which involves the large discrepancies in GH measurements among methods and laboratories. Some reports have proposed 10 μg/L as the acceptable GH cutoff value (1), but other values have also been used.

GH immunoassays show poor interassay agreement. These assay discrepancies may occur for several reasons: (a) the use of different GH calibrators (2); (b) the heterogeneity of the GH molecule in human serum (3); (c) the interference of endogenous GH binding protein (4); (d) the various GH epitope specificities of anti-GH antibodies; and (e) the addition of serum to GH calibrators. Because all of these reasons are closely interrelated, their interactions may also accentuate interlaboratory differences in results. The use of methods able to quantify the 22-kDa form of GH exclusively has been proposed as a means of approaching uniformity in results (5). The 22-kDa form is the major circulating fraction and carries the dominant bioactivity.

We analyzed serum GH cutoff values, using different immunoassays. We defined the commercial assay SER 66/217 as the Reference Method, and 10 μg/L as the reference cutoff value for this test, based on several years of clinical experience. In the present study, we analyzed 80 samples to compare the Reference Method with nine other commercially available assays. In addition, results of all assays were compared with those of the Delfia

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