biotinidase-deficient specimen in the autosampler. A sample cup containing phosphate buffer should be inserted between each sample suspected of having deficient or very low activity.

Interfering substances such as sulfonamides, if present in a sample, can cause color development, such that a biotinidase-deficient specimen could exhibit color corresponding to normal activity and a normal specimen could exhibit color corresponding to activity exceeding the upper limit of normal. Therefore, samples tested by either method also should be tested in the absence of substrate for the presence of other chromogenic compounds.

The applicability of the automated method to clinical, genetic, and epidemiological studies depends on the relative contributions of analytical and biological variation to total variation in activity, and on the relative contributions of intra- and inter-individual variation to total biological variation. Although there is no clear definition of "acceptable" analytical variation, Tonks (5) suggested that total analytical variation should not exceed one-fourth of the total biological variation. The analytical variation of the automated assay is sufficiently low (less than one-sixth of biological variation) to assure that true differences between subjects will not be obscured and that abnormal laboratory results will be identified reliably. The differences among individuals accounted for two to four times more variation than did variation within a single individual and, therefore, the automated system is acceptable for use in genetic and epidemiological studies.

This automated assay provides a reliable means for conveniently determining biotinidase activity in a large number of serum specimens, and results are more precise than with the manual assay. Thirty samples can be analyzed in an hour, whereas about 3 h is required to analyze an equivalent number of samples by the manual method. Furthermore, because handling of the samples and reagents is minimized, there is less possibility of human error. The speed, precision, and reliability of the present procedure make it the preferred method for large population-based studies of biotinidase activity in human serum.

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Statistical Interpretation of Concentrations of Magnesium, Zinc, Calcium, Potassium, Cholesterols, and Creatine Kinase Isoenzymes in Men at Different Stages of Ischemic Heart Disease

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We present a statistical interpretation of plasma (P1) and (or) erythrocyte (Erc) concentrations of magnesium, zinc, calcium, potassium, and total high-density lipoprotein (HDL) cholesterol, as well as of the activity of total creatine kinase (CK) and its CK-MB isoenzyme, in 28 men with pre-infarction syndrome (PIS) and 34 men with acute myocardial infarction (MI). Discriminant analysis allowed overall comparison of both groups and determination of the most significant variables: CK and P1-Zn. By non-hierarchical cluster analysis we defined three homogeneous subgroups among MI men, with CK, CK-MB, and P1-Zn differing significantly between the groups. In PIS men, P1-Zn was correlated with P1-Ca, whereas in MI men P1-Zn was correlated with P1-Mg. Stepwise regression indicated that P1-Zn was the most significant regressor of CK in PIS men and of CK-MB in MI men. All these statistical interpretations support a special role of P1-Zn in diagnosis and perhaps prognosis. After MI, interleukin-1 release may possibly mediate observed hypozincemia via formation of a heart Zn-metallothionein.

In previous papers, we studied the concentrations of various trace elements and biological variables at day 1 in two groups of men: one with pre-infarction syndrome (PIS) and the other with acute myocardial infarction (MI) (1, 2). We now present a statistical study involving cluster analysis and stepwise regression to show possible relationships among the variables investigated and changes according to the severity of ischemic heart diseases. We studied the following plasma (P1) and erythrocyte (Erc) variables: magnesium (P1-Mg, Erc-Mg), zinc (P1-Zn, Erc-Zn), calcium (P1-Ca), potassium (Erc-K), and total and high-density-lipoprotein-
tein (HDL) cholesterol, as well as the activity of total creatine kinase (CK; EC 2.7.3.2) and CK isoenzyme MB (CK-MB).

Subjects and Methods

Subjects. Samples were obtained from 60 white men (26 patients with pre-infarction syndrome, ages 46 to 82 y; and 34 patients with acute myocardial infarction, ages 32 to 82 y), all residents of the Nantes area in France. The 60 patients were admitted to an intensive-care unit within 10 h of the first clinical signs. These patients received six tablets of isosorbide dinitrate and three injections of calcium heparinate each day. Differential diagnosis between the two groups was made in subsequent days on the basis of biological and electrocardiographic signs. Subjects with prior therapy or who had not fasted for several hours were excluded from the study.

Assay techniques. Blood specimens (5 mL) were drawn into Venoject Tubes containing lithium heparin (Ref. T 206 LH, Code VT 050 HL 1; Terumo France, 78181 Saint-Quentin-en-Yvelines Cedex, France), then, without delay, centrifuged at 3500 × g for 8 min at 10 ºC.

Concentrations of Mg, Ca, and Zn were measured by flame atomic absorption spectrometry with Zeeman effect (Hitachi Model 180-60; Skalar Analytique, 75015 Paris, France); potassium was measured by emission spectrometry, with the same apparatus (3). HDL-cholesterol (Precipitant method 400 971: phosphotungstic acid–Mg2+; Boehringer Mannheim, Mannheim, F.R.G.) and total cholesterol were determined by an enzymatic colorimetric cholesterol C-system ("CHOD-PAP" method; Boehringer Mannheim). CK activity and CK-MB were measured with the "R-CK NAC-activated Merckotest" (ref. no. 14 317 and 14 333, respectively; E. Merck, 6100 Darmstadt, F.R.G.) at 25 ºC and 334 nm.

Statistical analysis. We used Student's t-test for individual comparison of the variables.

We used discriminant analysis allowing overall comparison of both groups to determine the most significant variables.

Non-hierarchical cluster analysis was used to define homogeneous subgroups among MI men, after transforming the data into ranks, as was also done for stepwise regression (4).

All these statistical procedures were implemented with Systat software (Systat, Inc., Evanston, IL).

Results

Table 1 summarizes the differences in variables for the PIS and MI men; CK and PI-Zn differed significantly between the two groups.

As might be expected, the variables allowing the best discrimination between PIS and MI men (who were presenting cardiac ischemia at two different stages of severity) were CK followed by PI-Zn.

A procedure for assigning individuals to groups was defined by a computer program. A random sample drawn from both groups showed that all the PIS subjects and half the MI subjects were correctly ranked. Evidently the PIS group was very homogeneous and the MI group very heterogeneous. This led us to attempt to define subgroups of homogeneous subjects in the MI group by means of non-hierarchical cluster analysis (K-means procedure) (5). Classification of MI subjects into four subgroups produced one subgroup containing a single, apparently atypical subject: young (44 y), CK very much increased (976 U/L), high CK-MB (58.9 U/L), and PI-Zn very low (4.40 μmol/L). Excluding this subject from the study left three homogeneous subgroups of MI men (Table 2). The size of the necrotic area in these men increased from Group A to Group C (6), and the three variables studied—CK, CK-MB, and PI-Zn—differed significantly (P < 0.05) among the three groups.

The nonparametric stepwise regression equation for the 26 PIS men was: CK = 11.347 - 0.536 Pl-Zn + 0.318 Erc-Mg + 0.378 HDL-cholesterol (P < 0.01). For the 34 MI men at day 1 it was: CK-MB = 23.449 - 0.340 Pl-Zn (P < 0.05), and at day 2: CK-MB = 22.704 - 0.408 Pl-Zn + 0.429 total cholesterol + 0.293 Erc-Mg - 0.304 HDL-cholesterol - 0.306 Pl-Ca (P < 0.001). The equation obtained for MI subjects at day 2, given for information only, serves to confirm the role of Zn. PI-Zn appears to be the variable most strongly correlated (P < 0.05) with CK or CK-MB.

Discussion

Recently, many investigators (7–9) have sought to present significant information about the biochemical and pathobiological pathways of trace elements and electrolytes in humans.

In our study, comparison of means between PIS and MI men showed a single significant difference in Pl-Zn (P < 0.05) in addition to the expected difference in CK (normal CK values for PIS men). The importance of Pl-Zn was also revealed in non-hierarchical cluster analysis and stepwise regression. Recent papers point out the role of CK-MB data in evaluating the extent of a myocardial infarct (6), and the importance of serum Zn in the acute-phase response to stress (10). Early peaking of CK-MB activity is also associated with MI extension (6). Zinc, an essential trace element required for DNA and RNA synthesis and the functioning of more than 200 metalloenzymes, is involved in numerous metabolic pathways of clinical importance. Certain cyto-

| Table 1. Results for Men with Pre-Infarction Syndrome or Acute Myocardial Infarction |
|---------------------------------|-----------------|-----------------|
|                                 | Mean and SD     |                 |
| Mean                           | 26              | 34              |
| Age, y                         | 64.4 (10.5)     | 61.1 (10.9)     |
| PI-Mg, mmol/L                  | 0.77 (0.09)     | 0.76 (0.09)     |
| Erc-Mg, mmol/L                 | 2.31 (0.27)     | 2.19 (0.25)     |
| PI-Zn, μmol/L                  | 9.12 (1.89)     | 8.07 (1.67)*    |
| Erc-Zn, μmol/L                 | 166 (27.7)      | 162 (20.6)      |
| PI-Ca, mmol/L                  | 2.10 (0.13)     | 2.08 (0.13)     |
| Erc-K, mmol/L                  | 8.86 (5.59)     | 8.63 (5.96)     |
| Total-chol., mmol/L            | 5.37 (1.10)     | 5.43 (1.07)     |
| HDL-chol., mmol/L              | 1.14 (0.46)*    | 1.07 (0.31)     |
| CK activity, U/L               | 40.9 (33.6)*    | 364 (250)*      |
| CK-MB activity, U/L            | 38.4 (29.3)*    |                 |

* Significantly different (Student's t-test) from results for PIS men:
  a P < 0.05; b P < 0.001. * Nonnormal distribution.

| Table 2. Non-Hierarchical Cluster Analysis of 33 MI Patients (Mean and SD) |
|--------------------------------|-----------------|-----------------|-----------------|
|                                | Group A (n = 15) | Group B (n = 8) | Group C (n = 10) |
| Age, y                         | 59.5 (12.2)     | 65.0 (5.81)     | 62.1 (9.73)     |
| CK, U/L                        | 134 (62.1)      | 351 (66.5)      | 659 (98.6)      |
| CK-MB, U/L                     | 15.3 (7.13)     | 35.5 (7.07)     | 73.4 (25.3)     |
| PI-Zn, μmol/L                  | 8.81 (1.16)     | 7.94 (1.67)     | 7.43 (1.55)     |
kines such as interleukin-1 are known to cause a decrease in serum Zn concentration as part of the acute-phase response to stress and (or) inflammation. Interleukin-1 stimulates production of metallothionein, a zinc-binding protein in tissue (10). After MI, a significant decrease in PI-Zn has been consistently observed, and several hypotheses have been advanced to account for it (2, 11). Our own studies of men and women who died after MI of the left ventricle indicate that only the Zn concentration in the necrotic area was decreased (P <0.01), as compared with that of the left ventricle of reference subjects (12); in the right ventricle and the non-necrotic left ventricle, Zn concentrations were even slightly higher than those of control groups. Accordingly, in view of the decrease in PI-Zn and the negative significant correlation between PI-Zn and CK or CK-MB on the first days after MI (13), it would seem feasible to propose that circulating Zn is taken up by non-necrosed myocardial tissue, in proportion to the extent of the necrotic area, as part of the reparative process (5, 11). Release of interleukin-1 might mediate this observed hypozincemia by stimulating production of a heart Zn-metallothionein.

Our findings here show the well-known relationship discussed in our previous works (13, 14) between PI-Zn and CK or CK-MB as well as other correlations, particularly, in PIS men, between PI-Zn and PI-Ca, and between PI-Zn and PI-Mg in MI. In PIS men, the correlation between PI-Zn and PI-Ca is similar to what we found in 58 control men (3). The concentration of Zn in serum, like that of Ca, is maintained within a narrow range in healthy humans (15, 16). Even though the metabolism of Ca and Zn differs substantially, Ca-regulating hormones are known to affect Zn metabolism (15). In MI men, the relationships are modified, and PI-Zn is correlated with PI-Mg.

All these statistical interpretations indicate both the differences among the relationships of these variables, according to the severity of ischemia, and the special role of PI-Zn in diagnosis and perhaps prognosis. Our current investigations are continuing along these lines.

References

A New Commercial Method for the Enzymatic Determination of Creatinine in Serum and Urine Evaluated: Comparison with a Kinetic Jaffé Method and Isotope Dilution–Mass Spectrometry

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We evaluated a new, simple, enzymatic kinetic method from Wako Chemicals GmbH in comparison with a kinetic Jaffé method by using isotope dilution–mass spectrometry (ID-MS) as a reference method. An ID-MS-calibrated serum standard was used. Both the enzymatic and the Jaffé method correlated well with ID-MS, except for sera with high concentrations of bilirubin. Ethyl acetoacetate, acetone, and glucose in serum interfered somewhat with the Jaffé method but not with the enzymatic method. We conclude that the present enzymatic method has merit as compared with a Jaffé method for routine work, but is more expensive.

Creatinine is measured in serum and urine to estimate renal function. The classical Jaffé technique is the method most commonly used for this measurement, even though it is not specific for creatinine. Different modifications of the