management. Because the bias is consistent it can be allowed for by subtracting the value of 1.54 mmol/L from each derived bicarbonate value; and because the differences between the methods are normally distributed we can be confident that in 95% of cases there will be agreement to within ±2 SD, i.e., ±3.3 mmol/L. We regard these limits as clinically acceptable.

Our findings are clearly at variance with those of Rosan et al. (8), who found errors in the calculation of bicarbonate of between −55% and +170% in sick newborns. Still, we believe that our population was similar to that studied by Rosan et al., both in terms of diagnosis and severity of illness. They attributed their large errors to a state of disequilibrium not accounted for in the Henderson–Hasselbalch equation. Karłowicz et al. (9), however, could find no alteration in pK⁺ when the acid–base status of newborn lambs was acutely disturbed. Re-analysis of the data presented by Rosan et al. shows that, although errors ranged from −55% to +72% (not 170% as stated by the authors), almost all could be explained on the basis of variance between the two machines used to measure total CO₂ content. Far from the negligible inter-instrument variance claimed, we calculated the standard deviation for the paired measurements to be 2.17 mmol/L, enough to account for all but three of the reported errors.

We also are unconvinced by the data presented by Natelson and Nobel (2), whose dissatisfaction with the calculated bicarbonate value was the foundation for the Rosan study. This often-cited Letter presented a frequency distribution curve for pK⁺ in which the cumulative frequency exceeded 140%! The scattergram on which this was based did not appear to have enough discrepant points to account for the range of pK⁺ values stated.

We conclude that the values for bicarbonate calculated by a ward-based blood-gas analyzer agree sufficiently closely with the total CO₂ measured in the chemical pathology laboratory for the former to be a clinically useful (and safe) method of characterizing acid–base disturbances in sick newborns. Also, the speed with which a result can be obtained from the ward-based instrument is a major advantage in a situation where derangements of acid–base status are so common and develop so rapidly.

We acknowledge the help of Dr. R. B. Payne.

References

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Influence of Hypertension and Antihypertensive Drugs on the Biological Intra-Individual Variation of Electrolytes and Lipids in Serum

Wieland G. E. Hözel

Biological intra-individual CVs for Na⁺, Cl⁻, K⁺, calcium, cholesterol, high-density lipoprotein cholesterol, triglycerides in serum, and hemoglobin in blood were estimated in men with essential hypertension (EH) treated with beta-blockers and diuretics, and compared with those of normotensive men. Although in EH the mean concentrations of Na⁺, Cl⁻, K⁺, hemoglobin, and triglycerides were increased and that of HDL cholesterol was decreased, the average intra-individual CVs did not significantly differ between the two groups. The mean concentration of cholesterol, as well as the average intra-individual CV for it, was significantly higher in EH. There was no correlation between the intra-individual CVs for the analytes and the mean blood pressure of the individuals. Individual values were normally distributed for all analytes. There was no increase of the intra-individual CV with the lapse of time between consecutively measured values. The estimated average biological intra-individual CV was used to derive decision-making criteria for interpretation of test results observed in monitoring EH.

Judging the clinical significance of changes between consecutively observed values in monitoring diseases requires decision-making criteria that are derived from the random intra-individual variation of the analytes. This random variation has two components, biological and analytical. The biological component must be specifically studied for each disease, because the disease, or the drugs used in its treatment, may influence the magnitude of variation

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(1-3). Subjects with essential hypertension (EH) have a high risk for development of cardiovascular diseases. They need long-term treatment by antihypertensive drugs to lower this risk (4).

Hypertension, and drugs used for its treatment, may influence the intra-individual variation of biochemical analytes in serum. It is well known that essential hypertension is associated with an increased intracellular concentration of sodium and calcium (5), and that beta-adrenergic blocking agents and thiazide diuretics, which are the antihypertensive drugs most widely used, influence the electrolyte and lipid metabolism (6-12). Therefore the intra-individual variation of these analytes is of both theoretical and practical interest. We studied the intra-individual variation of Na⁺, Cl⁻, K⁺, calcium, cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in serum and of hemoglobin in blood of a group of patients with EH, treated long term, to get information about the following:

- magnitude of biological intra-individual variation
- correlation between blood pressure and intra-individual variation
- time dependence of intra-individual variation
- variability of intra-individual variation between subjects
- type of distribution of the individual values

Information as to these characteristics is a prerequisite for deriving decision-making criteria for assessing changes noted in test results when EH is being monitored.

Materials and Methods

Subjects

All subjects were volunteers. During the course of the study no further illness or injury was observed, nor was the treatment of the patients with EH changed.

Normal men. This group consisted of 10 normal-weight white men, ages 31-50 years (average 41.8 y). All individuals were apparently healthy and were taking no drug.

Essential hypertension. This group consisted of 14 white men, ages 36-59 years (average 46.8 y). The mean values for blood pressure ranged from 13.1 kPa (98 mmHg) to 14.7 kPa (110 mmHg) diastolic and from 20.5 kPa (154 mmHg) to 24.6 kPa (184 mmHg) systolic. All patients were treated with the beta-adrenergic blocking agent propranolol hydrochloride, and seven patients were additionally treated with a diuretic containing hydrochlorothiazide and triamterene (potassium-sparing component).

Specimen Collection

No special restrictions were imposed on the subjects. Blood was drawn by an experienced phlebotomist between 0630 and 0800 hours. The serum was stored at -196 °C until analysis. For hemoglobin determinations, blood was collected into tubes containing EDTA, then also frozen. Blood was sampled once a week for eight weeks.

Analytical Procedures

All samples from the same individual were analyzed at least twice within one run. Comparability between runs was ensured by stringent quality control. The analytical methods were standard for the medical laboratories of the German Democratic Republic (13). The basic principles and instruments involved were given in previous papers (1, 2).

Statistical Analysis

The total intra-individual variance, $s_i^2$, was considered to be the sum of the biological intra-individual variance, $s_b^2$, and the analytical variance, $s_a^2$. For estimating the average CV, the analyte values for each individual were transformed into a percentage of the arithmetic mean for that analyte and individual. Differences between mean values were checked by Student's t-test. Differences between variances were checked by the F-test. Variability of the intra-individual variances in the groups were estimated by an approach suggested by Harris (14, 15). The type of distribution of individual values was investigated by the chi-square goodness-of-fit test. Linear regression analysis was used to investigate whether the CV's depend on the mean blood pressure or the CV's of the blood pressure, and to investigate the time dependence of the intra-individual variation.

Results and Discussion

Analytical Variation

To minimize random variations in the estimation of $CV_{bi}$, the analytical CV should be small. The analytical within-run CV's and the ranges within which they are valid are listed in Table 1. Because the analytical CV's are smaller than the computed $CV_{bi}$'s, it can be assumed that the estimations of $CV_{bi}$ are valid.

Mean Concentrations and Average Intra-Individual Variations

Table 2 gives the overall mean concentrations and the average $CV_{bi}$'s of patients with EH and of normal men. We found no significant differences, either between the mean values or between the average $CV_{bi}$'s for patients being treated with beta-blockers and patients being treated with

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<th>Table 1. Within-Run Analytical Coefficients of Variation</th>
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<th>Table 2. Overall Mean Values and Average Biological Intra-Individual Variations in Men with Essential Hypertension (EH) and in Normotensive Men (N)</th>
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$\bar{x}$, mean concentration of analyte in all individuals of that group. Q, CV ratio of EH patients to normal men. *Overall mean values and ratios statistically significant at $P <0.01$. 
both beta-blockers and diuretics. Therefore we combined the results.

Sodium and chloride. Despite antagonistic therapy, the overall mean concentrations of sodium and chloride in serum were significantly higher in the group of men with EH. Superficially, the increase seems small, but the sodium increase (2 mmol/L) exceeds by twofold the biological intra-individual standard deviation. The average CVs's for sodium and chloride were as low as in the group of normotensive. This suggests the hypothesis that some individuals with EH have not only a higher intracellular concentration of sodium but also a higher set point for its extracellular concentration, whereas the random fluctuations around this higher set point are as close as in the normal state. To confirm this hypothesis, we studied the sodium concentration in serum of 50 more men with EH. Again we found a significant increase in the mean concentration in serum of about 2 mmol/L (142.96 ± 2.47 mmol/L). There was a significant correlation between the concentration of sodium in serum and the systolic blood pressure (r = 0.630, P <0.05). Perhaps a small change in the set point of the well-regulated extracellular sodium concentration plays a role in the genesis of EH.

Potassium. Both the overall mean concentration and the average CVs were slightly higher in the group with EH, but the CV's did not differ significantly. Neither hypertension nor the drugs used for treatment, including diuretics, substantially influenced the intra-individual variation of potassium in serum.

Calcium. Although essential hypertension is associated with an increase of the intracellular calcium concentration (5) and beta-blockers and diuretics influence its intracellular concentration (9), there was neither a significant difference in the overall mean concentration in serum nor an increase of the intra-individual variation.

Cholesterol, HDL cholesterol, and triglycerides. The mean values for cholesterol, HDL cholesterol, and triglyceride concentrations were changed towards those associated with a higher risk for cardiovascular diseases. The increase in triglycerides and the decrease in HDL cholesterol may be caused by the drugs used in treatment (10, 11). The CVs of cholesterol of men with EH was significantly higher than that for normotensive men, whereas the CVs of triglycerides and HDL cholesterol were of the same order as those of normotensive men.

Hemoglobin. The intra-individual variation in hemoglobin in blood is a good indicator for intra-individual variation that is caused by random effects of hemodilution and hemo-concentration. There was no significant difference between the average CVs for hypertensive and normotensive men. Hypertension, as well as drugs used for its treatment in this study, did not increase these random fluctuations. The overall mean hemoglobin concentration in blood was slightly but significantly higher in the group with EH.

Correlation between Blood Pressure and Intra-Individual Variation

I found no statistically significant correlations between either the CV for the analytes and the mean systolic or diastolic blood pressure or between the CV for the analytes and the intra-individual CV for the systolic or diastolic blood pressure of the individuals. These findings suggest that the intra-individual variation of the analytes investigated is uninfluenced by hypertension.

Variability of Intra-Individual Variation between Subjects

The variability of the intra-individual variation between the subjects of the EH group was zero or very small for sodium, chloride, potassium, calcium, cholesterol, and hemoglobin. For these analytes the average CVs's are good estimates for those of the subjects. The CVs's for HDL cholesterol and triglycerides varied substantially among subjects. Therefore the average CVs's are only rough estimates for those of the individuals.

Time Dependence of the Intra-Individual Variation

The intra-individual variation of all analytes investigated showed no significant increase with the time lapse between two observations. This is of practical importance, because it allows the same decision-making criteria to be taken independently of different spans of time between consecutively observed values.

Type of Distribution

Although the concentrations of many analytes were abnormal in EH patients, the chi-square test showed a good agreement for all analytes with a normal distribution. This is also valid for triglycerides, known to have a log-normal distribution of group reference values for healthy subjects. Decision-making criteria can be derived under the assumption of a normal distribution.

Criteria for Decision Making

The estimated biological intra-individual variation can be combined with estimates of analytical precision to judge the significance of changes in the concentration of analytes observed during monitoring the course of EH. The magnitude of random changes can be estimated according to the formula

\[ CV_i = \sqrt{CV^2_{a} + CV^2_{l}} \]

CVa is the analytical CV of the laboratory, and CVl is the total CV of intra-individual variation. A difference, D, between two consecutively observed values is statistically significant (P <0.05) if it exceeds 2.8 CVi. This is exactly valid if there is no or only a small between-subject variability in the biological intra-individual variation and if the individual values are normally distributed. The last condition is fulfilled for all analytes, but HDL cholesterol and triglycerides have a remarkable between-subject variability in the CVi's. For these analytes we get only a rough estimate by this approach. Nevertheless, for practical pur-

| Table 3 | Differences, D, Calculated to Be Significant (P <0.05) in Two Consecutive Values Monitored In Patients with Essential Hypertension |
|--------|-----------------|-----------------|-------|
| **Analytes** | **CVa (%)** | **CVl (%)** | **D (%)** |
| Sodium | 1.0 | 1.17 | 3.2 |
| Chloride | 1.0 | 1.52 | 4.2 |
| Potassium | 2.0 | 4.9 | 14 |
| Calcium | 2.0 | 2.6 | 7.1 |
| Cholesterol | 5.0 | 7.4 | 21 |
| HDL cholesterol | 7.0 | 11.2 | 31 |
| Triglycerides | 5.0 | 22.6 | 63 |
| Hemoglobin | 2.0 | 3.0 | 8.5 |

CVa assumed long-term precision CV, CVl, average total intra-individual CV in EH, D, %, statistically significant (P <0.05) difference between two consecutive values in a percentage of the first value.

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poses, such criteria should be considered with this fact in mind. Derivation of criteria on the basis of subject-specific intra-individual variation is more complicated, and in practice it suffers from the lack of a sufficient number of individual values. Calculated differences, D(%), are given in Table 3. The values were calculated under the assumption of a long-term analytical precision that represents the present state of the art.

I thank Dr. Haseler (Poliklinik Plauen) for organizing the blood collection of the EH group, and C. Schneider, G. Lochbaum, and P. Junghans (Bezirkskrankenhaus Plauen) for technical assistance.

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The SimuTRAC™ FT₄/TSH Assay Evaluated as a First-Line Thyroid-Function Test

We evaluated the SimuTRAC TRAC FT₄¹⁵⁷Co/TSH¹²⁵I dual-isotope assay for the simultaneous measurement of free thyroxin (FT₄) by radioimmunoassay analog techniques and of thyrotropin (TSH) by immunoradiometry. Inter- and intra-assay CVs were <10% over the entire range tested except for 15.9% at the lowest FT₄ concentration. Results obtained by the SimuTRAC assay allowed complete differentiation of 85 hypothyroid patients and 35 hypothyroid patients from normal subjects. However, such estimations of FT₄ or TSH concentrations occasionally were misleading for assessing thyroid status in various clinical conditions. We conclude that the SimuTRAC assay has the same inherent disadvantages possessed by FT₄ analog and TSH immunoradiometric assays; however, where results of one of the simultaneous assays may be misleading, the results provided by the other may indicate the underlying pathology without requiring an additional assay.

Additional Keyphrases: free thyroxin · thyrotropin · monoclonal antibodies · immunoradiometric assay · radioimmunoassay · pregnancy · nonthyroidal illness · goiter

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Recent developments in thyroid-function testing have allowed measurement of thyrotropin (TSH) by immunoradiometric assay (IRMA) (1). We and many others (2–6) have found TSH measurements by IRMA to be a reliable and convenient screening test for the biochemical assessment of thyroid status, but others have noted that TSH measurements by assays with more sensitive detection limits may be misleading in certain conditions (7–12).

With the development of a dual-isotope assay that allows simultaneous determination of "free" thyroxin (FT₄) by radioimmunoassay (RIA) analog techniques and determination of TSH by IRMA, we decided to evaluate the analytical characteristics of this assay, and to compare it with FT₄ and TSH assays currently in use in our laboratory for evaluating several clinical conditions.

Materials and Methods

Assays. SimuTRAC™ FT₄¹⁵⁷Co/TSH monoclonal antibody¹²⁵I kits were supplied by Becton Dickinson, Rutherford, NJ 07070. Standards, supplied in a human serum matrix, contained both TSH (range: 0–155 mili-int. units/L) and FT₄ (range: 0–127 pmol/L) and were calibrated by

¹ Nonstandard abbreviations: TSH, thyrotropin; FT₄, (free) thyroxin; FT₃, (free) triiodothyronine; IRMA, immunoradiometric assay.