been found in patients with disorders such as myocardial infarction, various forms of cancer, liver disease, etc. that affect the general condition.

Because of the differences in Se intake, it is not adequate to compare serum Se values recorded in the U.S.A. with others from Finland. If the patients reported by Moore et al. are studied further, their dietary Se intake should be most carefully assessed.

Serum Se concentration is influenced by dietary Se intake, which in turn depends on the availability of Se from the soil.

In Finland, as in New Zealand and certain parts of China, the mean values for Se are low, in contrast to other countries such as Canada and the U.S.A., where dietary Se intake and serum Se concentrations are much higher (2). These differences are independent of analytical method used. The method we have used to measure Se has been found accurate in several (both domestic and global) intercomparison trials, one of which included up to six independent methods (5). Furthermore, we have used the same serum pool for the past five years as an internal quality control in addition to an external serum standard (Seronorm 103; Nyegaard & Co., Norway).

In the present study, the mean Se values were higher than in previous Finnish studies where the samples have been analyzed at the same laboratory (4). This is explained by the fact that during 1979–1981, when the present study was performed, the mean Se intake in Finland was temporarily increased, owing to the consumption of wheat imported from the U.S.A. (6).

References

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L-Alanine-4-nitroanilide as a Substrate for Microsomal Aminopeptidase

To the Editor:

Recently, a colorimetric method for assay of microsomal aminopeptidase (MAP, EC 3.4.11.2) activity in serum was described (1) in which a new substrate, L-leucyl-3-carboxy-4-hydroxyanilide (LCHA) is used. Two reagents are required and the product resulting from MAP activity at 37 °C for 20 min is measured at 535 nm, after 5 min of color development. Advantages of this method include simplicity, sensitivity of the substrate, high enzymatic hydrolysis rate, lack of the interference with 4-nitroanilide by serum components that is observed in other procedures, and the fact that LCHA is not affected by cystyl-aminopeptidase (EC 3.4.11.3).

For clinical reasons, we are interested in measuring MAP activity in urine. Because such activity is quite low (eight- to 10-fold less than in serum), we have tried different substrates, finally using, with some modifications, the method described by Jung and Schols (2), in which L-alanine-4-nitroanilide is the substrate. We have also adapted this modified method to the Hitachi 705 Analyzer, using it for the automated measurement of MAP activity in serum. In the latter case, by following the enzymatic hydrolysis of the substrate kinetically at 415 nm, we obviate any problem of interference by substances in serum with the 4-nitroanilide formed in the course of the reaction, thus making possible the assay of hemolytic, lipemic, or icteric samples. The rate of enzymatic hydrolysis of this substrate, under our conditions, is 201% of that with L-leucine-4-nitroanilide, a substrate commonly used in the measurement of MAP activity. This ratio, expressed as percentage, is close to the one found by Shimamoto et al. (1) for LCHA—about 230% for serum from normal subjects. L-Alanine-4-nitroanilide is readily soluble in aqueous solutions and is stable for at least 14 days at 4 °C (the light-yellowish color that appears is the result of some spontaneous hydrolysis of the substrate and does not alter the performance of the test). We use a phosphate buffer (100 mmol/L, pH 7.4) and a 2 mmol/L final concentration of L-alanine-4-nitroanilide (Table 1). The method is exceptionally simple, 180 determinations can be done per hour, and it can easily be automated in practical all types of chemical analyzers (in the Hitachi 705 we use two reagents, but a single reagent can also be used). In relation to the assay with LCHA, it has all the theoretical advantages of kinetic procedures over those in which a single measurement is made after a fixed time interval. For three MAP activities in serum—58, 93, and 285 U/L—the within-run CVs were 0.81, 0.80, and 0.63, the between-run CVs 0.93, 0.88, and 0.87, respectively. Color intensity is linearly related to enzymatic activity up to 1100 U/L. The regression equation for found (y) vs theoretical (x) values is $y = 0.944x + 17.9$ ($r = 0.999$). Our reference interval is 27–93 U/L for healthy males and nonpregnant women.

In respect to the susceptibility of L-alanine-4-nitroanilide to the action of cystyl-aminopeptidase, it can be stated that this substrate behaves much the same as LCHA. We have studied MAP activity in sera of women in their third trimester of pregnancy, using L-alanine-4-nitroanilide and L-leucine-4-nitroanilide. In all cases there were increases of MAP activity as measured.

<table>
<thead>
<tr>
<th>Test code</th>
<th>Assay code</th>
<th>Sample vol</th>
<th>Reagent 1, vol</th>
<th>Reagent 2, vol</th>
<th>Wavelength 1</th>
<th>Wavelength 2</th>
<th>Reagent blank absorb.</th>
<th>Reagent blank concn</th>
<th>Standard concn</th>
<th>Factor</th>
<th>Normal range, L</th>
<th>Normal range, H</th>
<th>Absorbance limit</th>
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</thead>
<tbody>
<tr>
<td>MAP 37 °C</td>
<td>Rate-19-31</td>
<td>20 μL</td>
<td>400 μL</td>
<td>660 nm</td>
<td>415 nm</td>
<td>—</td>
<td>0</td>
<td>0-0-0</td>
<td>0-3600</td>
<td>27</td>
<td>93</td>
<td>20 000</td>
<td></td>
</tr>
<tr>
<td>Reagent 1: phosphate buffer, 100 mmol/L, pH 7.4</td>
<td>Reagent 2: L-alanine-4-nitroanilide, 23 mmol/L, in the phosphate buffer.</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1. Conditions for the Measurement of Serum MAP Activity in the Hitachi 705 Analyzer
with either substrate, but the observed activity was 55.7% lower with L-alanine-4-nitroanilide, in contrast with what happens in sera of males and nonpregnant women. Evidently, L-alanine-4-nitroanilide behaves like LCHA in this regard, probably reflecting the relative resistance of these substrates to the action of cystein-aminopeptidase and perhaps other a-aminocacylpeptide hydrolases whose activity may be increased in the sera during pregnancy.

We thus find this method to be fast, kinetic, and readily amenable to total automation, and we believe it has practical and theoretical advantages over those in which LCHA is used as substrate.

References

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Concentrations of Trypsin, Elastase and Carbohydrate Antigen CA 19-9 in Serum of Cystic Fibrosis Patients

To the Editor:

A letter from Duffy et al. (1) has prompted us to report our findings. We measured concentrations of trypsin (EC 3.4.11.4), elastase (EC 3.4.21.11), and CA 19-9 in serum from nine patients with confirmed cases of cystic fibrosis (CF) and from 20 healthy persons as controls. CA 19-9 in serum from a patient with chronic pancreatitis (Figure 1) leads us to suggest that the pancreas may indeed be the source of the serum CA 19-9 concentration in CF, a chronic progressive disease.

References

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Lack of Effect of Isotretinoin on Thyroid-Function Tests

To the Editor:

With the increasing popularity of isotretinoin (Accutane; Hoffmann-La Roche) for the treatment of acne vulgaris, there is much interest in the effect of this retinoid on results of laboratory tests. There has been only one report of the effect on thyroid-function indices. Marsden et al. (1) studied seven patients who were receiving 1 mg/kg per day dosage of isotretinoin for 12 weeks and found a significant decrease in total thyroxin (TT4), free thyroxin index (FTI), and total triiodothyronine (TT3). They found no significant change in serum thyrotropin (TSH) in the basal state and after stimulation with thyroliberin.

We have studied thyroid function in 24 healthy male subjects who received 1 mg of isotretinoin per kilogram of body weight per day for 16 weeks for the treatment of acne vulgaris. They received no other medication and were instructed to abstain from ethanol and...