A Color Test for Methanol

To the Editor:

Accidental methanol poisoning in children and adults may result in severe metabolic acidosis, ocular toxicity, coma, and death (1). Chemical identification of the poison and analysis for methanol in a biological liquid are desirable before starting treatment (2). In modern laboratories these analyses are easily done with analytical instruments (3, 4) but many hospitals have no such facilities (5). We have developed a simple color test for the analysis of methanol in serum and poisonous solutions.

In the first reaction of the three-enzyme system (Figure 1), alcohol oxidase converts methanol to formaldehyde. This reaction is nonspecific. Specificity occurs at the second stage with formaldehyde dehydrogenase. The NADH generated in this step is required for the last color development stage.

For convenience, we stored the reagents as a crushed, dry, cold mixture (stable at −4 °C for ≥3 months) in small quantities, ready for dissolving in buffer just before analysis. The following reagent mixture would allow the analysis of 100 samples: alcohol oxidase (4.3 U), formaldehyde dehydrogenase (16 U), NAD+ (11 mg), tetranitroblue tetrazolium (12 mg), diaphorase (86 mg), bovine serum albumin (162 mg), and buffer (100 mmol/L potassium phosphate dibasic, adjusted to pH 8.35 with potassium phosphate monobasic), 50 mL.

Visual inspection and comparison with colored solutions gave semiquantitative results. We incubated 48 serum samples (20 μL each) containing added methanol with 0.5 mL of the reagent mixture for 4 min at room temperature (23 °C). The concentrations of methanol (mmol/L) in these samples were 0 (0+), 3.9 (1+), 7.8 (2+), 15.6 (3+), and 31.2 (4+). A comparison was made between the colored standards and the color developed in the unknown methanol-containing solutions. The results of the visual analysis had a correlation coefficient of 0.96, a slope of 1.05, and an intercept of 0.05. The standard error of the estimate was 0.41.

Because it is costly to analyze standards in each analysis, we offer two alternatives. First, as above, aqueous solutions can be made to correspond reasonably well to the colors developed with various concentrations of methanol: 0+ = potassium ferricyanide (A), 0.273 mmol/L; 1+ = 0.33 mmol/L A, 0.065 mmol/L methyl blue (B), 14.2 mmol/L cobaltous nitrate (C); 2+ = 0.43 mmol/L A, 0.084 mmol/L B, 18.3 mmol/L C; 3+ = 0.36 mmol/L A, 0.125 mmol/L B, 44.4 mmol/L C; and 4+ = 0.45 mmol/L A, 0.153 mmol/L B, 54.6 mmol/L C. Another approach is to use a chart made from commercial paint samples corresponding to the Pantone color numbers: 0+ = color 113, 10%; 1+ = 195, 30%; 2+ = 195, 50%; 3+ = 259, 40%; plus black, 40%; 4+ = 259, 60%, plus black, 40%. We prefer the first alternative because there is no difference in media between samples and references.

Formaldehyde generated from methanol in vivo may interfere with the analyses but most is contained within the liver (1). Also, we saw no interference from serum containing ethanol (174 mmol/L), isopropanol (133 mmol/L), acetone (15.8 mmol/L), or ethylene glycol (12.9 mmol/L). However, pure and 50% aqueous ethylene glycol solutions gave false-positive results; the same strength solutions of isopropanol and acetone did not.

References

H. Gwynne Giles
Maurice Hirst
Ewa Hoffmann
Bhushan M. Kapur

Addiction Research Foundation
Toronto, ON, Canada M5S 2S1
University of Toronto
Toronto, ON, Canada M5S 1A8

Serum Neopterin in Acute Rheumatic Fever

To the Editor:

Acute rheumatic fever (ARF) is a systemic inflammatory disease that results from untreated Group A streptococcal infections of the throat (1). Historically, ARF has been associated with poverty; however, a resurgence of this disease in developed countries occurred in the mid-1980s. Because immunological mechanisms are known to play a significant role in the pathogenesis of ARF, we anticipated that measurement of neopterin concentrations could be of value in monitoring patients with ARF (2). Increased neopterin concentrations have been shown to indicate cellular immune activation in patients with allograft rejection and various diseases, including autoimmune disorders (3). Recently we found that neopterin concentrations parallel
the course of disease in patients with congestive heart failure (4). Therefore, we examined serum neopterin concentrations in patients with ARF (Immunest Neopterin; Henning-Berlin, Berlin, Germany).

The nine patients were all young soldiers from the Moscow region (mean age 19 years). Clinical diagnosis was based on the revised Jones criteria (5). Three patients had previous rheumatic histories, one with mitral valve incompetence 4–7 days after the onset of symptoms. All patients received anti-inflammatory therapy (indomethacin 150 mg/day) for 28–35 days. At the end of the study, one patient had mitral and aortic incompetence; bacterial endocarditis in this case was excluded. One other patient had mitral prolapse. One patient was lost from follow-up because of transfer from the unit. The control group consisted of 24 healthy volunteers from the same geographic region.

The mean ± SD serum neopterin concentration in healthy control subjects was 5.2 ± 2.1 nmol/L, which is similar to values obtained from 399 healthy volunteers in Austria (6). The mean ± SD neopterin concentration in patients with ARF (15.1 ± 10.7 nmol/L) was higher than that observed in the control group (P <0.05; Wilcoxon rank test). Six of the nine (67%) patients with ARF had increased neopterin concentrations (Figure 1). The four patients with the highest neopterin concentrations had different forms of arrhythmias, and three of them had heart-valve lesions. There was no association between these findings and other laboratory markers such as erythrocyte sedimentation rate (ESR) or concentration of C-reactive protein, although all patients had an increased ESR (>20 mm/h). There was an inverse correlation between ESR and neopterin concentrations, indicating that a greater change of ESR was observed preferentially in patients with lower neopterin concentrations. However, the correlation was just below the level of statistical significance (r = −0.681, P = 0.06).

After therapy, seven of the eight remaining patients had lower neopterin concentrations than they had at study entry (P <0.05; Wilcoxon paired rank test; Figure 1), but neopterin concentrations in four patients were still outside the normal range for healthy control subjects. The neopterin concentration continued to be very high (25.9 nmol/L) in the patient who also had the highest value at the beginning of the study (patient L). The neopterin concentration remained unchanged in patient B, who had rheumatic mitral incompetence from childhood. A decrease of neopterin from 18.9 to 14 nmol/L was found in patient Z, whose mitral prolapse developed during the follow-up study.

In an earlier study, we found increased neopterin concentrations in patients with dilated cardiomyopathy and chronic myocarditis (4). Similarly, increased neopterin observed in ARF patients during the follow-up study was associated with heart-valve lesions. These data suggest a common immunopathogenetic mechanism of heart lesion in ARF and chronic myocarditis. However, an increase in neopterin is not specific for patients with myocardial inflammation. Neopterin changes indicate interferon-γ-dependent cell-mediated immune activation (3). Autoimmunity and viral diseases are commonly associated with an increased expression of major histocompatibility complex (MHC) antigens on targeted tissue induced by interferon-γ (7). In agreement, a recent report demonstrated aberrant increased expression of MHC class II antigens on heart-valve fibroblasts of rheumatic patients undergoing valvular surgery (8). Thus, cytokines such as interferon-γ, which increase expression of fibroblast class II antigens, may be very important for the disease process.

In patients with tuberculosis and with chronic bacterial pneumonia but not viral pneumonia, a significant correlation between neopterin and ESR has been described (9, 10). Such a correlation was absent in our patients with ARF; instead, we saw an apparent inverse relationship, indicating highly increased ESR preferentially in patients with normal neopterin concentrations. The small number of patients we examined may limit the relevance of these observations but may support the view that viral infections are more likely than bacterial infections to be involved in the development of valve lesions.

In conclusion, our data indicate that neopterin measurement may be useful in predicting the development of heart-valve lesions in patients with ARF. However, further studies are required to clarify this point.

This work was financially supported by the Bundesministerium für Wissenschaft und Forschung, Sektion Forschung, Austria.

References
days of life in mammals and confirming previous data obtained in humans (3, 4).

Moreover, we measured the EDLF concentrations in 30 milk specimens from 12 cows (from 6 cows, milk specimens were collected for 4 successive days); the mean (± SD) EDLF concentration found in milk specimens of these cows was 46.2 ± 10.8 ng/L digoxin equivalents (de) (range 20.5–60.0 ng/L de). We did not find any increase in EDLF concentrations after boiling the milk specimens, thus suggesting that EDLF are not (or weakly) bound to milk proteins, as also previously reported for human milk (4).

Finally, to determine whether industrial techniques for preparing artificial milk formulas for babies could affect the EDLF concentrations, we assayed three different types of milk formulas for neonates, choosing the most popular commercial products in Italy: five formulas for preterm babies (ESPGAN 1982), eight adapted formulas (ESPGAN 1977), and five cow-milk-based formulas (CODEX 1986, 1976) (all are powdered milk; reconstitution with water to the desired dilution was done as suggested by the manufacturers). No significant difference was observed among EDLF concentrations in the different formulas assayed: a mean EDLF value of 42.7 ± 16.9 ng/L de was found, which is very similar to that of native cow milk (46.2 ± 10.8 ng/L de), but lower than that previously observed in human milk (60.6 ± 4.9 ng/L de) (1).

In conclusion, our data indicate that (a) EDLF are normal constituents of milk from humans and several types of domestic animals; (b) industrial techniques for the preparation of artificial milk formulas for babies do not affect EDLF concentrations present in the native cow milk used.

References

Pascal Biver
Aldo Clerico
Maria Grazia Del Chicca
Gian Carlo Zucchelli
Carlo Cipolloni

Cattedra di Neonatologia
Università degli Studi di Pisa
1 Istituto di Fisiologia Clin. del CNR
Pisa, Italy

Cost-Effective Method for Detection of "Hook Effect" in Tumor Marker Immunometric Assays

To the Editor:

Under certain conditions, immunometric assays can give inaccurate measurements when the concentration of the analyte is greatly in excess of that of the antibody. This phenomenon, termed the "hook effect," occurs when the apparent concentration of an undiluted specimen with a high concentration of analyte falls within the calibration curve of the assay. Sandwich-type immunometric assays, in which all components are added simultaneously, are particularly susceptible to this effect. Although most analytes do not reach sufficient serum concentrations to be affected by the hook effect, some tumors secrete extremely large amounts of specific proteins, used as tumor markers, and give rise to this phenomenon, in which the serum concentration of the analyte may appear to be normal. In this laboratory we have previously experienced the hook effect with specimens analyzed for pro lactin and prostate-specific antigen (PSA) (1–4). For example, the concentration of PSA in a specimen that was 50 660 µg/L was initially found to have a concentration of 5.4 µg/L when undiluted (4).

Although underestimations of tumor marker concentration due to the hook effect occur infrequently, the consequence of such an error has serious medical implications. We have tried several approaches to detect samples that display the hook effect while maintaining cost-effectiveness. Our initial strategy was to analyze all patients' specimens at a minimum of two dilutions; however, this increased the cost of analysis by ≈100%. As an alternative, we use the following strategy: all specimens in a run are batched in groups of 10. As the specimens are prepared for analysis, an aliquot from