Effect of Ascorbic Acid on Leukocyte Esterase and Hemoglobin Test Strips

To the Editor:

During a recent evaluation of a new glucose test (1), we also evaluated the effect of ascorbic acid on results for 1-min leukocyte esterase (LE) and hemoglobin found on the new Chemstrip 8 (BM 33071 test strip; Bio-Dynamics, Boehringer Mannheim Diagnostics, Indianapolis, IN 46250). Ascorbic acid interferes with other tests on urine chemistry strips (2) and reportedly affects the 15-min leukocyte esterase test (3). Therefore, we attempted to clarify the effects of ascorbic acid on these two tests.

To 10 fresh, cell-free urines with negative ascorbic acid test results (C-Stix; Ames Co., Elkhart, IN 46514) and a relative density of 1.020 or greater, we added polymorphonuclear leukocytes to give a final concentration of 25 or 75 per microliter. Each urine was divided into four portions and ascorbic acid was added to give a final concentration of 0, 200, 400, or 800 mg/L. All portions were then tested by dipping the strip containing the LE pad into the urine and immediately withdrawing the strip along the rim of the container to remove excess fluid (3). The test pad was observed 1 min later.

To 10 fresh, cell-free urines with negative ascorbic acid test results and relative density of 1.020 or greater, we added erythrocytes obtained from fresh capillary whole blood. The cells, diluted in isotonic saline, were added to each urine to give a final concentration of 10 or 50 erythrocytes per microliter. Each urine was then divided as before and ascorbic acid was added to give concentrations of 0, 200, 400, or 800 mg/L. All portions were then tested by the same technique, with observation of the hemoglobin test pad at 1 min.

To establish a reference, we also tested the hemoglobin test pad on the "Hema-Combistix" (Ames). In this series, we observed the test pad at 30 s, as suggested by the manufacturer.

At the concentrations of ascorbic acid tested, we saw no interference with the LE reaction in comparison of the specimens containing no ascorbic acid vs those containing 200, 400, or 800 mg of ascorbic acid per liter. Evidently ascorbic acid in the clinically achievable concentrations will not affect the LE test.

Ascorbic acid also had no effect on the new Chemstrip 9 hemoglobin test, but the Ames strip showed partial interference at 400 mg/L and complete interference at 800 mg/L of ascorbic acid. These data are in harmony with those of a similar study (2). The hemoglobin pad on the BM 33071 appears to be resistant to interferences by ascorbic acid at the concentrations tested.

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References

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NMR Evidence for Multi-Component Spin Lattice Relaxation Time in a Case of Cryoglobulinemia

To the Editor:

Normal and pathological human sera generally present a single peak for H2O in the 3 H NMR spectra, and spin lattice relaxation times are adequately described by a single component (1, 2). In the context of our current studies we report here a case in which the H-1 spectra show two peaks in the chemical shift region of water; the application of a multi-exponential analysis is necessary to fit the obtained experimental results.

One of our patients had cryoglobulinemia. His serum became milky and sticky at about 30 °C. Electrophoresis on a close acetate showed a peak in the g2 zone, which was resolved into two mononuclear peaks by "high-resolution" electrophoresis on agarose gel. The more cathodic peak was due to a typical rheumatoid factor (IgM vs IgG), the more anodic was typified as a complex of IgM vs IgG and LDL (low-density lipoproteins). Despite the high relative (to H2O) viscosity of his plasma at 37 °C (3.43; our normal range 1.6–2.0), the plasma showed a low surface tension (51.8 dyn/cm; our normal range 81.5–63.9).

Serum, separated from the patient's blood without delay, was put in a NMR tube 3 mm in diameter that was supported coaxially within a 5-mm outer tube filled with a deuterated lock solvent. In separate experiments, acetone-d6 and benzene-d8 were used as lock solvents, with identical results. All measurements, completed within the next 5 h, were performed with both a Bruker WP-80-SY (80 MHz, Aspec 2000 computer system; Bruker Analytische Messtechnik GmbH, Karlsruhe, F.R.G.) and a Varian XL-200 (200 MHz, Univac V77-200 computer system; Varian Associates, Palo Alto, CA) NMR instrument.

Spin lattice relaxation times T1 were studied by applying the 180°–90°–90° (inversion recovery) pulse sequence (3). Samples were kept at 32 or 37 °C by use of a thermocouple control. In the applied pulse sequence, the 180° pulse width for the Bruker 80-MHz instrument was 8.8 μs, 13 μs for the Varian 200-MHz instrument. Relaxation curves were determined from experiments of four to 20 pulses, the pulse separation varying in the 80-MHz instrument from 0.1 to 10 s and in the 200-MHz instrument from 0.5 to 20 s. The sequence repetition time was chosen to be long enough to allow the spin system to reach equilibrium between sequences.

In the 180°–90°–90° inversion recovery experiment, T1 was obtained by fitting the following equation to the experimental data:

\[ M_s(t) = M_0 - (M_0 + M_w)e^{-t/T_1} \]

where \( M_0 \) = magnetization at \( t = 0 \), \( M_w \) = magnetization at equilibrium (\( t = \infty \)).

by using a three-parameter (\( M_0, M_w, T_1 \)) exponential least-squares fit of the peak amplitudes as a function of the time parameter \( t \).

The multi-exponential recovery function taken into account was:

\[ M_s(t) = \sum_{n=1}^{∞} M_w(n - e^{-t/T_n}) \]

where \( n = 2 \).

The multi-exponential analysis was carried out by using a modified program (4) for performing a four-parameter exponential least-squares fit.

Measurements of spin lattice relaxation time for water at different temperatures on the 80-MHz instrument gave the following results: (a) at 32 °C the uni-exponential model unsatisfactorily approximated the experimental data; (b) at 37 °C the decay could be fitted only by using a bi-exponential approach as reported in Table 1.

| Table 1. T1 Values for the Patient’s Serum at 80-MHz |
|------------------|-----------|-----------|-----------|-----------|
| Uni-exponential fit | Bi-exponential fit |
| T1    | M01 | T11 | M02 | T12 |
| At 32°C | 1.55 ± 0.04 | 4.03 | 0.95 | 1.65 | 2.45 |
| 1.69 ± 0.07* |
| At 37°C | 1.93 ± 0.19 | 4.63 | 0.73 | 2.76 | 4.75 |
| 1.93 ± 0.09* |

*Values for the patient’s serum after his complete recovery.

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