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Use of Bisulfite in the Streptococcal Anti-Nicotinamide Adenine Dinucleotidase Test

ELIA M. AYOUB[1] AND JOSEPH J. FERRETTI
Department of Pediatrics, University of Minnesota School of Medicine, Minneapolis, Minnesota

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ABSTRACT

AYOUB, ELIA M. (University of Minnesota School of Medicine, Minneapolis), AND JOSEPH J. FERRETTI. Use of bisulfite in the streptococcal anti-nicotinamide adenine dinucleotidase test. Appl. Microbiol. **14**:391–393. 1966.—A major drawback in the performance of the streptococcal anti-nicotinamide adenine dinucleotidase test on a large scale has been the hazard involved in the use of cyanide in assaying nicotinamide adenine dinucleotide. The use of bisulfite instead of cyanide in the performance of this test was investigated. The assays obtained with bisulfite parallel closely those obtained with cyanide, and justify the replacement of cyanide by the safer bisulfite reagent.

One of the more recently developed antibody tests for group A streptococcal extracellular products is the anti-streptococcal nicotinamide adenine dinucleotidase antibody test (2, 4, 7). This test was found comparable to the anti-streptolysin "O" (ASO) test in the frequency with which patients with uncomplicated streptococcal infections and patients with the nonpurulent complications of such infections developed antibodies to the respective streptococcal antigens (2, 4).

The procedure described for this test in the above reports prescribes the use of cyanide for the assay (6) of nicotinamide adenine dinucleotide (NAD). Because of the obvious hazard involved in the use of cyanide in a general laboratory, it was decided to test the use of bisulfite as a substitute for cyanide in this test. Bisulfite, like cyanide, forms a complex with N-substituted nicotinamide compounds (1, 6). This complex occurs best at a neutral *p*H and has an absorption spectrum at 325 mμ similar to that of the cyanide-NAD complex. The purpose of this report is to evaluate the use of bisulfite in the performance of the streptococcal anti-nicotinamide adenine dinucleotidase test.

MATERIALS AND METHODS

Sodium bisulfite reagent. Sodium bisulfite (1 M), adjusted to a *p*H of 7.3 to 7.5 with sodium hydroxide, was used. The solution, when stored in dark bottles at 4 C, was found to be stable for periods of up to 1 week.

[1] Established Investigator, Helen Hay Whitney Foundation.

Streptococcal nicotinamide adenine dinucleotidase activity and antibody titers to streptococcal nicotinamide adenine dinucleotidase were performed as previously described (2). Precautions were taken to use preparations which did not contain inactive enzyme, since previous studies in this laboratory have shown that this will result in falsely low antibody titers (3). In comparative studies, sodium bisulfite reagent was used in one set and cyanide was used in a duplicate set of reaction mixtures. Because of differences in absorption maxima, optical densities were measured at wavelengths of 325 mμ (OD_{325}) and 340 mμ (OD_{340}) for the bisulfite and cyanide reactions, respectively.

RESULTS

Figure 1 shows the optical density readings obtained by use of cyanide or bisulfite on duplicate preparations with varying amounts of NAD. These values when plotted show linear increments in optical density with increasing amounts of NAD up to the maximum of 0.4 mg used in the test. The plot obtained with cyanide and that obtained with bisulfite show a small divergence in their slope, which becomes more marked with increasing concentrations of NAD.

Table 1 shows the optical density readings and corresponding values for units of nicotinamide adenine dinucleotidase per milliliter obtained by assaying duplicate dilutions of a stock enzyme preparation by the cyanide and bisulfite techniques. The values obtained show a close correlation, the differences being within the acceptable range of the experimental error.

The effect of light on the time-dissociation of the bisulfite-NAD complex was tested. Duplicate

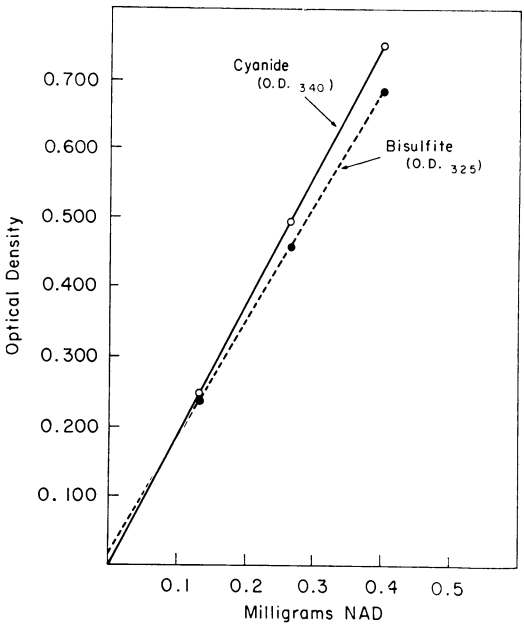

FIG. 1. *Proportionality between NAD concentration and optical density of NAD-cyanide and NAD-bisulfite complexes.*

TABLE 1. *Nicotinamide adenine dinucleotidase (NADase) activity values obtained in various dilutions of an enzyme preparation by the use of cyanide or bisulfite technique*

Enzyme dilution	Cyanide			Bisulfite		
	OD_{340}	NADase units	NADase units per ml	OD_{325}	NADase units	NADase units per ml
1:60	.170	345	20,700	.150	375	22,500
1:90	.240	240	21,600	.235	250	22,500
1:120	.285	175	21,000	.270	195	23,400
1:150	.300	150	22,500	.305	145	21,750
1:180	.320	120	21,600	.310	135	24,300

sets, each made up of a sample containing 0.4 mg of NAD in 0.5 ml of buffer and a sample containing the same amount of NAD in 0.3 ml of buffer plus 0.2 ml of a known dilution of streptococcal nicotinamide adenine dinucleotidase, were incubated simultaneously for 7.5 min at 37 C. After the addition of sodium bisulfite, one set was kept in the dark while the duplicate set was kept exposed to artificial (neon-fluorescent lamp) room light. The optical-density readings obtained at subsequent varying intervals of time are shown in Table 2. As can be seen, after a period of relative stability of about 15 min, dissociation occurs gradually in both systems at almost the same rate in light or in the dark. The dissociation appears to be higher in preparations containing the enzyme. This finding might be a result of both the dissociation of the NAD-bisulfite complex and the persistence of some enzymatic activity in these preparations.

A comparative study of the assay of the streptococcal nicotinamide adenine dinucleotidase antibody titers by the cyanide and bisulfite techniques was performed on 10 human sera. The antibody titers obtained by each technique on the tested sera are shown in Table 3. The differences in titers obtained by the cyanide and bisulfite methods are within the range of differences previously obtained by the cyanide test on a control serum.

TABLE 2. *Effect of light on the time-dissociation of the NAD-bisulfite complex*

Time	OD_{325}			
	NAD		NAD + enzyme	
	Light	Dark	Light	Dark
min				
0	.700	.705	.482	.460
2.5	.700	.705	.478	.460
5	.700	.702	.478	.460
10	.700	.700	.478	.458
15	.700	.698	.472	.452
20	.698	.698	.468	.451
25	.695	.692	.463	.448
30	.692	.692	.463	.447
40	.688	.690	.458	.442
50	.682	.685	.453	.438
60	.680	.682	.448	.432
75	.676	.680	.438	.426
90	.670	.679	.432	.420
120	.663	.662	.418	.402

TABLE 3. *Anti-nicotinamide adenine dinucleotidase (anti-NADase) titers obtained on different human sera by the cyanide and bisulfite techniques*

Serum	Anti-NADase titer (units/ml)	
	Cyanide technique	Bisulfite technique
1	94	83
2	96	105
3	109	109
4	139	128
5	255	260
6	260	275
7	340	340
8	370	363
9	510	550
10	930	930

DISCUSSION

The hazards of the use of cyanide on a large scale in a general laboratory have been recently stressed by Brandstein and his co-workers (5). Even with precautions taken with an awareness of these hazards, an appreciable degree of contamination of the laboratory atmosphere with hydrocyanic acid was found to be present.

As one of the tests for group A streptococcal extracellular antigens, the anti-nicotinamide adenine dinucleotidase compares favorably with the other tests. Moreover, it seems to be of slightly better value in patients with acute glomerulonephritis (2). The use of cyanide was a major drawback in its performance, particularly in application on a large scale. The results obtained in the above experiments indicate that sodium bisulfite is a satisfactory substitute for cyanide.

One possible disparity in the results is the dissociation of the bisulfite-NAD complex which occurs with time. In the regular performance of the test, we have found that this constitutes a minor factor, as the time lapse between reading the standard and the completion of the readings of the samples is usually within 30 min. The possible divergence in that period does not exceed 5% and is usually about 2%. This source of error can become exaggerated in the assay of a large number of sera, but can be minimized by rereading the NAD standards at intervals of 15 min and using the standard curve obtained at that interval for assessing the values obtained in the following period of time.

During the past 2 years, we have replaced cyanide with the sodium bisulfite reagent. Our precautions have consisted of storing the bisulfite at 4 C in a dark bottle and limiting the use of one batch to a period not exceeding 1 week. The results have proven quite satisfactory and justify the replacement of cyanide by the safer sodium bisulfite reagent.

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