Corrections to the Aminoglycoside Review

To the Editor:

The review by Maitra et al. (1) on determination of aminoglycoside antibiotics in biological fluids contains some imprecise and erroneous statements in the section on radioenzymatic assays.

The fact that plasmids code for the synthesis of several aminoglycoside-inactivating enzymes is firmly established (2–4). These plasmid-coded enzymes modify the antibiotics by only three known mechanisms: N-acetylation, O-adenylylation (nucleotidylation), and O-phosphorylation (4, 5). The specificity of the three categories of enzymes for various positions on the structure of the aminoglycosides was established, not by us, but by numerous other investigators (2, 4). Our article cited by Maitra et al. describes only a comparison between gentamicin concentrations as measured with an adenylating assay and a microbiological method (6)—we have never worked with aminoglycoside phosphotransferases.

The partial inhibition by chloramphenicol of measurement of serum amikacin (or gentamicin) by some acetylating enzymes was reported, not by Stevens et al. (7), who do not even mention chloramphenicol in that article, but in another paper from the same laboratory (8). The interference by chloramphenicol is ascribable to the presence in the bacterial extract that is used as a source of enzyme of both chloramphenicol acetyltransferase (9) and an aminoglycoside acetyltransferase competing for the radioactive cosubstrate acetyl CoA. This difficulty can easily be overcome by partly purifying the aminoglycoside acetyltransferase or with use of plasmid-bearing strains that are sensitive to chloramphenicol and therefore do not produce chloramphenicol acetyltransferase.

Contrary to what Maitra et al. say, the 6'-N-acetyltransferase does not act on acetylaminoglycosides (C15, 5), which does have a primary amine at position 6'-as indicated in their Figure 1. The enzyme does not act on N-acetyl gentamicin C1 (5). As Stevens et al. (7) correctly pointed out, this is of no practical significance, however, because the three minor gentamicin components that are sold commercially as a mixture differ little in their composition, and the concentrations found with use of the acetylated enzyme correlate well with those obtained by radioimmunoassays and microbiological assays.

We have used adenylating procedures for five years, but now strongly prefer aminoglycoside acetyltransferases for assays of aminoglycosides. The utilization of acetylated enzymes offers several advantages: much greater enzyme stability, recovery in higher yield from bacteria, and the requirement of more favorable cosubstrate/substrate mass ratios (10).

We did observe an interference of tetracycline on the adenylating procedure (6), but Holmes and Sanford (11), and Stevens and Young (7) did not. It is worth mentioning that tetracyclines are rarely used concurrently with an aminoglycoside, so this would not be a major drawback anyhow.

We consider the broad specificity of plasmid-coded enzymes a great advantage, not a major disadvantage (12). Because of their toxicity, the aminoglycosides are never administered simultaneously to patients. The analyst must therefore assay only one specific aminoglycoside for each patient. We have successfully measured seven aminoglycosides with use of the same reagents and a combination of 3'-N-aminoglycoside acetyltransferase and 6'-amino-N-glycoside acetyltransferase (13).

Finally, while it is true that the biologically inactive gentamicin-carbenicillin complex is not detected by the acetylating procedure, it is also not measured by radioimmunoassays (7).

References

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Detection of Abnormal Hemoglobin Variants during Glycohemoglobin Analysis

To the Editor:

It is possible to detect some abnormal hemoglobin variants (S, C, and D) during performance of glycohemoglobin analyses by the Fast Hemoglobin Test...
System (Isolab, Inc., Akron OH 44321). With this procedure, which we have found to be of value in estimating the long-term control of diabetic hyperglycemia (1), hemolyssates are applied to a cation-exchange column and eluted sequentially with no different buffered solutions. After the first fraction containing “fast” glycosylated hemoglobins ($A_1 + A_1B + A_1C$) has been eluted, a distinct dense band may be evident in abnormal variants, just below the top disc of the column, at the upper margin of the broad band of hemoglobin A, which is eventually eluted later by the second buffered solution (Figure 1). This difference, which may sometimes be visible before elution, has been seen only when hemoglobin S, C, or D were present, a fact verified by electrophoresis on starch gel in the laboratory of Dr. Titus Huisman (2). F (fetal) hemoglobin does not create a dense band inasmuch as it is not retained on the column but is eluted in the “fast” hemoglobin fraction. Hb F may thus lead to a falsely increased estimation of the presence of glycosylated hemoglobin (1, 3).

This indication of the presence of an abnormal hemoglobin should serve not only as an impetus to hematological investigation of the patient, but should also bring the validity of hemoglobin $A_I$ quantitation for the particular patient into question. Non-diabetic patients with AS genotypes have “fast” hemoglobin values that are below the normal range, and diabetics with sickle cell anemia or sickle trait have low “fast” hemoglobin values inconsistent with the amount of their hyperglycemia (4).

References

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False-Positive Solubility-Turbidity Test for Sickling Hemoglobin in a Case of Congenital Syphilis

To the Editor:
Of the several laboratory tests available for detection of hemoglobin-S, two commonly used ones are the solubility-turbidity test and tests exploiting the electrophoretic mobility properties of hemoglobin on proper supporting media. Solubility-turbidity tests, rapid and inexpensive, are the most widely used as screening tests. These tests work on the principle first described by Murayama: the stacking of hemoglobin-S molecules in a deoxygenated state (1).
One of these, the “Sickledex” (Ortho Diagnostics, Raritan, NJ 08869), is widely used for screening. Although false-positive Sickledex tests have been reported in association with several clinical or laboratory conditions, to our knowledge a false-positive Sickledex test for hemoglobin-S associated with congenital syphilis has not been reported. We wish to report such a case.
A three-month-old black girl was transferred to our institution from an outlying hospital because of unsuccessful efforts to treat a generalized skin rash, edema, and anemia. She had been born at term after an uneventful labor and delivery. The mother’s VDRL (Venereal Disease Research Laboratories) test was negative. The father had been treated for syphilis 35 years previously, but his disease was currently inactive. There was also an unclear family history of sickle cell disease in a distant relative.
When two months old, the patient developed a dry, scaly rash over her entire body. One week before admission she developed nosebleeds and generalized swelling of the extremities and abdomen.
At the time of admission to the hospital, the patient was in mild respiratory distress, with tachypnea and tachycardia. Dry, scaling skin, especially at the corners of the mouth; anasarca; 3+ to 4+ pitting edema of the back and extremities; and opisthotonic posturing were noted. The abdomen was taut and fluid-filled, with dullness on percussion. The liver edge was palpable 8 cm below the right costal margin, and the spleen was palpable 5 cm below the left costal margin. A complete blood-cell count at the time of admission showed: leukocytes 16 200/mm$^3$, hemoglobin 50 g/L, hematocrit 18%, mean cell volume 86 $\mu$L$^3$, and 12.5% reticulocytes. Serum total protein concentration was 54 g/L, albumin 19 g/L. Measurement of serum immunoglobulins showed IgA concentration to be 220 mg/mL (normal: 50–640), IgM 1.65 g/L (normal: 0.16–1.25), and IgG 8.5 g/L (normal: 1.42–9.30). The initial VDRL test on the infant was 4+ and the serum for PTA-ABS (Fluorescent Treponema Antibodies Absorbed) was 4+ reactive. The mother was weakly reactive to the VDRL test.
Initial sickle cell screen by Sickledex was positive; when repeated several times it remained positive, even when the hematocrit in the sample was increased twofold or decreased to half the initial value. Hemoglobin electrophoresis on cellulose acetate at an alkaline pH and on citrate agar gel with an acid pH showed hemoglobin A 78%, hemoglobin F 20%, but no hemoglobin S. Sickle cell screens repeated with Sickledex, Sickle Quick (General Diagnostics, division of Warner-Lambert, Morris Plains, NJ 07950), and the metabisulfite method were now negative. However, the patient had been transfused at that time to a hematocrit of 32%, and antibiotic treatment had been initiated. Results of sickle cell screen and a hemoglobin electrophoresis on the mother were also negative, and a sickle cell screen on the father was negative. The patient was treated with penicillin and blood transfusions, and symptomatically for her edema. She was discharged from the hospital, asymptomatic, in one month.