False Positive Reaction Due to Methocarbamol in the Screening Test for Vanilmandelic Acid (VMA)

D. J. Campbell, R. Sherbaniuk, and J. Rigby

Methocarbamol, a widely prescribed muscle relaxant, gives a positive result for increased vanilmandelic acid excretion with the useful screening test devised by Gitlow for evidence of pheochromocytoma. Evidence for the presence of this drug can be obtained from a positive color reaction with the 5-hydroxyindoleacetic acid test, used in the screening for carcinoid tumor.

In pheochromocytoma, 3-methoxy-4-hydroxymandelic acid (VMA), a metabolite of epinephrine and norepinephrine, is excreted in urine in abnormally large quantities. Gitlow et al. (1) have developed a semi-quantitative screening method for VMA that we have found to be useful and reliable. The procedure is particularly welcome because it permits screening of a large number of urines from hypertensive patients and is much more convenient than the measurement of catecholamines. Also, a reagent kit based on Gitlow's colorimetric reaction is commercially available.* We have performed more than 400 such urine tests and 2 patients in whom the tests indicated increased VMA were surgically proved to have pheochromocytomas.

Briefly, the test as described by Gitlow involves extraction of the acidified urine with ethyl acetate, evaporation of the solvent, and solution of the residue in dilute K$_2$CO$_3$. Diazotized p-nitroaniline is added and the mauve-colored azo-VMA product is extracted into amyl alcohol made alkaline with ethanolamine. Two readings are made in a

From the Biochemistry Division of the Department of Clinical Laboratory Services and the Department of Medicine, University Hospital, Edmonton, Alberta, Canada.

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*Pheoset, Scientific Products Division, American Hospital Supply Corporation, Evanston, Ill.
spectrophotometer: one at the azo-VMA peak at 550 m\(\mu\) and the other at the minimum at 450 m\(\mu\). Results are expressed as a ratio:

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R = \frac{\text{absorbance at } 450 \text{ m}\(\mu\)}{\text{absorbance at } 550 \text{ m}\(\mu\)}
\]

Any \(R\) value less than 1.2 reflects abnormal concentration of VMA.

The urine volume tested is that containing 0.5 mg. creatinine. The 13 cases of pheochromocytoma reported by Gitlow had \(R\) values from 1.16 to 0.45. Our two proved cases had values of 0.68 and 0.91. However, some specimens were encountered that had borderline \(R\) values between 1.1 and 1.4. On performing the quantitative procedure of Sunderman et al. (2) for VMA, these all proved to be in the normal excretion range of 1-7 mg. per day.

It was noted that several of these false-positive specimens gave a mauve color on addition of the diazo reagent and that this color was extracted partially or completely into the amyl alcohol. Further investigation revealed that these specimens came from patients taking any one of the following drugs; methocarbamol, mephenesin carbamate, mephenesin, and a cough preparation containing glyceryl guaiacolate. Interestingly, the first two drugs were reported by Honet et al. (3) to give false positives in the widely used screening method for increased 5-hydroxyindoleacetic acid (5-HIAA) for suspected carcinoid tumor.

Results

In patients free of hypertension who were being given these drugs, 24-hr. urine collections were made and the VMA screening test was carried out on a unit volume containing 0.5 mg. of creatinine. On 4 gm. per day of methocarbamol, normal \(R\) values were found in the first 24 hr. The next 24-hr. specimen gave a purple reaction, and on the fourth day a strong purple was produced that extracted into the amyl alcohol to give an \(R\) value of 0.83. When using the commercial reagents and procedure, this gave a quantitative result of 15.3 mg. VMA per day, an abnormal value. Quantitative VMA by the procedure of Sunderman gave a normal VMA output of 2.9 mg. per day. Two days after the drug was discontinued, the \(R\) value returned to normal. With a recording spectrophotometer, we found that the urine color reaction had a peak absorption of 510 m\(\mu\) and a minimum at 450 m\(\mu\). Although the azo-VMA absorption peak is at 550 m\(\mu\), the substance in the patients’ urine had enough absorption at 550 m\(\mu\) to give a low \(R\) value. Tablets containing the drug were dissolved in water and put through the test.
No reaction occurred, so it must be assumed that the false-positive test is due to metabolite of the drug soluble in amyl alcohol.

Urides from patients taking the other three drugs were similarly checked. All gave purple to red reactions, but fortunately the color did not extract into amyl alcohol when care was taken to centrifuge the solvent layer clear of water. Thus, normal $R$ values were obtained when this was done. Addition of VMA to urine of patients on methocarbamol gave an increased color reaction and decreased the $R$ value further. Apparently the diazo-VMA color reaction is not inhibited, as was reported by Ross et al. (4) in the case of the 5-HIAA, sodium nitrite, nitrosonaphthol reaction in urines of patients on chlorpromazine. In connection with this, a number of urines from patients on phenothiazine derivatives, as well as reserpine, were run through the VMA screening test. No spurious color reaction of any sort was observed. Also, with addition of VMA to urine of patients taking phenothiazine drugs, the azo-VMA color product was formed, showing that inhibition did not take place.

**Discussion**

Since all four drugs giving color reactions have a similar structure, it is not surprising that their metabolites in urine produce a similar color reaction. As reported by Sjoerdma (5), urines from patients on these drugs give a bright cherry-red color reaction with the sodium nitrite, nitrosonaphthol reagent in the simple test for increased 5-HIAA.

It should be mentioned that Gitlow recommends that patients abstain from all drugs, coffee, fruits, etc. 48 hr. prior to undergoing the VMA test. However, the problems encountered if this regimen was not followed were not identified. In our case, where many specimens are received on an out-patient basis, we found this requirement difficult to meet. Thus, the need arose to identify such problems.

**Summary and Conclusions**

Urine from patients taking the muscle relaxant, methocarbamol, gave false-positive results, indicating increased VMA levels when the useful and convenient screening method of Gitlow as well as the commercially available reagent kit were employed. Other mephenesin derivatives gave a similar color reaction, but did not interfere with the final measurement. Phenothiazine derivatives and reserpine do not
react. To identify this problem, we now carry out the 5-HIAA screening test (as slightly modified by one of us (6) to increase sensitivity) on all specimens giving an \( R \) value less than 1.4. If a cherry-red color is obtained, we know that the VMA color reaction is probably due to methocarbamol or a related compound, rather than increased VMA. Also, although it is somewhat lengthy to perform, we have found that the quantitative VMA procedure devised by Sunderman is not affected by these drugs and gives correct results.

Just prior to submitting this report, we encountered a specimen that gave an \( R \) value of 0.9 and no color reaction with the 5-HIAA screening test. Quantitative VMA values were normal. It was found that this patient was on the analgesic, anileridine dihydrochloride, a compound not related in structure to the mephenesin compounds, but having an aromatic amino group that can couple with a diazo salt. As the pure drug did not give an interfering reaction, again a metabolite must be involved. No other patients on this drug were available to confirm this result.

References