Recently, a urine specimen to be analyzed for morphine, barbiturates, and methadone tested negative by EMIT, but positive for all three drugs by thin-layer chromatography. Further investigation revealed that the patient had added sodium chloride to the urine specimen. We undertook a preliminary investigation on the EMIT system by supplementing urine specimens known to be positive for morphine, barbiturates, and methadone with sodium chloride to concentrations up to 200 g/liter. When concentrations exceeded 50 g/liter, all specimens became negative. Thus, one should be alert for the possibility of addicts clandestinely placing salt in their urines to escape detection. Fortunately, the added salt appears to nullify all EMIT tests, so that all drugs tested will be negative, which in itself may be suspicious.

Thin-layer chromatographic results are not affected (2).

pH and ionic strength play a definite role in the mechanism of enzymatic reactions (3), a role that becomes more complex in the case of EMIT (4). The effect we report here is probably attributable to an increase in ionic strength above a critical point, at which so many ions congregate at one or more charged sites that they prevent the necessary interactions. If so, the effect is nonspecific and we would expect any salt solution that contributes a high ionic strength to work in a similar manner.

References

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Thin-Layer Chromatographic Detection of Quinine, Morphine, and Poly-Drugs

To the Editor:
We read with great interest the letter [Clinical Chemistry 22, 393 (1976)] by Wilkinson et al. in which they discussed the findings of a service laboratory that had mistakenly reported the presence of morphine and cocaine in an individual’s urine. We believe that the authors’ point with regard to the use of more than one analytical procedure for confirming positive results was a valid one. Another article, by McIntyre and Armande, which appeared in the same issue, discussed their ability to detect free morphine at a sensitivity of at least 0.5 mg/liter.

We wish to call the attention of readers of this journal to the thin-layer chromatographic technique used in this laboratory. It is capable of detecting free morphine in a concentration of 100–190 μg/liter of urine. It is used to analyze 3000 urine specimens per week for opiates (morphine, codeine, methadone, and quinine), and more than 1000 specimens for poly-drugs (15 drugs— opiates plus amphetamine, methamphetamine, phenmetrazine, methylphenidate, phenothiazines, sedatives, and hypnotics). The technique has been described elsewhere (1,2). The following modifications have been introduced: (a) urine containing ion-paper is shaken for 20–30 min, (b) ratio of chloroform and isopropanol used is 5:2, and (c) drugs are extracted by shaking for 20 min. The sensitivity of this ion-exchange paper technique was described at the Sixth International Congress of Pharmacology (3).

The use of this single-step extraction and two-stage thin-layer development system enables us to measure the entire array of drugs of abuse in urine concurrently in the following minimum concentrations (mg/liter, expressed as base): morphine, 0.1 (volume of urine, 50 ml) and 0.15–0.19 (volume urine, 20 ml); amphetamine, 0.87; methamphetamine, 0.4; phenmetrazine, 0.41; methylphenidate, 0.87; codeine, 0.35; methadone, 0.45–0.9; phenobarbital, 0.5; seconobarbital, 0.36; propoxyphene, 0.90; and cocaine, 0.89. The volume of urine required for these sensitivities is 20–50 ml. We recommend that positive results obtained for barbiturates be confirmed by resorbing the residue and developing in another solvent. A technician can analyze 120 urine specimens for opiates and 80–90 specimens for poly-drugs per day. The cost of analysis for performing at least 4–5 tests (opiates) per urine specimen is $0.58 and for performing 9–15 tests (poly-drugs) is $0.82 per specimen (4), including labor, chemicals, and supplies. Our current total cost of analysis, including supervisory and administrative salaries (one chief toxicologist, one laboratory manager, one chief chemist), chemicals and supplies, laboratory rental, technical and support services, is $1.38 per specimen for monitoring 3500–4000 specimens per week. Set-up costs of a toxicology laboratory facility with thin-layer chromatography and various detection procedures currently used in drug-abuse screening programs are discussed elsewhere (5,6).

References


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Study of Contaminants and Metabolites during Therapy with High Doses of Methotrexate

To the Editor:

Several methods have been described for determination of methotrexate concentrations in body fluids, and the specificity of the methods has been stressed (1, 2).

It has long been known that clinically used methotrexate preparations contain contaminants, including 4-amino-4-deoxy-N\(^{\text{10}}\)-methylpteroyl acid, amionopterine, methopterin, and other conjugated pteridines (3, 4). These clinical preparations of methotrexate are only 93 to 97% pure (5, 6). However, possible interference of the contaminants and metabolite(s) with analytical measurements has not been tested or discussed.

We attempted to do so. Methotrexate (250 \(\mu\)g) used clinically was applied to a thin-layer chromatographic plate (Eastman Kodak Co., cat. No. 13256) and developed two-dimensionally. For the first developer we used n-propanol and 1% aqueous ammonium hydroxide, 2:1 by volume. As the second, we used 0.1 mol/liter ammonium bicarbonate (pH 8.3).

At least seven spots were located consistently under both shortwave (254 nm) and longwave (366 nm) ultraviolet sources. Their \(R_f\) values were as follows: I (1st phase, 0.173; 2nd phase, 0.239), II (0.202, 0.568), III (0.277, 0.890), IV (0.328, 0.801), V (0.386, 0.806), VI (0.549, 0.561), and VII (0.636, 0.761). Additional spots were visible only under the longwave ultraviolet light. Of the seven spots, six inhibited tetrahydrofolate dehydrogenase (EC 1.5.1.3) isolated from Lactobacillus casei. Spots I, II, IV, V, and VII contained 0.9, 0.13, 53.3, 119.9, 43.3, and 2.0 \(\mu\)g of methotrexate equivalent. However, spots IV and V could not consistently be differentiated well. A third developing system, 0.1 mol/liter \(\text{Na}_2\text{HPO}_4\) gave similar results.

In humans, it has been generally accepted that methotrexate is not metabolized significantly, most of the drug being excreted unchanged. However, a metabolite or a degradative product isolated from human urine after high-dose methotrexate treatment (50 to 150 mg/kg body weight) also inhibits tetrahydrofolate dehydrogenase. Column-chromatographic analyses revealed that there are several methotrexate metabolites or degradative products in urine after therapy with methotrexate. The elution pattern for a reconstituted lyophilized urine sample (100 ml) through a column containing diethylaminoethyl-cellulose (DE-32, Whatman) with an ammonium bicarbonate gradient (0.1 to 0.4 mol/liter) at pH 8.3 is shown in Figure 1. Usually one of these peaks (peak 1) appears on diethylaminoethyl-cellulose column chromatography of urine samples before the subject has received methotrexate treatment. There are usually three peaks (peaks 1, 2, and 3 of Figure 1) for urine samples collected during the 24 h of methotrexate infusion. Peak 3 is methotrexate. Peak 2, 4, and 5 are metabolites and (or) degradative products of infused methotrexate. Peak 2 has more than one component, one of which inhibits tetrahydrofolate dehydrogenase. Its chemical and physical nature is still under investigation, but it is not 4-amino-4-deoxy-N\(^{\text{10}}\)-methylpteroyl acid or 7-hydroxymethotrexate, although results with thin-layer chromatography suggest that both may be present in some samples. In addition, the presence of methotrexate metabolites or degradative products in plasma has been previously demonstrated (7). Thus the specificity of the enzymic kinetic method (1) for methotrexate is limited. Moreover, the specificity of radioimmunoassay and radioassay needs to be further evaluated for these methotrexate contaminants and metabolite(s).

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References


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Interpretation of Laboratory Results

To the Editor:

The chemical clinist, in addition to “analyzing,” should also know the clinical significance of his test results and be able to interpret the results in a meaningful way to the physician. Some physicians, including some pathologists, may object to this and say we are practicing medicine. A number of clinical chemists do this, and we would prefer to confine their activities to the analytical aspects of clinical chemistry.

The trouble lies in confusing interpretation with diagnosis. The chemical significance of test results is well elaborated in text books and in the medical literature. If we are familiar with this material, we can be helpful in inter-