infections theory has no satisfactory experimental pathological basis. When a patient contracts chronic tonsillitis together with systemic disorders suspected to be derived from focal infection, the most important problem is to determine whether the tonsillitis is indeed focal or not and to decide on proper treatment (13).

Individual variation was large in this study and no comparison were done by t-test in the age groups (Table 1). As the concentration of at least some of the amino acids appeared to differ with age we could reach no conclusion as to the significance of amino acid metabolism in human tonsils from comparison with the pathological groups. However, such information may eventually prove helpful in the study of tonsillar function and focal chronic tonsillitis, and further inquiry into the amino acid metabolism of the tonsil seems warranted, because abnormal amino acid metabolism caused by pathological conditions of the tonsil could assist in diagnosis of focal tonsillitis.

We thank Dr. T. Sasaki, Central Clinical Laboratory, Sapporo Medical College Hospital, for his advice on the analytical procedure.

References

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Comparison of Results for Morphine Urinalyses by Radioimmunoassay and Thin-Layer Chromatography in a Narcotic Clinic Setting

Robert J. Kokoski and Mishrilal Jain

Radioimmunoassay (RIA) and thin-layer chromatography (TLC) were compared for morphine detection in an actual narcotic clinic setting. A choice of urine from all those screened by TLC allowed a critical comparison as to actual use or non-use of narcotic drugs, rather than a sampling at random in which the question of possible false positives or negatives cannot be conclusively answered. Although RIA is more sensitive than TLC, its advantage is apparent only in those cases where urine specimens are difficult to obtain frequently regularly or where the use of morphine is suspected by the positive identification of quinine in urine that was morphine-negative by TLC. In a selected group of negative and positive specimens chosen without conscious bias, the two methods gave consistently similar results, indicating that the modified TLC method provided few or no false positives or negatives if the negatives were from those cases that were not positive anytime up to 3–4 days before urine collection. We conclude that RIA can be of significant value as a supplement to a TLC screening program, without sacrificing the many advantages that TLC has to offer.

Various analytical procedures for detecting drugs in urine are reviewed by Kaistha (1) and Finkle (2). One of the more sensitive methods for detection of narcotics is immunoassay by, e.g., the Free Radical Assay Technique ("FRA'T")1, the Enzyme Multiplied Immunoassay Technique ("EMIT"),1 hemagglutination inhibition, and radioimmunoassay. Several investigators (3–5) evaluated...

...
these methods and found that, because of its greater sensitivity, radioimmunoassay detects more morphine-positive urines than do the widely used thin-layer chromatographic procedures.

In the present study we compare morphine detection by radioimmunoassay (RIA) and by thin-layer chromatography (TLC) in a clinical setting. Possible interference by oral naloxone administration was also investigated. The study was done by superimposing RIA on selected urine specimens obtained from a clinic population that had initially been screened by TLC.

Materials and Methods

Materials: All chemicals used were of reagent grade. Test kits for RIA ("ABUSCREEN™") were obtained from Roche Diagnostics, Division of Hoffmann-La Roche, Inc., Nutley, N. J. 07110. Naloxone was obtained from Endo Laboratories, Inc., Garden City, N. Y. 11530.

Collection of urine: A total of 2375 urine samples were collected under direct observation for a period of four weeks from a group of 161 patients. The patients were paroled from Maryland Correctional Institutions participating in an out-patient abstinence and (or) naloxone-antagonist clinic program. Frequency of urine collection varied from daily to once a week, depending on the results of previous urinalyses; i.e., patients whose specimens gave negative results were able to earn "days off" from an initial routine of daily testing. All urines were analyzed by the TLC procedure described below, which is routinely used for screening all specimens obtained from the program.

Thin-layer chromatography: To 10 ml of urine, 1 ml of hydrochloric acid (12 mol/liter) was added and the mixture autoclaved at 120 °C for 15 min. The hydrolyzed urine was cooled, made alkaline with 1.5 ml of ammonium hydroxide (15 mol/liter) and extracted by shaking with 40 ml of chloroform–isopropanol (9:1 by vol). The extract was acidified with a few drops of HCl in methanol (1 ml/dl) and evaporated. The residue, dissolved in methanol, was transferred to a TLC plate coated with Silica Gel G, and the plates were developed in ethyl acetate–methanol–ammonia (85: 10:5 by vol) to an ascending distance of 10–12 cm. The dried plates were first sprayed with ninhydrin (3 g/liter of butanol) for amphetamine detection, then with H2SO4 (5 ml/liter) for quinine detection, and finally with iodoplatinate reagent for morphine detection. The morphine area on the plate was protected from the acid spray by a cover glass plate. A more detailed description of the spraying and detection techniques for morphine appears elsewhere (6).

The sensitivity of this procedure for detection of morphine added to urine was 0.5 µg/ml.

RIA of selected urine samples: From the group of urines analyzed by TLC, selected samples were tested by RIA by the "Abuscreen" procedure (Roche), using the suggested 60 ng/ml cutoff concentration for a positive determination.

Based on the results of TLC, the criteria used for the selection of urines for RIA were as follows:

- Negative urines on any Thursday or Monday for patients who had a morphine-positive on any day up through the previous three or four days, respectively.
- Morphine-positive urines in this group.
- Morphine and (or) quinine-positive urines on any Thursday or Monday from patients having mostly negative urines on the remaining days of the four-week test period.
- Negative urines, selected without conscious bias.

Results and Discussion

The results obtained were classified into the following groups:

1. Random negative urines by TLC,
2. Morphine-positive urines by TLC,
3. Morphine-negative but quinine-positive by TLC, preceded by a morphine-positive urine up through three days if collected on a Thursday or up through four days if collected on a Monday.
4. Morphine-negative and quinine-negative by TLC, preceded by a morphine-positive urine up through three days if collected on a Thursday or up through four days if collected on a Monday.
5. Morphine-negative by TLC preceded by no morphine-positive urines up through three days if collected on a Thursday or up through four days if collected on a Monday.
6. Quinine-positive urines by TLC, preceded by no morphine-positive urines during the preceding three days if collected on a Thursday or the preceding four days if collected on a Monday.

Table 1 lists results of RIA as compared with TLC. The data indicate that our TLC procedure is just as reliable as RIA with respect to randomly selected morphine-negative (Group 1) and morphine-positive (Group 2) samples. All 27 urines that were morphine-positive by TLC were also positive by RIA, and of 71 randomly selected TLC-negative urines, only two were positive by RIA. One of these was from a patient whose urine was consistently negative by TLC for four previous days, but gave several morphine and (or) quinine-positive specimens for a period of two weeks before the negative specimens were obtained. Therefore, we suspect that this patient was probably using morphine, but not at a dosage detectable by TLC. The other sample was from a patient who gave 16 negative and no positive urines during the four-week test period, but failed to give a specimen on the day before the RIA-positive urine was collected. Whether or not this RIA result was a true or false positive could not be determined.

For TLC morphine-negative urines (Groups 3 and 4) from patients showing morphine use any time up through three to four days before the negative urine, the RIA test was positive in 17 of 28 urines (61%). For those patients (Group 5) whose urine was morphine-positive by TLC only between three to four and seven days before the negative urine, the RIA test was positive in 3 of 17 cases (17%). This indicates that RIA detects morphine longer after its use (i.e., is more sensitive) than TLC, as reported by others (3–5).

It is generally recognized that to detect resumption of drug use by a patient in a treatment program, urinalyses by TLC must be made on specimens collected no less fre-

<table>
<thead>
<tr>
<th>Group</th>
<th>RIA No. samples</th>
<th>RIA</th>
<th>TLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71</td>
<td>9</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* See description of groups in text.
quently than every other day and preferably daily. Use of RIA decreases this frequency to twice weekly. For programs that may require only one specimen per week, random scheduling of collection is recommended, because, if random testing is not followed, even RIA will detect only a small percentage of the sporadic narcotic users. It is interesting that in a group of 15 patients with TLC-negative urines, who were on a once-weekly schedule, three had RIA-positive urines. One of these patients was RIA positive for two consecutive weeks and a morphine-positive by TLC was not obtained until the third week. Another was RIA positive a week before the first TLC morphine-positive and the third was RIA positive for two consecutive weeks, never showing a TLC positive. Thus use of drugs by these three patients could thus have been detected much earlier and perhaps necessary therapy or counseling initiated, including more frequent urine testing.

Quinine-positive urines: Because quinine is often used in diluting illicit heroin or morphine, quinine-positive urines in a drug clinic signal possible narcotic use. Eight of 20 (40%) quinine-positive urines (Group 6) from patients who gave morphine-negative or no specimen up to three or four days before such samples were collected were found to be positive for morphine by RIA. This is different from the comparative results obtained with the randomly chosen negative urines, only 2.8% of which were RIA positive. This illustrates the utility of quinine detection in urinalyses as an alerting signal indicating the need for more frequent testing. The possibility of a urine that, by TLC, is quinine-positive but morphine-negative can be morphine-positive by RIA is further demonstrated by comparing the results of group 3 in Table 1.

Naloxone patients: Some patients in the treatment program were on a contingency naloxone study, i.e., upon showing morphine-positive urines by TLC, naloxone was administered in single, daily oral doses starting at 500 mg and increasing to 2000 mg. On testing urines from these patients by TLC and RIA, we found that 10 of 13 such samples from patients receiving naloxone 24 h before urine collection were negative for morphine both by RIA and TLC. This suggests that the three urines found to be positive by RIA may be explained by previous narcotic use rather than by cross-reactivity of naloxone or its metabolites. Mulé et al. (3) showed that naloxone at a concentration of 290 μg/ml was equivalent to 1 μg of morphine per milliliter in its reactivity with RIA. It is very unlikely that 24 h after administration, sufficient naloxone would be present in the urine to cross-react and indicate morphine-positivity by RIA. We find that after a single 2000-mg oral dose of naloxone is administered (with no other drugs being taken) urine collected at 2, 5, and 12 h will be morphine-positive by RIA, but at 25.5 h after naloxone administration, it will be morphine-negative (<60-ng) (Table 2). This indicates that the possibility of naloxone cross-reactivity with RIA in a urine collected 24 h after administration of the drug is remote, but if no urine has been excreted for several hours before the collection, a positive RIA may result. Because naloxone or its metabolites do not interfere in the TLC test, we suggest that TLC confirmation is necessary when such urines collected 24 h after naloxone administration are morphine-positive by RIA. The TLC procedure for detection of naloxone and its metabolites has been described elsewhere (7).

Mulé et al. (3) suggest that the choice of any analytical technique or method for detection of drugs of abuse must consider the purpose for which the analysis is performed—e.g., routine urine screening, toxicology, medico-legal, or hospital emergencies. Our data indicate that for routine urine screening, TLC after acid hydrolysis, if used frequently (e.g., at least three times a week on any patient) can detect narcotic drug use for most patients, perhaps missing the few cases where drug use is sporadic. Superimposing RIA on a well-established, reliable TLC system can enhance the effectiveness of the detection program. We suggest that for a clinical setting such as we used in this study, RIA testing for morphine be performed only on those patients reporting less frequently than three times a week. If a case is RIA positive, but not confirmed by TLC because of its lesser sensitivity, then the patient can be required to report for urinalyses by TLC on a daily basis, to assist the clinician in determining what additional or alternative treatment measures could be instituted. In this way the versatility of low-cost TLC testing for a wide range of drugs of abuse can be combined with the sensitivity of RIA without undue increase in overall patient cost, an important factor in the administration and public support of such greatly needed programs.

The technical assistance of Myron Shiple, Samuel Hamner, and Elizabeth Bakutis is gratefully acknowledged. Thanks are also due to Friends Medical Science Research Center, Inc., for the use of laboratory facilities.

### Table 2. Effect on Results of Radioimmunoassay for Morphine of a 2000-mg Single Oral Dose of Naloxone

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Hours after naloxone administration</th>
<th>CPM</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0 (before drug)</td>
<td>1881</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>4408</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4064</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>3684</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>25.5</td>
<td>3589</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>37</td>
<td>2559</td>
<td>—</td>
</tr>
<tr>
<td>Negative urine (control)</td>
<td>—</td>
<td>1773</td>
<td>—</td>
</tr>
<tr>
<td>60 ng/ml morphine (positive control)</td>
<td>—</td>
<td>3640</td>
<td>+</td>
</tr>
</tbody>
</table>

RIA was performed according to the instructions for “Abusscreen-H1" (Roche Diagnostics). “Instagel" (Packard) was used as the scintillation fluid.

### References