Table 1. Effect of iodoacetate on Interference of Free-Sulphydryl-Containing Drugs with the Chemstrip Ketone Test

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc, mmol/L</th>
<th>Iodoacetate, g/L</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril</td>
<td>2</td>
<td>0</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mesna</td>
<td>20</td>
<td>0</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NAC</td>
<td>20</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>20</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Acetoacetate + NAC</td>
<td>4</td>
<td>0</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Acetoacetate + captopril</td>
<td>1</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

or urine to form a stable S-carboxymethyl derivative (7). Thus, iodoacetate may eliminate the interfering effect of free-sulphydryl-containing compounds by removing the free-sulphydryl group through forming such covalent derivatives. Because, in the presence of iodoacetate, free-sulphydryl-containing drugs initially form a color on the ketone pad that gradually fades, we suggest that the iodoacetate may also break up the color complex formed between the sulphydryl group and nitroprusside.

Our iodoacetate method does not work well with the Ames Multistix ketone test; there, the false-positive colors fade slowly and incompletely. We also caution users that urine containing iodoacetate should be used only for the ketone tests on Chemstrip, because iodoacetate interferes with the pH test and may also interfere with some of the other tests.

References

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Steroid Profile for Urine: Reference Values

To the Editor:

Weykamp et al. (Clin Chem 1989; 35:2281–4) established reference ranges for urinary steroid excretion rates by capillary column gas chromatography (GC). They found no difference between the results in groups of adults at ages 16–70 years, then showed a decline in steroid excretion rates in the elderly subjects. These results are to be expected from what is known about the changes in adrenal function with age. They discussed excretion rates of individual steroids but made no comment about total steroid excretion. Moreover, the steroid excretion rates for children, combined in a single group for ages three months to 13 years, are misleading. The difficulties in interpreting steroid profiles in the newborn were appreciated but not addressed. Because the study was organized through a number of centers in Holland, it was surprising that Weykamp et al. did not report comparability of the data for samples analyzed in several laboratories.

The data are also misleading with respect to the identification of steroid because, in some circumstances, the chromatographic method chosen does not separate steroids with different origins. For example, the peak for 11-
hydroxyandrostenedione (primarily a metabolite of cortisol) contains a variable quantity of 17α-hydroxyprogrenolonone (a metabolite of 17α-hydroxyprogesterone). The latter steroid may be marker for late-onset congenital adrenal hyperplasia (LOCAH)—a potential cause of polycystic ovaries, for which the authors have defined a discriminant function analysis based, on what they see it, on the excretion rates of androgen and 11-hydroxyandrostenedione. However, 11-hydroxyandrostenedione may also be a metabolite of 11α-hydroxyandrosterone, which is usually considered to be a specific adrenal androgen. Thus these laboratories could miss LOCAH unless the excretion of pregnantriol and 11-oxo-pregnaneoltriol is also considered.

I question where the authors see the relevance of their normal ranges in clinical practice. My conclusion is that the urine steroid metabolites assessed by this technique reflect principally the multiple products of steroids secreted by the adrenal cortex. My reasons for this are as follows:

1. Gonadal steroids produced in puberty add little to the high concentrations of androgens excreted by a child at the end of adrenarche who is going into puberty. The excretion rates of androgen and etiandrosterone and etiocholanolone rise in childhood and reflect the increased adrenal secretion of dehydroepiandrosterone sulfate.
2. There is little difference between the androgen excretion rates of men and women.
3. The steroid excretion rates of males with hypogonadotropic hypogonadism can be in the normal range.
4. The decrease in rates of androgen excretion in the elderly reflects a decline in adrenal function.

We are not informed what importance the Dutch laboratories assigned to a urine steroid profile (screening a complementary to other tests). In my opinion the object of a steroid profile is to display evidence for abnormalities of steroid metabolism when other tests may have given equivocal data. Because adrenal disorders lead to clinical signs of steroid excess or absence, with, in some cases, life-threatening
The main use of steroid profiles will be in childhood. In the age group 13 to 70 years a steroid profile as limited value. So-called nonfunctioning adrenal tumors are commonly seen post-mortem in elderly subjects. In unusual steroid products (e.g., pregnanolone metabolites) may be found in the urine of elderly subjects. A survey of the applications of steroid profile analysis (1) included few cases in adults. The situation has not markedly changed since that study. Evidence can be produced in a chromatogram for ste-onset adrenal hyperplasia (2), although without mass spectrometry this may require the preparation of a different derivative before analysis by GC. A useful application of profile analysis is in defining and monitoring steroid-secreting tumors, which often secrete unusual steroids for which assays of plasma hormones may not exist. Although some disorders of terminal metabolism have been identified in the search for known and unknown putative mineralocorticoids in some hypertensive diseases, the interpretation of the GC data has needed mass spectrometric confirmation, which the Dutch group do not report.

For the diagnosis of congenital adrenal hyperplasia (CAH), the assays of plasma steroids in the newborn require special expertise and careful interpretation in relation to gestation age, weight, and stress (3). The diagnosis of AH can now be made with urine steroid profile analysis for urine collected as soon as three days after birth; indeed, this is probably the most important application of a steroid profile (4). Results can be obtained within 30 h of receiving the sample in the laboratory. Childhood is characterized by dramatic changes both in excretion rates and relative excretion of related metabolites (5). Furthermore, because frenal disease can affect growth (androgens stimulate growth, cortisol suppresses growth), we have found that interpretation, steroid excretion sites need to be corrected for body size. So, we have made some adjustments for body size, and I have found that cortisol excretion is constant with age, whereas excretion of androgens increases from age four to seven years, immunsurates with adrenal growth and differentiation of the zona reticularis (6). The pattern of metabolites from cortisol and cortisone shows striking changes. In the newborn, the main metabolites are of cortisone, including me very polar products not seen in the adult. Ratios of tetrahydrocortisone to tetrahydrocortisol and tetrahydrocortisol to allotetrahydrocortisol also change in childhood. Weykamp et al. make no mention of how clinical situations, such as liver, kidney, and thyroid disease as well as medications and diet alter the metabolism of steroids.

Most of the adrenal disorders presenting in childhood lead to a dramatic change in the appearance of the normal GC profile, with gross increases of abnormal metabolites. Premature adrenarche, however, exhibits a subtle change in steroid excretion rates. The diagnosis is supported by showing increased rates of androgen and cortisol excretion for age, data that are consistent with accelerated growth of the adrenal gland. Because the normal ranges are 95% limits, 5% of results are expected to be outside the normal range. In some cases the steroid excretion is appropriate for body size, reflecting the benign nature of the condition.

I hope these comments will help the Dutch group and the use steroid profiles. I encourage them to establish normative data for children.

References

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An author of the article in question replies:
To the Editor:
With reference to Dr. Honour’s letter it is appropriate to explain the background of the group that performed the project on the reference values for steroid profile for urine.

When our group started the discussion on steroid profiles in urine, there was a Babylonian confusion of tongues. In fact this was not surprising: no standards were available, there were no internal control materials, no external quality-assurance program existed, we all had our own analytical procedures with the resulting nonstandard reference values, and there was no clear guide to clinical application and interpretation.

We started to tackle the problem in four steps:
1. We prepared a standard for all members of the group and we developed a lyophilization procedure of the preparation of stable urinary control materials. The lyophilized specimens are available as internal control material and are applied in the external quality-assurance program we started. The fact that we can produce samples "à la carte" enables us to set up programs in which accuracy, precision, and linearity are tested and in which specimens are entered as interpretation exercises. (The external quality-assurance program is open to laboratories outside the Netherlands. Standards and control materials are available to all who are interested.)
2. The second step was a serious study of the analytical procedures. Though we did not develop a standard method—this appeared to be impossible, given the differences in chromatographic equipment of the participants—we adopted the same methodological principles.
3. After the introduction of standards, control materials, external quality assurance, and analytical standardization, the comparability of the analytical results improved dramatically. This seemed to be the appropriate moment to set up the project to determine uniform reference values. The results were published in the article Dr. Honour refers to.
4. By now we have constructed our tools, and work is in progress to finish the job: a review on clinical importance and applications of the steroid profile in urine.

We consider that Dr. Honour’s comments and statements are really to the point but are more relevant to step 4 than to step 3 of our approach to the problem.

Writing this review on the clinical importance and applications of the steroid profile in urine is as important as difficult. I appreciate any contribution and invite Dr. Honour and other experts to contact me if they want to cooperate in this project.

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