muscle tissues, a minimum of 37, 13, 32, and 20 mg of tissue (wet weight) would be sufficient to accurately quantify UDP-Gal, UDP-Glc, UDP-GalNAc, and UDP-GlcNAc, respectively.

Aliquots of muscle homogenate could be kept at −80 °C for at least 3 months, and freeze-thawing of the muscle homogenate pool did not lead to diminished concentrations of UDP-sugars. The fractions obtained after strong ion-exchange chromatography could be kept at room temperature overnight without degradation.

In 12 normoglycemic subjects the following concentrations, mean (SD), of UDP-sugars were found: UDP-Gal, 1.19 (0.75) nmol/g of tissue; UDP-Glc, 13.9 (5.4) nmol/g of tissue; UDP-GalNAc, 3.99 (1.1) nmol/g of tissue; and UDP-GlcNAc, 6.61 (3.4) nmol/g of tissue. These amounts did not differ between male and female subjects and exhibited gaussian distributions. In muscle tissue from the five diabetics, we found similar (P > 0.01) UDP-Gal concentrations [1.05 (0.35) nmol/g of tissue], but lower (P < 0.001) UDP-Glc concentrations [5.81 (3.81) nmol/g of tissue]. UDP-hexosamines did not differ significantly from concentrations in normoglycemic patients [UDP-GalNAc, 4.40 (0.61) nmol/g of tissue; UDP-GlcNAc, 8.20 (1.96) nmol/g of tissue; P > 0.1 for both compared with concentrations in normoglycemic subjects]. These preliminary data on UDP-hexose and UDP-hexosamine concentrations in human skeletal muscle differ from earlier studies performed in rats and mice (2–7).

In contrast to most reported HPLC-based assays (3, 6, 7), all hexosamine biosynthesis pathway metabolites of interest are separated in this method. The method is sensitive enough to accurately quantify UDP-hexoses and UDP-hexosamines in small samples of human skeletal muscle, e.g., those obtained by percutaneous muscle biopsy. We currently are collecting muscle tissue from a large number of diabetics and controls to confirm and extend the preliminary data described here.

Liver-Type Arginase Is a Highly Sensitive Marker for Hepatocellular Damage in Rats, Masaki Ikemoto,1* Shoji Tsunekawa,2 Yoshinobu Toda,3 and Masayuki Totani4 (1 College of Medical Technology, Kyoto University, Kyoto 606-8507, Japan; 2 Department of Surgery, NTT West Kyoto Hospital, Kyoto 601-8441, Japan; 3 Study Center for Human Remains, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan; 4 The National Institute of Health and Nutrition, Shinjuku-ku, Tokyo 162-8636, Japan; * author for correspondence: fax 81-75-751-3945, e-mail mmas@kuhp.kyoto-u.ac.jp)

Hepatic enzymes in serum, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are routinely measured in serum for the diagnosis of hepatic disease; however, these enzymes are not liver specific because they are widely distributed in nonhepatic tissues. In contrast, urea cycle enzymes, i.e., liver-type arginase (ARG), ornithine carbamoyltransfere (OCT), and arginosuccinate synthase (AS), exist almost exclusively in the liver (1–3) and may serve as more specific markers of liver

### Table 1. Interassay CVs* for UDP-sugars.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>15 mg</th>
<th>30 mg</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>4000</th>
<th>Intraassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-Gal</td>
<td>5.9</td>
<td>6.6</td>
<td>5.5</td>
<td>9.9</td>
<td>6.2</td>
<td>7.2</td>
<td>3.0</td>
<td>5.0</td>
<td>6.4</td>
</tr>
<tr>
<td>UDP-Glc</td>
<td>3.3</td>
<td>6.9</td>
<td>4.7</td>
<td>4.4</td>
<td>1.6</td>
<td>7.8</td>
<td>5.8</td>
<td>5.4</td>
<td>5.0</td>
</tr>
<tr>
<td>UDP-GalNAc</td>
<td>1.3</td>
<td>12</td>
<td>9.2</td>
<td>6.6</td>
<td>3.1</td>
<td>4.2</td>
<td>3.6</td>
<td>3.7</td>
<td>5.4</td>
</tr>
<tr>
<td>UDP-GlcNAc</td>
<td>6.1</td>
<td>3.9</td>
<td>2.8</td>
<td>5.6</td>
<td>3.1</td>
<td>3.2</td>
<td>3.0</td>
<td>3.6</td>
<td>5.8</td>
</tr>
</tbody>
</table>

* CVs for five separate determinations in a 15- or 30-mg wet weight muscle tissue pool and six added concentrations. Intraassay CV is the residual variance at physiological concentrations for 30-mg tissue pool.

### References


injury. It has been reported that some of the urea cycle enzymes leak rapidly from hepatocytes when liver cells are damaged (4–11). Although there are several “hepatic marker” enzymes, including the urea cycle enzymes, it is not known which one of them is the most suitable enzyme for early detection of hepato cellular injury. To confirm the most suitable enzyme for this purpose, it is important to verify changes in the serum concentrations of urea cycle enzymes after liver damage, in comparison with enzymes in routine use. Two rat experimental models were designed: (a) a chemical liver injury model induced by carbon tetrachloride, and (b) an ischemia-reperfusion liver injury model. We measured the urea cycle enzymes ARG, OCT, and AS in sera, using procedures that we described previously (9, 12). Anti-OCT and AS IgGs were conjugated with N-hydroxysuccimidobiotin, essentially as described by Akhoundi et al. (13). These conjugates were used as second antibodies. Evaluation was based on the limited localization of the urea cycle enzymes in hepatocytes and the high specificity of our antibody. Serum activities of AST and ALT were measured by an automated blood chemistry analyzer (Hitachi 736-60E).

Chemical liver injury was induced by intraperitoneal injection of carbon tetrachloride in 4-week-old male Wistar rats (n = 14). The rats were anesthetized with ether and killed at time points 0 (before treatment; control), 15 min, 30 min, 45 min, 1 h, 3 h, and 5 h for the short-interval experiments (two rats at each time point). Blood samples were collected and incubated for 1 h at 37°C; the serum was then separated. In the ischemia-reperfusion injury model, 5-week-old male Wistar rats (n = 10) were used. Each rat was anesthetized with ether, and total liver ischemia was determined by clamping the hepatoduodenal ligament, which contains the hepatic artery and portal vein, for 30 min using a small clamp unit; thereafter, the liver was reperfused by declamping the ligament. At time points 0 (before treatment), 5 min, 10 min, 20 min, and 30 min after reperfusion of the liver, blood samples were drawn quickly from the inferior vena cava; serum samples were then separated as described above.

We measured three urea cycle enzymes (liver-type ARG, OCT, and AS) and two conventional hepatic markers (AST and ALT) in the sera of rats after intraperitoneal injection of carbon tetrachloride. We then compared the patterns of increase and fluctuations of AST and ALT >5 h after treatment with the chemical agent. Serum liver-type ARG increased dramatically immediately after the injection, and within 30 min, it was ~45-fold higher than the concentration before treatment (Fig. 1A). In comparison with ARG, the concentrations of all other hepatic markers tested in this study remained low and were, at most, ~10-fold higher than before treatment. In every case, the liver-type ARG increased more on a relative basis than any of the other hepatic enzymes. An ischemia-reperfusion liver injury model was designed to confirm the leak-prone character of liver-type ARG. After reperfusion, we measured serum liver-type ARG and determined the activities of AST and ALT. A large amount of liver-type ARG leaked immediately into the blood stream ~5 min after the reperfusion. The concentration was 60-fold higher than before treatment and gradually increased thereafter (Fig. 1B). Thirty minutes after the reperfusion, the ARG concentration was 100-fold higher than the control concentration, whereas the serum AST and ALT concentrations were never >10-fold higher than in the controls throughout the course of the experiment. It has been reported that AST and ALT increase dramatically in acute liver congestion or acute occlusion of the hepatic artery (4). In our study, the increase of liver-type ARG was more rapid than increases in AST or ALT, indicating a potential clinical usefulness of ARG as a hepatic marker.

We previously described the potential importance of

![Fig. 1. Changes in serum liver-type ARG, OCT, AS, and other hepatic enzymes in rats with liver damage induced by carbon tetrachloride during a short period after the injection of carbon tetrachloride (A) and after the reperfusion of ischemic liver (B).](image-url)
measuring human serum liver-type ARG in diagnosis of hepatic disorders (9). However, a lack of knowledge about the turnover of ARG has hampered a wider application of this enzyme for clinical diagnosis. As shown in Fig. 1, our results indicate that liver-type ARG is increased substantially in the acute phase of liver disorders.

We suggest that after induction of liver damage with carbon tetrachloride and ischemia-reperfusion injury, liver-type ARG, in comparison with the other enzymes tested in this study, leaks most profusely into the blood from the damaged liver. In an immunohistochemical study (data not shown), massive disappearance of liver-type ARG from hepatocytes immediately after liver damage was demonstrated. This further supports our hypothesis that liver-type ARG is an enzyme that reflects damage to hepatocytes regardless of the cause. The release of liver-type ARG into the bloodstream appears to differ from the release of AST and ALT (Fig. 1), supporting the leak-prone character of ARG.

ALT and AST are markers of liver cytolysis (4, 14, 15). During a short period after the injection of the chemical agent, liver-type ARG increased more rapidly than ALT (Fig. 1), indicating that the clinical value of the enzyme could be equal or superior to that of ALT, which supports an earlier study indicating that after partial hepatectomy, liver-type ARG increased more dramatically than ALT and AST (5).

OCT leaks out of hepatocytes into the bloodstream through the mitochondrial and plasma membranes, whereas ALT, which is a cytosolic enzyme, and liver-type ARG leak only through the plasma membrane. The difference in the pattern of increase of ARG compared with OCT and the transaminases may be attributable to their differential localization in hepatocytes, as well as the fact that liver-type ARG has a smaller molecular mass than other hepatic-marker enzymes (13, 16).

Our results indicate that among the hepatic enzymes tested in the present study, the release of liver-type ARG may be unique, particularly in the acute phase of hepato-cellular injury, and that the measurement of ARG has its own clinical merit for detecting hepatic lesions.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (to M.I.). We thank Kiyoyuki Takahashi of Kyoto University for technical instruction and helpful advice for immunohistochemical staining.

References

6. Yu Y, Terada K, Nagasaki A, Takiguchi M, Mori M. Preparation of recombi-

Cerebrospinal Fluid Cytokeratins for Diagnosis of Patients with Central Nervous System Metastases from Breast Cancer, György Sélétormos* and Flemming Bach* (*Department of Clinical Biochemistry, Hillerød Hospital, Helsevej 2, DK-3400 Hillerød, Denmark; 2 Department of Oncology, Ålborg Hospital, DK-9000 Ålborg, Denmark; *author for correspondence: fax 45-48-24-00-67; e-mail ges@fa.dk)

In cases of breast cancer, the diagnoses of parenchymal brain metastases and leptomeningeal carcinomatosis are often delayed (1). New inexpensive biochemical diagnostic initiatives have suggested measurement of cytokeratin tumor markers in the cerebrospinal fluid (CSF) (2, 3). Each type of epithelial cell can be characterized by its cytokeratin polypeptide content because the expression pattern varies with the type of epithelium (4–6). Malignant epithelial tumors and their metastases maintain the cytokeratin expression typical for the tissue of origin (4, 7, 8). Cytokeratins 8, 18, and 19 are abundantly expressed in adenocarcinoma of the breast, only weakly expressed in epithelial cells of the choroid plexus, and not expressed in nerve, glial, ependymal, and meningial cells (9). Because neither healthy nor malignant epithelial cells are known to secrete cytokeratins, how they appear in the CSF remains unexplained. Some authors have suggested that cytokeratins are released from the large fraction of dead and dying cells in growing tumors (5), whereas others have suggested that cytokeratins are markers of cell proliferation released during mitosis (10). In either