the microsomal mixed-function oxidase system responsible for biotransformation of numerous endogenous and exogenous chemicals, including toxins and carcinogens (5). This decrease in cytochrome P450 concentrations in cirrhosis was accompanied by an increase in activity of 5-aminolevulinate synthase (EC 2.3.1.37), the first and rate-controlling enzyme of hepatic heme biosynthesis (2–4). In normal human livers, we had found previously that activity of this synthase was positively correlated with iron concentration (7).

To determine whether there is such a decrease in hepatic cytochrome P450 in humans with iron overload, we have now measured hepatic iron and P450 concentrations in portions of needle biopsies. We have also studied in vivo metabolism of antipyrine, a drug metabolized by cytochrome P450-dependent oxidases (6).

Fifty-five patients were studied, all of whom had fasted for at least 12 h. None had ingested potent cytochrome P450-inducing drugs (e.g., phenobarbital, phenytoin, glutethimide, or rifampicin) within seven days of the study. In virtually all, use of alcohol or tobacco was moderate (≤3 drinks and/or ≤1 pack of cigarettes per day) or nil. Aspiration liver biopsies were performed under local anesthesia, for a variety of clinical indications, most commonly to evaluate patients for possible iron overload. A core of tissue at least 2 cm in length was sent for histological interpretation. The remainder (5–30 mg) was used for determinations of iron (7) and, when possible, cytochrome P450 (8), the latter done in duplicate in a DW-2 spectrophotometer. Antipyrine metabolism was as described (9). Concentrations of unchanged drug were measured (10) in specimens of saliva collected 3, 6, 9, 12, and 24 h after administration of antipyrine.

Patients were grouped according to the nature and severity of liver injury or the presence of heavy iron overload (3+ to 4+ by Perls’ stain and >500 μg of Fe per 100 mg of dry liver). Two of the patients with iron overload had cirrhosis. As shown in Table 1, the presence of heavy iron loading was associated with only small, statistically insignificant increases in concentrations of cytochrome P450 and rates of clearance of antipyrine. For all patients considered together, or for patients in each sub-group considered separately, there was no correlation between any of the variables studied.

Table 1. Liver Iron and Cytochrome P450 Concentrations and Clearances of Antipyrine in Patients Studied

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>n</th>
<th>Liver iron, μg/100 mg dry</th>
<th>Liver P450, pmol/mg prot.</th>
<th>Antipyrine clearance, L/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or mild changes</td>
<td>31</td>
<td>133 ± 15</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Severe hepatitis or cirrhosis</td>
<td>13</td>
<td>116 ± 27</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Iron overload</td>
<td>10</td>
<td>1850 ± 372</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

Thus our findings fail to show that hepatic iron overload in humans leads to a decrease in hepatic cytochrome P450 or mixed function oxidase activity. Interpretation should be tempered by the realization that there is a rather wide range of cytochrome P450 concentrations in livers of unrelated human subjects, which may have obscured an effect of iron overload. It is also possible that, with chronic iron loading, only very high concentrations of hepatic iron (above those observed here) are capable of decreasing concentrations of cytochrome P450, as has recently been reported in a new experimental model of dietary iron loading (11, 12).

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Incidence of Increases in β-Glucuronidase as Compared with Increases of γ-Glutamyltransferase and Other Serum Enzymes during Long-Term Anticonvulsant Therapy

To the Editor:

Plum (1) reported that, in comparison with findings for a normal population and patients suffering from different mental and neurological diseases, serum β-glucuronidase (ED 3.2.2.21) activity in epileptic patients was distinctly increased, regardless of seizure type; they could not explain this observation. Varma et al. (2) reported that the increase of β-glucuronidase activity in epileptic patients was due to active seizures and unrelated to anticonvulsant medications, and concluded that this increase has diagnostic value and prognostic implications during therapy of epileptic patients.

Recently, after statistical analysis of data from 158 patients, we reported (3) that the increase in this enzyme could not be correlated with seizure frequency. Its mean increase in patients seizure-free for longer than one year was not statistically different from the mean of patients with uncontrolled seizures. Rather, we found that these increases were linearly and directly correlated with anticonvulsant medications.

Here we report the incidence of increased β-glucuronidase activity in se-
rum, as compared with that for \( \gamma \)-glutamyltransferase (GGT; ED 2.3.2.2), alkaline phosphatase (ALP; EC 3.1.3.1), alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), and lactate dehydrogenase (LD; EC 1.1.1.27) in a large group (n = 100, 47 of whom were women) of randomly selected, chronically medicated, institutionized adult (mean age 35.5 years, range 20–71 years) epileptic patients. We also report statistically significant correlations with other parameters. Albumin and total bilirubin were within normal limits for all patients; clinically, no liver dysfunction was suspected. To establish the normal reference interval for \( \beta \)-glucuronidase activity, we obtained sera from 18 apparently healthy employee volunteers, mean age 41 years, and assayed these concurrently with sera from the patients. The activities of GGT, ALP, ALT, AST, and LD in serum were determined by Continental Bio-Clinical Laboratories, Inc., Grand Rapids, MI; these results were retrieved from patients’ files.

Serum concentrations of phenytoin, phenobarbital, primidone, carbamazepine, and valproic acid were determined either with XMT® reagents (Syva Co., Palo Alto, CA) and a Stasar III spectrophotometer (Gilford Instrument Labs., Oberlin, OH) attached to Syva AutoCarousel and Lab Processor 6000, or with Abbott Diagnostics reagents and a TD Automated Fluorescence Polarization Analyzer (Abbott Labs., N. Chicago, IL). Ultrafilters for determination of free phenytoin were obtained with XMT FreeLevel tracers from the manufacturer.

For quantitative colorimetric determination of \( \beta \)-glucuronidase activity, we used phenolphthalein glucuronide as the substrate with assay reagents from Sigma Chemical Co., St. Louis, MO 63178. In brief, we add 0.2 mL of serum to a mixture of acetate buffer (pH 4.5) and phenolphthalein glucuronide solution, then incubate at 56 °C for 1 h. A reagent blank (excluding serum) and a serum blank (excluding substrate) are prepared similarly. After addition of 2-amino-2-methyl-1-propanol (pH 11), we measure the absorbance of the reagent blank, serum blank, and test specimen at 550 nm vs water as reference. The absorbances of the reagent and serum blanks are subtracted from the test reading, and the \( \beta \)-glucuronidase activity, expressed in terms of phenolphthalein concentration (\( \mu \)g/mL of serum), is obtained from the calibration curve.

Table 1 shows the incidence of increases of serum enzymes, expressed as multiples of the upper limit of normal values. GGT, \( \beta \)-glucuronidase, ALP, ALT, AST, and LD were increased in 79, 68, 49, 23, 13, and 5% of the patients, respectively. It is interesting to note that this order is the same as the order of the magnitude of the increases. Furthermore, the incidence as well as the magnitude of ALT, AST, and LD increases were relatively small. For 27 patients, activities of GGT, \( \beta \)-glucuronidase, and ALP were all increased, and in no case was ALP the only enzyme with increased activity. We emphasize that this random screen refers to chronically medicated (for more than three years) patients, so that results are likely to differ from the serum enzyme status of patients who are just beginning anticonvulsant therapy.

Linear regression analysis for patients with increased \( \beta \)-glucuronidase activity (n = 68) showed a direct correlation with free phenytoin concentrations in ultrafilters from patients cotreated with valproic acid (\( r = 0.509, n = 33, p = 0.0025 \)), with concentrations of phenobarbital in serum (\( r = 0.445, n = 30, p = 0.0137 \)), and with increased GGT activity (\( r = 0.541, n = 50, p = 0.0001 \)).

Increases in \( \beta \)-glucuronidase in epileptic patients can apparently be related mainly to anticonvulsant medications, in confirmation of our earlier conclusion (3); moreover, after GGT, \( \beta \)-glucuronidase is the enzyme most frequently increased.

Among other pathological conditions (such as alcoholic cirrhosis), GGT activity in serum is known to be increased in patients medicated with anticonvulsants (4). As with GGT, the \( \beta \)-glucuronidase increase probably reflects increased hepatic synthesis in response to chronic load of anticonvulsants presented for metabolism. It remains to be established to what extent (if any) monitoring serum \( \beta \)-glucuronidase activity can clarify the subject of anticonvulsant drug interactions and enzyme induction or slowly progressing clinically unrecognized microscopic liver damage (5).

References

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Improved Liquid-Chromatographic Determination of Catecholamines in Platelets

To the Editor:

Many procedures for measuring catecholamines in human plasma have been described, principally radioenzymic or "high-performance" liquid chromatography (HPLC) with electrochemical detection (see 1 and its references). Determination of catecholamines in plasma can aid in the diagnosis of pheochromocytoma; however, such measurements may lack diagnostic specificity, because catecholamine concentrations can also be increased in subjects who are stressed. It has therefore been suggested that measurement of catecholamines in platelets may be a better indicator of pheochromocytoma because platelet catecholamines do not appear to subject to the rapid changes in concentration seen in plasma (2).

In previous studies (2, 3) in which platelet catecholamines were examined, the radioenzymic assays used were both complicated and time consuming and hence open to some degree of experimental error. In this study we used HPLC with electrochemical detection. Having evaluated several methods of platelet extraction, we opted for the following procedure.

Blood from 13 rested and supine healthy volunteers was collected, with EDTA as anticoagulant. Platelet-rich plasma (PRP) was prepared by low-speed centrifugation and the volume...