phane bond was not affected by temperature.

Analysis of the data displayed in Table III by the Student–Newman–Keul's test (7) showed that none of the observed differences in separation force for both end and side samples was statistically significant.

Seal strength measurements of samples from the longer dimensions showed that parting took place at the polyethylene–polyethylene heat seal for all temperatures studied except 175°. At 175°, parting was at the foil–cellophane lamination. These data suggest that, except for the 175° treatment, the seal was less strong than the lamination. At 175°, the seal strength must surpass the lamination strength, causing parting at the latter site. This finding indicates that the seal strength increased as temperature increased. Miller (8) found a similar correlation.

The seal strength test was not pursued after Experiment I since a definitive discrimination among sealing temperatures was not shown.

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Comparison of Observed and Predicted First-Pass Metabolism of Imipramine in Humans

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Abstract □ The first-pass metabolism of imipramine was calculated based on the dose, hepatic blood flow, and total area under the plasma–time curve after oral administration of 0.71 ± 0.03 mg/kg of imipramine to four individuals suffering from mild depression. The predicted values of first-pass metabolism ranged from 37 to 68%, consistent with experimentally derived estimates.

Keyphrases □ Imipramine—comparison of observed and predicted first-pass metabolism □ Metabolism, first pass—imipramine, comparison of observed and predicted values □ Antidepressants—imipramine, comparison of observed and predicted first-pass metabolism

The bioavailability of imipramine following oral administration in humans was recently reported (1). A comparison of the total metabolites excreted following intravenous and oral administration shows that the absorption of impramine from the solution dosage form was complete. The low bioavailability of imipramine following oral administration was attributed to the first-pass metabolism, mainly to desipramine, as evidenced by the higher levels of this metabolite following administration of an oral dose compared to an equal intravenous dose.

DISCUSSION

The following equation was proposed by Gibaldi and coworkers (2, 3) to predict the degree to which a drug is subject to first-pass metabolism:

$$f = \frac{\text{flow rate}}{\text{flow rate} + \left(\text{dose} \Big/ \int_0^\infty C_0\, dt\right)} \qquad \text{(Eq. 1)}$$

where f is the fraction of orally administered dose that actually reaches the systemic circulation, the flow rate is the hepatic blood flow rate, and $\int_0^\infty C_0\, dt$ is the total area under the plasma concentration–time curve after oral administration. This equation is applicable, however, only if the absorption is complete, as was evidenced for imipramine (1). A correction factor is needed(2) if the dose administered orally is not completely absorbed.

Therefore, the percentage of drug metabolized during the first pass can be expressed as:

$$\%\ \text{first pass} = \frac{\left(\text{dose} \Big/ \int_0^\infty C_0\, dt\right) \times 100}{\text{flow rate} + \left(\text{dose} \Big/ \int_0^\infty C_0\, dt\right)} \qquad \text{(Eq. 2)}$$

EXPERIMENTAL

The area under the plasma concentration–time curve following oral administration was calculated from reported (1) values of apparent hepatic blood flow and apparent clearance (Table I). A mean blood flow of 1.53 liters/min (3) was used for calculations using Eq. 2.

RESULTS

As reported in Table I, the predicted first-pass metabolism, 58.25 ± 14.38, correlated very well with the experimentally determined value, 52.75 ± 21.36, obtained by comparison with the intravenous data. Therefore, the reliability of Eq. 1 for the prediction of first-pass metabolism following oral administration was confirmed.

The variability of blood flow rates does not seem to affect the first-pass metabolism estimates significantly. For example, the reported apparent hepatic blood flow rates were 1.3–3.5 liters/min (1), whereas a constant value of 1.53 liters/min was used for the calculation of first-pass metabolism in all individuals; both values resulted in fairly good agreement.

Table I—Predicted and Observed First-Pass Metabolism of Imipramine in Four Subjects

Subject	Sex	Age	Dose, mg	Body Weight, kg	$\int_0^\infty C_0\,dt$, mg min/liter	First Pass Predicted, %	First Pass Observed[a], %
A.B.	f	28	35	52	11.97	66	71
G.A.	f	28	40	55	44.24	37	23
U.F.	m	25	50	68	15.13	68	52
P.L.B.	m	59	50	71	19.84	62	65
Mean ± *SD*						58.25 ± 14.38	52.75 ± 21.36

[a] Reference 1.

Another significant aspect of the correlations presented here is that the area under the plasma concentration–time curve described the first-pass metabolism adequately, signifying that red blood cell transport is not significant in the first-pass metabolism of imipramine. This result may be due either to almost equal partitioning between blood cells and plasma or to quick equilibrations between red blood cells and plasma.

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High-Pressure Liquid Chromatographic Determination of Acetaminophen in Biological Fluids

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Abstract □ A method for the rapid estimation of free acetaminophen in biological fluids is described. The assay involves ether extraction and high-pressure liquid chromatographic analysis on a 10-μm particle-size silica gel column, using a mobile phase of 10% chloroform in tetrahydrofuran. The procedure was used to determine acetaminophen levels in urine from two healthy volunteers who ingested 650 mg of ^{14}C-acetaminophen (20 μCi), and the accuracy of the method was compared with the carbon-14 determination. The limit of detectability for acetaminophen is 1 μg/ml.

Keyphrases □ Acetaminophen—high-pressure liquid chromatographic analysis, biological fluids, compared to radioisotope method □ High-pressure liquid chromatography—analysis, acetaminophen, biological fluids, compared to radioisotope method □ Analgesics—acetaminophen, high-pressure liquid chromatographic analysis, biological fluids

For the determination of free acetaminophen [*N*-(4-hydroxyphenyl)acetamide] in biological fluids, a GLC procedure (1) has been used frequently (2–4). The method requires a silylation step to convert acetaminophen into a volatile disilyl derivative before it is suitable for GLC determination. Although GLC analysis of phenolic drugs (*e.g.*, acetaminophen) is possible by this technique, the silylation step often lengthens the analysis time and also introduces another variable. Liquid chromatography appears to be more suitable for the quantitation of these drugs. It usually requires no derivatization of samples, and nonvolatile compounds can be analyzed just as easily as volatile compounds. The main requirement is that the sample be soluble in the mobile solvent.

This paper describes a simple high-pressure liquid chromatography (HPLC) method for the rapid estimation of acetaminophen in biological fluids using a UV absorption detector. A high-performance liquid chromatographic method for the quantitation of acetaminophen was reported recently (5). The technique, which employs a less common but highly sensitive electrochemical detector, does not involve high pressure in its operation. Determination of acetaminophen in the picogram level using this technique has been reported. However, its sensitivity is limited when applied to the analysis of the drug in biological samples due to the interference of endogenous, electrochemically reactive materials.

EXPERIMENTAL

Reagents and Materials—Acetaminophen was obtained commercially[1]. ^{14}C-Acetaminophen (uniformly labeled), with a specific activity of 17.24 μCi/mg, was custom synthesized[2]. Chloroform[3], methanol[3], and acetic acid[2] were all reagent grade and were used as received. Tetrahydrofuran[4] was freshly distilled prior to use.

Instrumentation—A liquid chromatograph[5] equipped with a positive displacement pump capable of developing a pressure of 5000 psi, a stop-flow injection port, a variable wavelength UV absorbance detector operated at 247 nm, and an integrator[6] was employed. The

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