with tetramethylsilane as the internal standard, is shown in Fig. 1 along with the spectra of V-acetate and IV. The signals of the methylene protons at the C_3 position of IV at δ 3.84 (doublet, 1H) and 4.90 (doublet, 1H) ppm turned into a singlet signal (1H), shifting toward lower field to 5.05 ppm, by the metabolic transformation to V. The integration of the broad signal of V at 2.30 ppm, which could be replaced by deuterium adding CD_3OD, counted as two protons. These facts indicated that the structural change, *i.e.*, hydroxylation, had occurred at C_3 of IV. Further evidence was that the spectrum of V-acetate showed two acetyl signals at δ 1.83 and 2.26 ppm; the former was assigned to the N_1-2-acetoxyethyl group and the latter to the C_3-acetoxy group. The signal of the C_3 proton of V shifted toward lower field to 6.00 ppm by acetylation. Thus the structure of the metabolite was determined to be as shown in V.

This compound was then synthesized in a separate experiment, and its psychotropic activity was studied. Its CNS-depressant activity in DDY strain male mice was at the 50-mg/kg dose level (oral), which was approximately the same potency as unchanged I. It is of interest that V was found to be one of the metabolites in humans, and details of this work will be reported.

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Pithed Rat Blood Pressure in the Assay of Norepinephrine

Keyphrases □ Norepinephrine—analysis in plasma and cerebrospinal fluid of rats by pithed blood pressure method □ Sympathomimetics—norepinephrine, assay, pithed rat blood pressure

To the Editor:

Measurement of rat blood pressure is a standard method in biological estimations of acetylcholine (1–5), angiotensin (6), norepinephrine (6, 7), epinephrine (6, 7), and vasopressin (8). Preparations reported for norepinephrine assay show a sensitivity of 1–2 × 10^{-7} g/ml. The pithed rat blood pressure in 250 preparations, with some modifications used in our study for the estimation of norepinephrine in plasma (9) and cerebrospinal fluid (10), was sensitive from 1 × 10^{-9} to 5 × 10^{-10} g/ml. The changes followed in these preparations are described here since a highly sensitized preparation could be mounted with the additional operations.

Albino Wistar rats of either sex, 200–350 g, were used. The rats, after being fasted overnight, were injected intraperitoneally with 0.4 ml (0.4% in 0.85% saline) of atropine sulfate[1] 20 min before anesthetization. Anesthesia was induced by spraying ethyl chloride into the glass funnel initially and was maintained with anesthetic ether until the rat became unconscious.

The anesthetized rat was opened for tracheotomy, and a polyethylene cannula with a side opening was immediately introduced into the trachea and tied in position. The rat was then turned on its back to one side and pithed as described previously (6). Once the brain and spinal cord were destroyed, as seen by spasticity of contralateral muscles, the rat was immediately ventilated mechanically by connecting the tracheal cannula to a mini-respiratory pump. The regulator was adjusted to ensure sufficient ventilation so that inflation and deflation of the chest and heart rate were 30–40 strokes/min.

Both carotid arteries were ligated at the central end. Then a laparotomy was done, and the viscera were removed from the esophagus to the rectum, including the spleen; the liver was left *in situ* as per the method of Venkatakrishna-Bhatt and Haranath (3), and the bleeding points were carefully ligated. The left carotid artery and the right femoral vein were cannulated and connected to a manometer[2] and saline stand, respectively. Heparin[3], 0.5 mg, was injected into the femoral vein to prevent clotting. Then 1–3 ml of saline was injected to compensate for the loss of blood volume during evisceration.

Initially, the response with a low concentration of norepinephrine[4] (10^{-11} g/ml) was recorded on a kymograph. Sensitivity increased on repeated injections of standard norepinephrine into the femoral vein at regular intervals. Thus, when the blood pressure reached a steady level, usually after 10–15 trials, the test solution was injected. The volume of fluid injected was always kept constant, usually being 0.1 ml followed by 0.1 ml of saline. Care was taken to prevent leakage or passing of air bubbles from the syringe or leaking points.

This preparation was frequently found to be sensitive to a concentration of 10^{-9}–10^{-10} g/ml of norepinephrine when prepared in saline with 5 × 10^{-6} g/ml of ascorbic acid. Ascorbic acid in saline as a control, when injected in a way similar to the test and standard norepinephrine solutions, did not elicit a peak response as did the test solutions. Although skill and precision are essential for handling the preparation,

[1] E. Merck.
[2] Condon.
[3] Biological Evans, India.
[4] Fluka.

the method is convenient and sensitive for the estimation of norepinephrine. The preparation lasts for 1–3 hr if the assay is commenced soon after mounting.

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Estimation of Hepatic First-Pass Effect of Acetaminophen in Humans after Oral Administration

Keyphrases □ Acetaminophen—estimation of hepatic first-pass effect, oral administration, humans □ Metabolism—acetaminophen, estimation of hepatic first-pass effect, oral administration, humans □ First-pass effect, hepatic—acetaminophen, estimation, oral administration, humans

To the Editor:

Acetaminophen is one of the most frequently used analgesics and antipyretics today. It is primarily metabolized in humans after oral administration. In one study, only about 3–4% of the dose was recovered in urine as intact compound after dosing (1). Recently, Cohen *et al.* (2) reported that its availability to the systemic circulation after intraperitoneal administration to rats was only 34% of that after intravenous administration, indicating an extensive hepatic first-pass metabolic effect. This finding prompted them to speculate (2) that a marked variation in peak acetaminophen plasma concentrations in humans with different stomach emptying rates (3) might be caused to a varying degree by the hepatic first-pass effect.

The purpose of this communication is to estimate theoretically the extent of the hepatic first-pass effect of acetaminophen in humans, using an equation derived recently (4, 5). The following assumptions were made in the derivation: (*a*) the metabolism takes place only in the liver; (*b*) the excretion of intact drug occurs only from the kidney and/or lung; (*c*) the liver, kidneys, and lungs are part of the central compartment; (*d*) the elimination and distribution processes between the central and other peripheral compartments are all first-order processes; and (*e*) the administered drug is all absorbed after oral or intraperitoneal administration.

The fraction of the dose metabolized during the first passage to liver, f_m, can be estimated by the following equation:

$$f_m = \frac{(F_m)(\text{dose})}{\text{dose} + (HFR)(AUC)} \qquad \text{(Eq. 1)}$$

where F_m = fraction of the administered dose metabolized at infinite time, HFR = hepatic blood flow rate, and AUC = total area under blood concentration *versus* time curve in infinite time.

A search and evaluation of past studies related to acetaminophen oral absorption in humans revealed that some work reported by McGilveray *et al.* (1) was suitable for this pharmacokinetic prediction analysis. That article reported the acetaminophen blood level data up to 6 hr after oral administration of 1 g of the drug dissolved in 200 ml of water to 10 normal subjects 1.5 hr after a light breakfast. Based on the urinary excretion data and the solution dosage form administered, one could reasonably assume that all of the dose was completely absorbed (1, 6, 7).

An AUC of 5626 (min) (μg/ml) was obtained by the summation of the area from 0 to 6 hr calculated by the trapezoidal rule and of the area from 6 hr to infinity calculated by the extrapolation method (5, 7). An average biological half-life of 3.26 hr (1) was used in the calculation for the extrapolated area. This half-life is similar to (7), but different than (8), the values from studies reported by other workers, indicating the possibility of intersubject variation. The F_m was assumed to be 0.96. By substituting a value of 1500 ml/min for HFR (4, 5) and other appropriate data into Eq. 1, one obtains an f_m value of 0.102. This value is obviously much lower than those observed (f_m = 0.66) and predicted (f_m = 0.60) for rats (2).

These results indicate that there is a marked interspecies variation in the extent of the first-pass effect. Although absorption of several drugs from intraperitoneal administration has been shown to occur predominantly *via* the portal circulation in dogs and rats (9), this has not been proven for acetaminophen.

The f_m value estimated by Eq. 1 generally represents a maximum value. If the hepatic metabolic process deviates from pseudo first order as the absorption rate of drug increases (based on Michaelis–Menten enzyme kinetics), the observed f_m value would be lower than that predicted by Eq. 1. In other words, the maximum fraction of the orally administered acetaminophen dose metabolized in the liver during the first pass is only about 0.10, and the effect of the