of them the DBH activity was increased significantly. The magnitude of increase in urinary excretion of VMA was not directly related to that of the increase in serum DBH activity when compared in each subject. Since recent studies demonstrate the stoichiometric release of DBH and NE,[19] and the considerable intersubject variation of urinary excretion of NE and E before and after delivery,[17] the discrepancy in our results seems to be due to intersubject variation in the enzymatic conversion of NE and/or E to VMA and in the rate of elimination of VMA from the kidney.

The present study demonstrates that the emotional and physiological stress of delivery produced the increase in serum DBH activity and urinary excretion of VMA, and that the increase in serum DBH activity was the result of increased release of DBH from the sympathetic nervous system. This study is compatible with other data[9] and again suggests that, if compared in individual subjects, the increase in enzyme activity might be an index of the increase in release of NE and/or E from the sympathetic nervous system.

Department of Medicine,
Osaka Teishin Hospital,
Osaka
YUICHI HASHIMOTO

Department of Pharmacology,
Osaka Medical College,
Takathuki, Osaka
YOSHIKO KUROBE

Department of Obstetrics and Gynecology,
Suita City Hospital,
Suita, Osaka, Japan
KEIZO HIROTA

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Hepatocyte suspensions as a model for demonstration of drug hepatotoxicity

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PREVIOUS studies in this laboratory have attempted to test the hypothesis that some instances of drug-induced hepatic injury, which appear to be the result of hypersensitivity to the respective drugs, may reflect

intrinsic toxicity of the agent coupled with hypersensitivity.[1–6] These studies have employed models *in vitro* to demonstrate the potential for hepatic injury of drugs known to produce liver damage in humans. Chlorpromazine (CPZ) and several other phenothiazines, incriminated as causes of jaundice in patients, have been found to produce more injury to suspensions of Chang cells (harvested from tissue culture)[1–2] and rabbit liver slices[3] than promazine and promethazine, drugs that very rarely produce recognizable hepatic injury in humans. Erythromycin estolate (EE), an agent known to cause jaundice in patients,[7] also has been reported to be much more toxic for Chang cells (as measured by enzyme leakage) than erythromycin base or erythromycin stearate, drugs which have not been recognized to produce hepatic injury in humans.[8] Since Chang cells, originally of human embryonic liver origin, have undergone extensive dedifferentiation in tissue culture,[9] the validity of using them as a model of the reaction of the liver to injury may be open to question. Efforts to evaluate the relevance of models *in vitro*, therefore, have included the demonstration that perfusion of the rat liver, *in vitro*, with erythromycin[6] and phenothiazine[4,5] derivatives leads to impaired function of the organ.

The present report is concerned with further studies to develop a model *in vitro* for measurement of the potential for hepatotoxicity of various drugs. For this purpose, suspensions of rat hepatocytes, prepared according to a modification of the method of Berry and Friend,[10] have been employed. In this method hepatocytes are isolated by continuous recirculating perfusion of the rat liver with a calcium-free Hanks' solution containing 0·05% collagenase and 0·10% hyaluronidase. As described by Berry and Friend,[10] the liver is perfused *in situ*. In the method employed in this laboratory, the liver is removed and promptly (within 3 min) perfused *in vitro* in a perfusion chanber (Metalloglass, Boston). All other steps in the isolation of the parenchymal cells are those described by Berry and Friend.[10]

The experiments were conducted immediately after harvesting and counting the cells. To a 1·0 ml suspension of rat hepatocytes in normal saline, containing 10^5 cells, was added 1·0 ml saline (control) or 1·0 ml ethanol (U.S.P.) in saline, calculated to provide a final concentration of 1·7 mM/ml of ethanol (ethanol control) or 1·0 ml of 1·7 mM/ml of ethanol containing EE, erythromycin-base (EB) or proprionate (EP) or 1·0 ml saline containing CPZ. Concentrations of EE, EB and EP employed ranged from 5×10^{-6} to 5×10^{-4}M (Fig. 1); the concentration of CPZ was 5×10^{-4}M.

The cell suspensions were incubated for 30 min at 37°, then centrifuged and the supernatants assayed for activity of glutamic oxalacetic transaminase, (aspartate aminotransferase; GOT). In some of the experiments, enzyme activity residual in the cells also was measured. Data were analyzed for statistical variability and Student's t[11] was used to test the significance of differences between the means

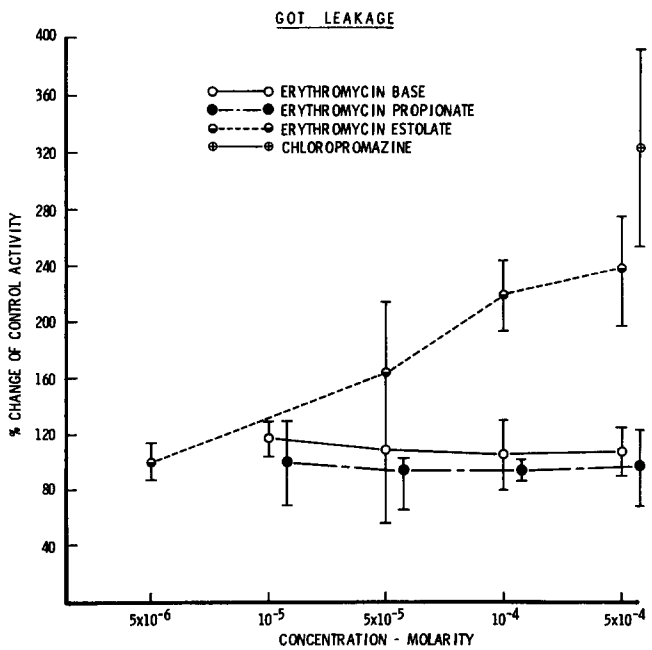

FIG. 1. Effects of three erythromycin derivatives and of chlorpromazine on hepatocytes as reflected by leakage of enzyme.

Leakage of GOT into the medium was induced only by EE and by CPZ. A dose-related effect of EE was demonstrated (Fig. 1) with the maximum effect apparent at a concentration of 5×10^{-4}M and the least at 5×10^{-5}M. Concentrations of 10^{-5} and 5×10^{-6}M led to leakage no different from that of controls. EB and EP, however, at all concentrations tested led to no greater enzyme leakage from cells than that of the control preparation. CPZ, at a concentration of 5×10^{-4}M, led to a degree of enzyme leakage approximately equal to that induced by EE at that concentration.

The results of the present study, like those employing Chang cells,[8] have demonstrated a dose-related cytotoxicity of EE, an agent known to produce jaundice in patients, but no apparent cytotoxicity of EB and EP, agents that apparently produce no hepatic injury in humans. Studies of the effects of phenothiazines in models *in vitro* also demonstrate a parellel between adverse effects on Chang cells and the reported ability of a drug to produce jaundice in humans.[1,2]

Implicit in the employment of cells separated by the use of proteolytic enzymes is the recognition that the plasma membranes of the separated cells would differ from those of cells in the intact liver. Nevertheless, the demonstration that low concentrations of drug lead to enzyme leakage no greater than that observed in controls and that higher concentrations lead to a dose-related effect supports the validity of the model Furthermore, the concentrations of drugs that lead to leakage from hepatocytes are approximately the same as those of CPZ[1,2] and EE[8,12] that were found to produce leakage of enzymes from Chang liver cells in suspension and to interfere with bile flow and dye excretion by the perfused rat liver *in vitro*.[4,6]

The lowest concentration of EE shown to have an effect on this model *in vitro* (5×10^{-5}M) is approximately 20 times as high as the concentrations found[13] in the blood of patients receiving therapeutic doses of the drug. Animal studies[14] indicate, however, that concentrations of EE in the liver are 15- to 150-fold those in the blood. Accordingly, if observations in the rat[14] can be extrapolated to humans, concentrations of EE in the liver of patients who received therapeutic doses of the agent might be at least as high as those employed in the experiments *in vitro* of the present study.

These observations suggest that toxicity *in vitro* may be relevant to an understanding of hepatic injury *in vivo* (in humans) and are consistent with the hypothesis[15] that apparent hypersensitivity-dependent drug-induced hepatic injury also involves a direct, adverse effect of the drug on the liver.

The demonstration that similar concentrations of drugs lead to a similar effect in several different models *in vitro* supports the biological validity of the several models. Hepatocyte suspensions, in their enzyme content and metabolic activity, are similar to the intact liver,[10] while cells grown in tissue culture are not.[9] Accordingly, hepatocyte suspensions may be a more pertinent model for further exploration of models of drug-induced hepatotoxicity *in vitro*.

Medical Service,
Veterans Administration Hospital, and
Department of Medicine,
George Washington University,
Washington, D. C 20422, *U S.A*

HYMAN J. ZIMMERMAN
JEAN KENDLER
SAM LIBBER
LORINC LUKACS

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