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# Kinetics of glyburide metabolism by hepatic and placental microsomes of human and baboon

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ABSTRACT

Glyburide (glibenclamide) is under investigation for treatment of gestational diabetes. Two metabolites of glyburide have been previously identified in patients, namely, 4-*trans*-(M1) and 3-*cis*-(M2) hydroxycyclohexyl glyburide. Recently, the metabolism of glyburide by microsomes of liver and placenta from humans and baboons revealed the formation of four additional metabolites: 4-*cis*-(M2a), 3-*trans*-(M3), and 2-*trans*-(M4) hydroxycyclohexyl glyburide, and ethyl-hydroxy glyburide (M5). The aim of this investigation was to determine the kinetics for the metabolism of glyburide by cytochrome P450 (CYP) isozymes of human and baboon placental and hepatic microsomes. The metabolism of glyburide by microsomes from the four organs revealed saturation kinetics and apparent $K_m$ values between 4 and 12 μM. However, the rates for formation of the metabolites varied between organs and species. M1 was the major metabolite (36% of total), formed by human hepatic microsomes with $V_{max}$ of 80 ± 13 pmol mg protein$^{-1}$ min$^{-1}$, and together with M2, accounted for only 51% of the total. M5 was the major metabolite (87%) formed by human placental microsomes with $V_{max}$ of 11 pmol mg protein$^{-1}$ min$^{-1}$. In baboon liver, M5 had the highest rate of formation ($V_{max}$ 135 ± 32 pmol mg protein$^{-1}$ min$^{-1}$, 39% of total), and in its placenta, was M4 ($V_{max}$ 0.7 ± 0.1 pmol mg protein$^{-1}$ min$^{-1}$, 65%). The activity of human and baboon hepatic microsomes in metabolizing glyburide was similar, but the activity of human and baboon placental microsomes was 7% and 0.3% of their respective hepatic microsomes. The data obtained suggest that more than 1 CYP isozyme is responsible for catalyzing the hydroxylation of glyburide.



## 1. Introduction

Glyburide (glibenclamide) is a second-generation sulfonylurea drug that has been used for treatment of type 2 diabetes and is currently being used in a clinical trial by the Obstetric-Fetal Pharmacology Research Units (OPRU) Network for treatment of gestational diabetes. In the United States, approximately 14% of pregnant women suffer from gestational diabetes, and 20–60% of those women require therapy to control their glucose levels [1]. A clinical trial revealed that glyburide was as effective as insulin for treatment of gestational diabetes; thus, glyburide use has been advocated

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*Abbreviations:* M, metabolite; IS, internal standard; CYP, cytochrome P450; HPLC, high performance liquid chromatography; MS, mass spectrometry

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because of its additional advantages that include longer shelf-life and lower cost [2].

Pregnancy is accompanied by changes in maternal physiology that alter the pharmacokinetics of administered drugs [3]. Moreover, the developing fetoplacental unit contributes to drug disposition as a new compartment. To better understand the role of human placenta in the disposition of glyburide, our investigations have focused on its in vitro transfer [4] and metabolism of the hypoglycemic agent [5]. However, the activity of placental enzymes changes with gestation [6] and its metabolism of glyburide cannot be determined at the different preterm gestational ages. Therefore, an animal model was necessary to obtain this type of data. The baboon (*Papio cynocephalus*) was chosen as a nonhuman primate on the basis of its placental structure and possible manifestation of gestational diabetes as well as its 95% DNA homology with humans [7–9].

In vivo, glyburide is metabolized in the liver by its hydroxylation and the formation of two metabolites, 4-*trans*-hydroxy- and 3-*cis*-hydroxycyclohexyl glyburide, that were identified in plasma, urine, and feces [10–14].

In vitro metabolism of glyburide by human hepatic microsomes resulted in the formation of five [15,16], six [5], or seven [17] monohydroxylated glyburides. Our recent investigation comparing the glyburide metabolites formed by hepatic and placental microsomes from humans and baboons revealed the formation of six metabolites. Five metabolites were identified by comparison with the synthesized standards and included 4-*trans*-hydroxy- and 3-*cis*-hydroxycyclohexyl glyburide. The remaining three were identified, for the first time, as 4-*cis*-, 3-*trans*-, and 2-*trans*-hydroxycyclohexyl glyburide. The sixth metabolite was identified as ethyl-hydroxylated glyburide on the basis of its mass spectrometry (MS) fragmentation pattern [5]. This proposed structure is in agreement with previous reports [15–17]. However, the pharmacology of the newly identified metabolites is unknown, but recent investigations in healthy volunteers and diabetic patients revealed that the two metabolites, 4-*trans*- and 3-*cis*-, were as potent as the parent compound in their glucose-lowering effect [18–20]. These two metabolites accounted for approximately half of the total amount of glyburide metabolites formed in vitro by human liver microsomes [21]. On the other hand, there were differences in the amounts of the metabolites formed between the liver and placenta of the same species (human or baboon) as well as for the same tissue (placenta or liver) between humans and baboons [5]. Moreover, investigations of the hepatic enzymes responsible for the metabolism of glyburide revealed that several cytochrome P450 (CYP) isozymes are involved [21–23] but the kinetics of the reaction remain unclear, to the best of our knowledge.

Therefore, the aim of this work is to investigate the kinetics for biotransformation of glyburide by hepatic and placental human and baboon microsomes. The information obtained will contribute to: (1) identification of the CYP isozyme(s) responsible for the metabolism of glyburide and (2) validation of the baboon placenta and liver for investigations of the effects of glyburide in human at all gestational ages.

## 2. Materials and methods

### 2.1. Chemicals

Acetonitrile (HPLC-grade), dichloromethane, and hexane were purchased from Fisher Scientific (Fair Lawn, NJ). Glyburide (glibenclamide; *N*-4-[β-(5-chloro-2-methoxybenzamidoethyl) benzenesulfonyl]-*N*′-[cyclohexyl] urea) and all other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Metabolites of glyburide: 4-*trans*-, 3-*trans*-, 2-*trans*-, 3-*cis*-, and 4-*cis*-hydroxycyclohexyl glyburides were synthesized according to the procedure reported by Hill et al. and made available to our laboratory [24].

### 2.2. Clinical material

Human term placentas were obtained from uncomplicated pregnancies immediately after delivery according to a protocol approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston. Placentas of baboons (*P. cynocephalus*) were obtained by cesarean section. Adult baboon livers were obtained from animals that were sacrificed for herd reduction. All animal tissues were obtained according to a protocol approved by the Institutional Animal Care and Use Committee of the Southwest National Primate Research Center, San Antonio, TX.

Baboon livers and placentas, as well as human placental villous tissue, were homogenized in 0.1 M potassium phosphate buffer pH 7.4. The homogenates were used to prepare crude subcellular fractions by differential centrifugation as previously described [25]. The microsomal pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4) and stored at −80 °C until used. Protein content of the microsomal fraction was determined by a commercially available kit (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as a standard. Pools of microsomes were made of 12 human and 11 baboon placentas and used in all experiments. The pool of 15 human liver microsomes was purchased from CellzDirect (Austin, TX).

### 2.3. Experimental conditions

#### 2.3.1. Stock solutions of glyburide and its metabolites

Glyburide was dissolved in dimethylsulfoxide (DMSO) (40 mM) and diluted in 0.1 M potassium phosphate buffer pH 7.4 as needed. Each of the following standards (4-*trans*-, 3-*trans*-, 2-*trans*-, 3-*cis*-, and 4-*cis*-hydroxycyclohexyl glyburide) was dissolved in a solution of acetonitrile:water (50:50, v/v) to a final concentration of 1 μg/ml. The internal standard [IS], estrone, was dissolved in acetonitrile at a concentration of 10 μg/ml.

#### 2.3.2. Glyburide metabolism by human and baboon microsomes

The activity of the four microsomal preparations in metabolizing glyburide was determined in a total reaction volume of 1 ml of 0.1 M potassium phosphate buffer (pH 7.4). Each reaction solution contained 1 or 0.5 mg protein of placental or hepatic microsomes, respectively. Glyburide was added, in increasing concentrations (highest was 120 μM), to the

solution and preincubated for 5 min at 37 °C. The reaction was initiated by the addition of NADPH regenerating system (0.4 mM NADP, 4 mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, and 2 mM $MgCl_2$) and incubated for 15 min at the same temperature. The reaction was terminated by adding 10 μl of 10% (w/v) trichloroacetic acid and icing the test tubes. Estrone (20 μl) was added to each tube as an internal standard. The precipitated protein was separated by centrifugation at 9500 × *g* for 12 min. The metabolites formed were extracted from the supernatant by 3 ml of dichloromethane–hexane (1:1, v/v). The organic layer was siphoned and evaporated to dryness, and the residue was reconstituted in 150 μl of the HPLC mobile phase. An aliquot of 100 μl was injected in the HPLC column to determine the amount of each metabolite formed. All data are reported as the mean of three to five experiments or as otherwise indicated.

#### 2.3.3. *Extraction and recovery of glyburide metabolites*

Glyburide and its metabolites were extracted from the reaction solution according to a reported method for extraction of the 4-*trans*- and 3-*cis*-hydroxycyclohexyl glyburide from urine and plasma [13]. The recovery of the synthesized standards from the reaction solution was as follows: 4-*trans*-(M1), 86%; 4-*cis*-(M2a), 94%; 3-*cis*-(M2b), 91%; 3-*trans*-(M3), 94%; and 2-*trans*-hydroxycyclohexyl glyburide (M4), 91%.

There is no synthesized standard available for either M or M5. Therefore, the recovery of M5 by the extraction procedure used was determined as follows: glyburide was incubated with hepatic baboon microsomes. The resulting supernatant and the solution of standards were divided into two equal aliquots. One of the aliquots was extracted and the other was not. The amounts of each metabolite formed, including M5, were then determined (as described in Section 2.4) in both aliquots. The amounts were within experimental variations, indicating that the recovery of the extracted M5 is in agreement with that of the synthesized metabolites.

### 2.4. *Identification and quantitative determination of glyburide metabolites*

The metabolites formed were identified by their retention time and mass spectrum using HPLC/MS. The separation of glyburide metabolites was achieved by a reverse phase $C_{18}$ column (Symmetry 3.5 μm, 4.6 mm × 75 mm, Milford, MA). The mobile phase was made of acetonitrile:water (33:67, v/v) and the pH was adjusted to 3.5 with acetic acid. The flow rate for the initial 40 min was 1.2 ml/min followed by a linear increase to 1.5 ml/min to 60 min; this rate was then maintained for the remaining 30 min (total 90 min). The injected sample volume was 100 μl. The effluent was monitored at a wavelength of 203 nm with a third of it directed to the mass spectrometer (Waters EMD 1000 single-quadrupole; Milford, MA). Details on the conditions for mass spectrometry were previously described [5]. Briefly, selected ion monitoring at *m*/*z* 510 $[M + H]^+$ was utilized for quantitative analysis of the metabolites formed. For the estrone IS, the selected ion monitoring was set at *m*/*z* 272. In each experiment, the ratio of the peak areas of the metabolites to that of the internal standard (M/IS) was used to determine the amount of each metabolite using standard curves for the synthesized compounds. A mean for the standard curves was calculated and used to determine the amounts of metabolites M and M5. The amounts of each of the metabolites (M1, M2a, M2b, M3, and M4), determined using this protocol, differed by 5–10% of those calculated from individual standard curves.

### 2.5. *Data analysis*

The apparent $K_m$ and $V_{max}$ values were calculated from the saturation curves for glyburide metabolites using the Michaelis–Menten equation and nonlinear regression analysis (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL).

## 3. Results

### 3.1. *In vitro metabolism of glyburide*

The metabolism of glyburide by microsomal fractions of human and baboon livers and term human placentas resulted in the formation of seven metabolites and occasionally six for baboon placental microsomes. The reaction required the presence of an NADPH regenerating system, indicating that it is catalyzed by one or more CYP isozymes. The metabolites formed were identified and their amounts determined by HPLC/MS as described in the Section 2. All seven metabolites were monohydroxlated glyburides with the ion 510 $[M + H]^+$ prevailing in their mass spectrum (Figs. 1 and 2). Five of them were identified by comparing their retention time and mass spectrum with the following synthesized compounds (the number in brackets corresponds to the order of elution): (M1) 4-*trans*-, (M2a) 4-*cis*-, (M2b) 3-*cis*-, (M3) 3-*trans*- and (M4) 2-*trans*-hydroxycyclohexyl glyburides. One of the remaining two metabolites, M, was the least in amount, eluted before M1, and was detected in reactions catalyzed by the pool of baboon placental microsomes but not in all of the individual placentas.

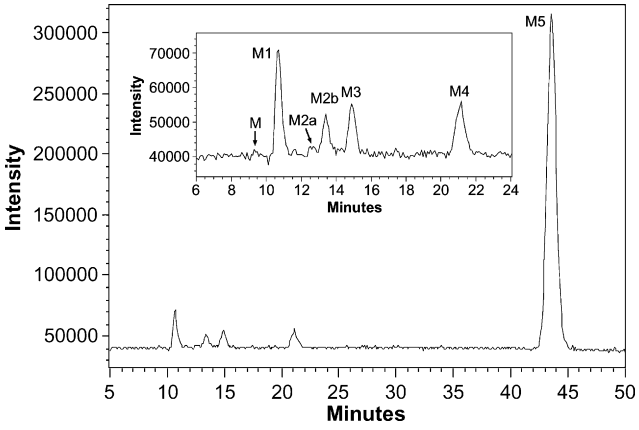


**Fig. 1 – The reconstructed ion chromatogram (*m*/*z* 510) of the glyburide metabolites formed by human placental microsomes obtained by HPLC–MS. The reaction solution was made of glyburide (60 μM), microsomal protein (1 mg), and NADPH regenerating system. Other experimental conditions are described in the text.**

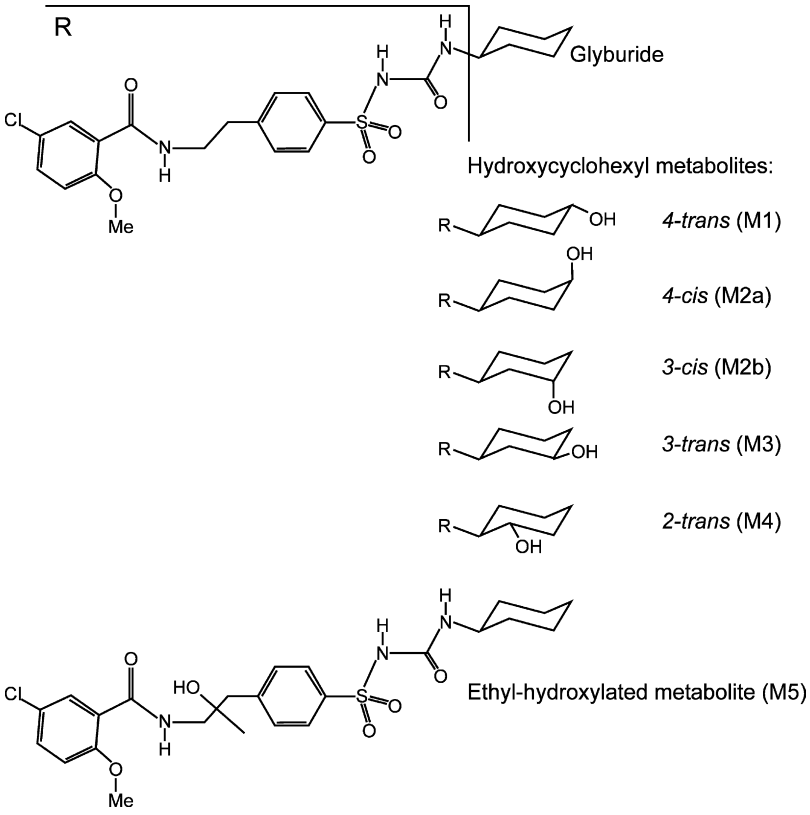


**Fig. 2 – Structure of glyburide and its metabolites on basis of the synthesized compounds except for M5. The structure of M5 is based on its fragmentation pattern by mass spectrometry hence the ambiguous position of the hydroxyl group in the ethyl bridge.**

Metabolite M was a hydroxycyclohexyl glyburide as revealed by its prevailing ions (*m*/*z* 510 and 369). The seventh metabolite, M5, had the longest retention time but less than that for the parent compound. The mass spectrum and fragmentation pattern of M5 suggested its hydroxylation in the ethylene bridge connecting the cyclohexyl ring to the rest of the molecule [5].

The metabolism of glyburide by microsomal fractions from the four organs revealed typical saturation kinetics (Fig. 3). The rates of metabolites formation, however, varied widely between the liver and placenta of the same primate and, to a lesser extent, between the same organs (liver or placenta) of each species. The rate of M5 formation was significantly higher in baboon liver than human liver and lower in baboon placenta than human placenta (Table 1). Moreover, the rate of M4 formation by human placental microsomes was biphasic (Fig. 3A).

### 3.1.1. *Metabolism of glyburide by human placental microsomes*

The amount of the major glyburide metabolite M5, formed by human placental microsomes, represented 87% of the total and its $V_{max}$ was $11.1 \pm 0.6$ pmol mg protein$^{-1}$ min$^{-1}$ (Fig. 4B). The rates for formation of the remaining six metabolites were lower than that for M5 (Fig. 3, Table 1). The amount of each metabolite, as a percent of the total formed, occurred in the following descending order: M1, 4%; M4, 4%; M2b, 2%; M3, 2%; and M2a, 0.3% (Fig. 4B). When detected, the metabolite M represented approximately 0.1%.

The apparent $K_m$ for glyburide, calculated from its saturation curve and the rates for formation of each metabolite except M4, ranged from 10 to 16 μM (mean $11.6 \pm 2.4$ μM). The rate of formation of M4 exhibited biphasic kinetics (Fig. 3A) with the corresponding apparent $K_m$ values of $1.7 \pm 0.5$ and $55 \pm 11$ μM.

### 3.1.2. *Metabolism of glyburide by human liver microsomes*

The metabolism of glyburide by human liver microsomes exhibited typical saturation kinetics and an apparent $K_m$ of $4.5 \pm 0.8$ μM. The amount of the major metabolite M1, formed by human liver microsomes, was 36% of the total and its $V_{max}$ was $80 \pm 13$ pmol mg protein$^{-1}$ min$^{-1}$. The amounts of the remaining metabolites were (in decreasing order): M5, 22%; M2a, 15%; M2b, 15%; M3, 7%; M4, 4%; and M, 1% (Fig. 4A). The rate of metabolites formed by the human liver was approximately 10 times that formed by human placenta (Table 1).

### 3.1.3. *Metabolism of glyburide by baboon placental microsomes*

The metabolism of glyburide by baboon placental microsomes exhibited monophasic saturation kinetics with a mean apparent $K_m$ of $9.6 \pm 1$ μM, except for the formation of M4 ($K_m$ of $43 \pm 17$ μM). The $V_{max}$ values for the formation of the metabolites by baboon placentas were lower than those by

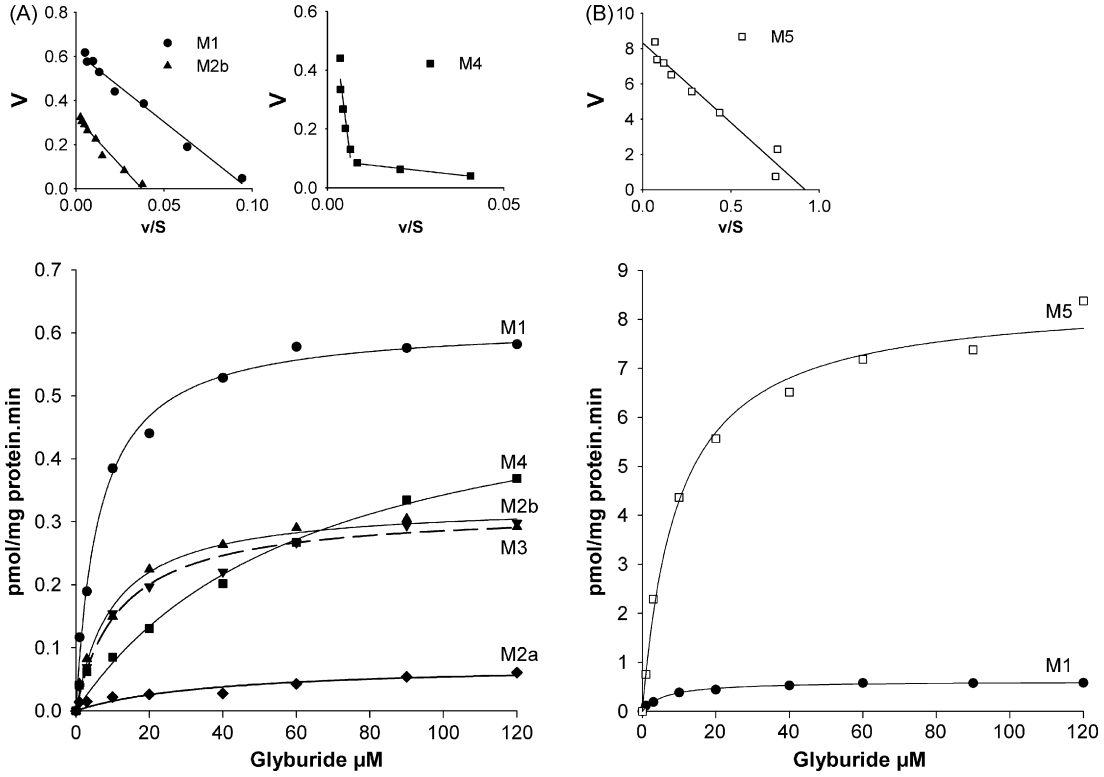


**Fig. 3 – The effect of glyburide concentration on the rates of metabolites formed by a pool of placental microsomes. A plot of the data obtained revealed saturation kinetics and the formation of six metabolites. Eadie–Hofstee plots of the data revealed monophasic kinetics for metabolites M1, M2a, M2b, M3, and M5, and biphasic kinetics for M4. Each data point represents the mean of four experiments, and the data of a typical saturation curve are presented as follows: (A) rates of M1–M4 formation. (B) Rate of M5 formation, approximately 10 times that of the other metabolites, represented with M1 for comparison. The mean of the apparent $K_m$ values was 12 ± 2 μM for the formation of all metabolites except for M4, which were 1.7 ± 0.5 and 55 ± 11 μM.**

human placenta (Table 1). The major metabolite formed by baboon placental microsomes was M4 (65% of the total amount of metabolites). The amounts of metabolites M5 and M1 represented 14% and 12% of the total, respectively. The contribution of the remaining metabolites to the total was less: M2b, 4%; M2a, 3% and M3, 2% (Fig. 4B). Metabolite M (less than 0.1%) was not detected in all of the placental microsomal preparations.

### 3.1.4. *Metabolism of glyburide by baboon liver microsomes*

The apparent $K_m$ of glyburide determined for baboon liver microsomes was 6.4 ± 1.5 μM, and the $V_{max}$ values for formation of the metabolites are shown in Table 1. The major metabolite was M5, with 39% of the total metabolites formed. The amounts of M1, M2a, M2b, and M3 were almost equal and represented, respectively, 17%, 15%, 12%, and 13% of the total amount formed. M4 and M contributed only 3% and 1% of the total, respectively (Fig. 4A).

The difference between human and baboon hepatic microsomes was in the formation of M1 and M5. The amount of M1 formed by human hepatic microsomes was the highest, while M5 was the major metabolite formed by baboon hepatic microsomes (Fig. 4A).

**Table 1 – Rates of formation of glyburide metabolites by human and baboon microsomal fractions**

| Source of microsomes | $V_{max}$ (pmol mg protein$^{-1}$ min$^{-1}$) | | | | | | | $V_{max}$, all metabolites |
|---|---|---|---|---|---|---|---|---|
| | M | 4-*Trans*-M1 | 4-*Cis*-M2a | 3-*Cis*-M2b | 3-*Trans*-M3 | 2-*Trans*-M4 | M5 | |
| Human liver | 2 ± 0.5 | 80 ± 13 | 49 ± 6 | 30 ± 4 | 15 ± 2 | 8 ± 2 | 49 ± 14 | 213 ± 37 |
| Baboon liver | 3 ± 1 | 57 ± 8 | 49 ± 10 | 41 ± 8 | 47 ± 11 | 10 ± 2 | 135 ± 32 | 342 ± 64 |
| Human placenta | 0.02 ± 0.003 | 0.6 ± 0.1 | 0.05 ± 0.01 | 0.3 ± 0.05 | 0.3 ± 0.1 | 0.5 ± 0.1 | 11.1 ± 0.6 | 13 ± 0.8 |
| Baboon placenta | 0.01 | 0.13 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.02 ± 0.004 | 0.7 ± 0.1 | 0.15 ± 0.02 | 1.1 ± 0.1 |

Data are mean ± standard error of the mean (S.E.M.) of several experiments: human liver (5), baboon liver (3), human placenta (4) and baboon placenta (5).

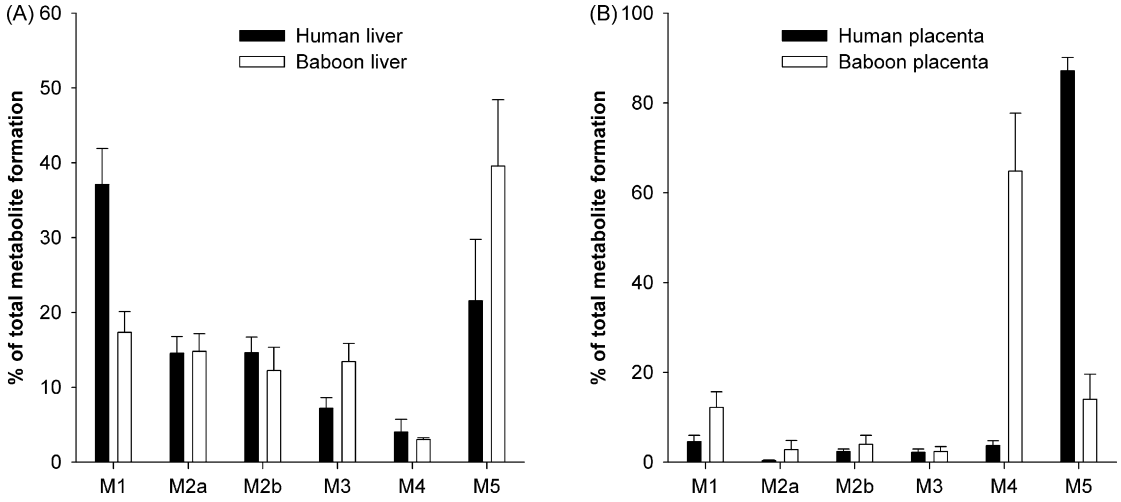


**Fig. 4 – Histograms illustrating the amount of each metabolite of glyburide as percent of the total formed by each of the four microsomal preparations: (A) human and baboon liver and (B) human and baboon placenta. Each histogram represents the mean for data obtained from several experiments as follows: human placenta (4), human liver (5), baboon placenta (5), and baboon liver (3). The data on metabolite M were not included because of its small amounts: human and baboon livers, 1%; human placenta, 0.1%; and baboon placenta, <0.1%.**

## 4. Discussion

The aim of this investigation was to determine the kinetics of glyburide metabolism by placental and hepatic microsomes from humans and baboons. Two monohydroxylated metabolites of glyburide have been identified in plasma, urine, and feces of patients under treatment and volunteers in a ratio of 4 for the 4-*trans*- to 1 of the 3-*cis*-hydroxycyclohexyl glyburide [10–15,26]. On the other hand, the metabolism of glyburide by human liver microsomes revealed the formation of five to seven metabolites [15–17], but the ratio of 4-*trans*- to 3-*cis*-hydroxycyclohexyl glyburide was close to one [27]. Our investigation of glyburide metabolism by microsomes prepared from human and baboon livers and placentas revealed the formation of seven hydroxylated metabolites; six of them were previously identified: namely, 4-*trans*-(M1), 3-*cis*-(M2b), 4-*cis*-(M2a), 3-*trans*-(M3), 2-*trans*-(M4) hydroxycyclohexylglyburides, and ethyl-hydroxylated glyburide (M5) [5]. The chromatograms presented in the reports by Fischer et al. [15] and Zhang et al. [16] revealed the formation of five metabolite peaks with no definitive identification. The difference in the number of metabolites is most likely due to our identification of two peaks (compounds), instead of one, for the 4-*cis*-(M2a) and 3-*cis*-(M2b) hydroxycyclohexyl glyburide (Fig. 1). The number of metabolites (seven), cited here for human liver microsomes, is in agreement with that reported by Tiller et al. [17]. In addition, the seventh metabolite, designated (M) in our data, occurred in small amounts and represented approximately 1% of the total formed by liver microsomes. The metabolite M was also formed by placental microsomes but at the detection limits of our MS method. The prevalent ions in the mass spectrum of metabolite M (510 and 369) are the same as for the other hydroxycyclohexyl glyburides, but the position of the OH group is yet to be determined.

Data on the rates for formation of glyburide metabolites are scarce but their amounts were reported [15,17]. Data cited here on the metabolism of glyburide by a pool of human liver microsomes revealed typical saturation kinetics with an apparent $K_m$ of $4.5 \pm 0.8$ μM, but the rates of formation of the seven metabolites were different and their $V_{max}$ values are in Table 1. The major metabolite formed in vitro by the human liver microsomes was the 4-*trans*-hydroxycyclogexyl glyburide (M1), which was identified in patients' plasma and urine. The second metabolite identified in vivo, the 3-*cis*-hydroxycyclohexyl glyburide (M2b), was also formed in vitro and its amount was 40% of M1. Therefore, the metabolites 4-*trans*- and 3-*cis*-(M1 and M2b), previously considered the major metabolites of glyburide, represent approximately 50% of those formed in vitro by human hepatic microsomes (Fig. 3A). It is interesting to note that the amount of metabolite M5 (i.e., glyburide hydroxylated in the ethyl bridge) had the second highest rate of formation, and its amount represented 22% of the total. Three explanations can be offered for the discrepancy between the in vivo and in vitro data: (1) M5 was not formed in vivo, (2) M5 was formed in vivo but was not detected, or (3) M5 was formed in vivo but was further metabolized and thus not detected.

At this time, it is unclear whether one or more CYP isozymes are responsible for the formation of all seven metabolites. However, our data on the kinetics of the formation of each metabolite by the microsomal preparations from human and baboon livers and placentas strongly suggest that more than 1 CYP isozyme is involved. This is also supported by reports indicating that CYP2C9, CYP2C19, and CYP3A4 are involved in the metabolism of glyburide by human liver [15,21–23].

In this investigation, the metabolism of glyburide by a pool of placental microsomes revealed typical saturation kinetics with an apparent $K_m$ of $11.6 \pm 2.4$ μM (similar to that for

human liver microsomes). Moreover, the metabolites formed in the presence of human placental and hepatic microsomes were identical. However, both the rates for metabolites formation by placental microsomes and their amounts were approximately 10% of those formed by hepatic microsomes. Therefore, it can be concluded that the CYP isozyme(s) metabolizing glyburide in human placenta might be similar to those in the liver, and the lower activity is due to decreased expression [6]. On the other hand, there are also differences between the formation rates of individual metabolites by hepatic and placental microsomes which could be attributed to lower expression, different CYP isozymes or both. The major metabolite formed by human hepatic microsomes is M1 (4-*trans*-hydroxycyclohexyl glyburide), which contributed 36% of the total; whereas, the major metabolite formed by human placenta is M5 (ethyl-hydroxy glyburide), which contributed 87% of the total. Another difference is the rate of formation of M4 (2-*trans*-hydroxycyclohexyl glyburide) by human placenta, which exhibited biphasic kinetics (with respective $K_m$ values of 1.7 and 55.0 μM) while it was monophasic for hepatic microsomes. These data suggest that the formation of M4 by human placenta is either by 1 CYP isozyme with high and low affinity binding sites or by 2 CYP isozymes. It is interesting to note that the amount of M4 formed by both human placental and hepatic microsomes was 4% of the total.

To the best of our knowledge, there are no reports on the metabolism of glyburide by baboons. In one investigation, the metabolites formed by hepatic microsomes from cats, dogs, rabbits, and monkeys were similar in their relative amounts and mass spectra [17]. Data cited in this report indicated several similarities between the metabolites formed by human and baboon microsomes and include saturation kinetics and apparent $K_m$ values. There were also similarities between the $V_{max}$ values and amounts of all of the metabolites formed by human and baboon livers except for M1 and M5. The major metabolite formed by human liver was M1, which represented 36% of the total; its $V_{max}$ value was $49 \pm 14$ pmol mg protein$^{-1}$ min$^{-1}$. The major metabolite formed by the baboon liver was M5 (39% of total and $V_{max}$ $135 \pm 32$ pmol mg protein$^{-1}$ min$^{-1}$). These data suggest that baboon liver CYP isozyme had the highest activity in hydroxylation of the ethyl moiety of glyburide, while in a human liver it was the isozyme responsible for hydroxylation of the cyclohexyl ring in the 4-*trans* position. On the other hand, the overall rates of glyburide metabolism by baboon and human microsomes were within close agreement ($342 \pm 64$ and $213 \pm 37$ pmol mg protein$^{-1}$ min$^{-1}$, respectively).

The metabolites of glyburide formed by baboon placental microsomes were identical to those formed by baboon liver, human liver, and placenta, but there were significant differences in the rates of metabolite formation. The rates for metabolite formation by baboon placenta were 10% of the formation rate by human placenta and <1% of the formation rate by baboon liver. Therefore, it appears that the metabolism of glyburide by baboon placenta is lower than that by human placenta. Moreover, the major metabolite formed by baboon placenta was M4 (2-*trans*-hydroxycyclohexyl glyburide), which represented 65% of the total, but its rate of formation was monophasic with an apparent $K_m$ value of $43.2 \pm 17.1$ μM. Therefore, there are apparent differences between the metabolites formed by baboon and human placentas, specifically M4 and M5. At this time, it can be speculated that the introduction of a hydroxyl group in the ethyl bridge of glyburide to form M5 is more likely by a CYP isozyme that is different from that introducing the same group in the 2-*trans*-position of the cyclohexyl ring of glyburide. Accordingly, baboon placentas, as determined by its in vitro metabolism of glyburide, may be a poor model for human placentas.

In summary, the metabolism of glyburide by human and baboon hepatic and placental microsomes revealed the formation of identical metabolites. However, the range of apparent $K_m$ values and the various rates of metabolite formation suggest that the reaction is catalyzed by more than 1 CYP isozyme. The amount of the newly identified metabolites accounted for approximately 50% of the total formed and raises the following questions. Are these newly identified metabolites formed in vivo and not previously detected? Are they pharmacologically active, and what are their effects on perinatal and neonatal outcome? Answering these questions will provide information that could improve maternal and neonatal outcome of gestational diabetics that are treated with glyburide.

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