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Passage of Bisphenol A into the Fetus of the Pregnant Rat

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We examined whether orally administered bisphenol A transfers from the maternal rat to the fetus. After oral dose of 10 mg/kg bisphenol A, it immediately appeared in maternal blood, and transferred into the fetuses. The concentration of bisphenol A in both maternal blood plasma and fetuses peaked within 1 h after administration. The values were approximately 34 ppb and 11 ppb, respectively. At 3 h, the concentration of bisphenol A in maternal blood plasma had decreased to approximately 10% of the peak value. The 3-h decrease in fetuses was only about 40% of the peak, and by 24 h, the fetal concentration had increased again to the nearly 70% of the peak value. The results suggest that bisphenol A might easily pass through the placental barrier, unlike sex hormones such as estrogen.

Key words —— bisphenol A, placental barrier, transfer, pregnant rat, fetus, plasma

INTRODUCTION

Bisphenol A (4,4'-isopropylidenediphenol) is a monomer used in the synthesis of epoxy resins or polycarbonates and serves as a stabilizing material or antioxidant for many types of plastics. Polycarbonates are commonly used to line food cans, and recent studies showed that bisphenol A can leach from lacquer coated cans[1,2] and baby feeding-bottles.[3] Bisphenol A stimulates α and β estrogen receptors (ERs) at concentrations of 100—1000 nM[4,5] and induces proliferation of MCF-7 human breast cancer cells.[6,7] Vom Saal *et al.* reported that bisphenol A, at 20 ng/g body weight, fed to female mice from gestation days 11—17 significantly reduced sperm production (daily sperm production per g testis) in male offspring by 20% relative to control males.[8,9]

The placenta is an effective barrier against fetal exposure to proteins or foreign bodies that might cause harm. It probably also protects the developing embryo against hormones circulating in the maternal blood that might adversely affect its development.[10,11] Thus, the placental barrier is impervious to most sex hormones, including estrogen. However, no studies have determined whether bisphenol A crosses the placental barrier. Therefore, we examined the transfer of orally administered bisphenol A from pregnant rats to their fetuses. (This research was presented in part at the Meeting for the Japan Society of Endocrine Disrupters Research held at Kyoto in December 1998.)

MATERIALS AND METHODS

Reagents —— All solvents and reagents were analytical grade, except d_{10}-fluoranthene, and were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The purity of bisphenol A was over 99.0%. D_{10}-fluoranthene, over 98.0% pure, was purchased from C/D/N Isotopes (Quebec, Canada). Water was distilled and then purified by a Milli-Q™ Type I Reagent Water System. GL-Pak PLS-2 cartridges (500 mg/ 6 ml) from GL Sciences Inc. (Tokyo, Japan) were used for the solid phase extraction (SPE) of bisphenol A. Prior to use, the cartridges were washed with 10 ml methanol, 10 ml Milli Q, and then 10 ml 0.01 N HCl.

Animals —— Pregnant Wistar rats, gestation days 12—14, were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The animals were kept in a room maintained at 23℃ with 50% relative humidity. The animals were housed two per cage, fed rat chow (CE-2) and offered tap water *ad libitum*. One day before administration, the rats were fasted and offered only tap water. We evaluated

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the passage of bisphenol A into the fetuses from pregnant rats given an oral dose of 10 mg/kg bisphenol A on gestation day 19. After 1, 3 and 24 h of exposure, maternal blood plasma was separated, and fetuses were removed from the pregnant animals.

Extraction and Acetylation of Bisphenol A —— Blood Plasma of Pregnant Rats: HCl (0.01 N) was added to the blood plasma of pregnant rats. Samples were then passed through SPE cartridges, and the organic compounds remaining on the disk were eluted with methanol. The eluates were evaporated, and pyridine and acetic anhydride were added to the residue and reacted for 20 min at 70℃.[12,13] Water and hydrochloric acid were added. Bisphenol A was extracted twice with toluene and the toluene layer was evaporated to near dryness.

Fetuses Removed from Pregnant Rats: Whole organs from rat fetuses were homogenized with acidic methanol (0.1 N HCl: methanol 1 : 9, v/v). The homogenate was centrifuged, and the supernatant was added to methanol and *n*-hexane to remove fat. The aqueous methanol layer was transferred to a glass tube and mixed with 0.01 N HCl. Samples were then passed through SPE cartridges, and subjected to the same procedures described above for extraction from blood plasma. A detailed description of the method is given in the flow sheet (Fig. 1).

GL-Pak PLS-2 cartridges (polystyrene divinyl benzene polymer) were used for SPE because of the high recovery of bisphenol A, relative to C18 (octadecyl) and CH (cyclohexyl), and because of their stability and low-contamination under acidic conditions. The residual water in the SPE cartridges was gently removed from the column using an evaporater for 30 seconds, since a low recovery of bisphenol A was observed when the cartridges were completely dried. Acetic anhydride acetylation in pyridine was performed because bisphenol A reacts even if water is present.

Determination of Bisphenol A by GC/MS-SIM —— An HP model 5890 II gas chromatograph from Yokogawa-Hewlett-Packard Ltd. (Tokyo, Japan) equipped with a JEOL Automass 50 from JEOL Ltd. (Tokyo, Japan) was used for qualitative and quantitative analyses. The analytical column was CP SIL 19CB, 25 m×0.25 mm i.d. and 0.20 μm film thickness, from Chrompack (The Netherlands).

Immediately prior to GC/MS analysis, 100 μl d_{10}-fluoranthene was added to each extract. The d_{10}-fluoranthene was used as a synthetic deuterated internal standard for quantitation.[12] Splitless injection (1 μl) into the GC/MS was performed at 250℃. The carrier gas was helium, and the flow rate was 1.0 ml/min. The oven temperature was initially 80℃ for 2 min and was then programmed to increase to 200℃ at 20℃/min and held for 2 min. It was then increased further to 270℃ at 5℃/min (for blood plasma) or 3℃/min (for rat fetuses), and held for 10 min. The electron impact ionization conditions were as follows: ion energy, 70 eV; ion source temperature, 200℃; mass range, m/z 50—500 full scan for qualitative analysis.

The acetylated bisphenol A was measured by GC/MS in the selected ion monitoring mode (GC/MS-SIM) using d_{10}-fluoranthene. The most stable and abundant ions, m/z 119, 213, 270, and 312 for acetylated bisphenol A and 212, and 213 for d_{10}-fluoranthene, obtained by electronic impact, were monitored for the analysis of bisphenol A. Ion m/z 213 for acetylated-bisphenol A and d_{10}-fluoranthene were used for the quantification of bisphenol A.

RESULTS

Firstly, we examined the recovery of bisphenol A during the extraction process. A standard solution of bisphenol A was added to maternal blood plasma and fetuses and then the entire analytical procedure was carried out. We obtained a good mean recovery: 94.0% with a relative standard deviations (RSD) of 4.4% for blood plasma, and 87.4% with an RSD of 7.9% for fetuses.

Although the retention time of acetylated-bisphenol A in blood plasma and fetuses was different due to the GC operating conditions (Fig. 2), the peaks appearing at 20.33 min (blood plasma) and 23.31 min (fetus) for m/z 213 of acetylated bisphenol A were used to determine the detection limit with a signal-to-noise ratio of 3 (Fig. 2). The detection limit of acetylated bisphenol A was approximately 30 pg when 1 μl of the extract (100 μl) was injected on to the GC column. Identification of the signal component at 20.33 min (blood plasma) and 23.31 min (fetus) was performed by comparison of the corresponding mass spectrum and retention time of a standard acetylated bisphenol A solution. We confirmed that there was no signal appearing at 20.33 min (blood plasma) and 23.31 min (fetus) for acetylated bisphenol A in an extract from a pregnant rat 1 h after oral administration of corn oil (control) (Fig. 2). Thus, we confirmed that bisphenol A could be efficiently extracted from

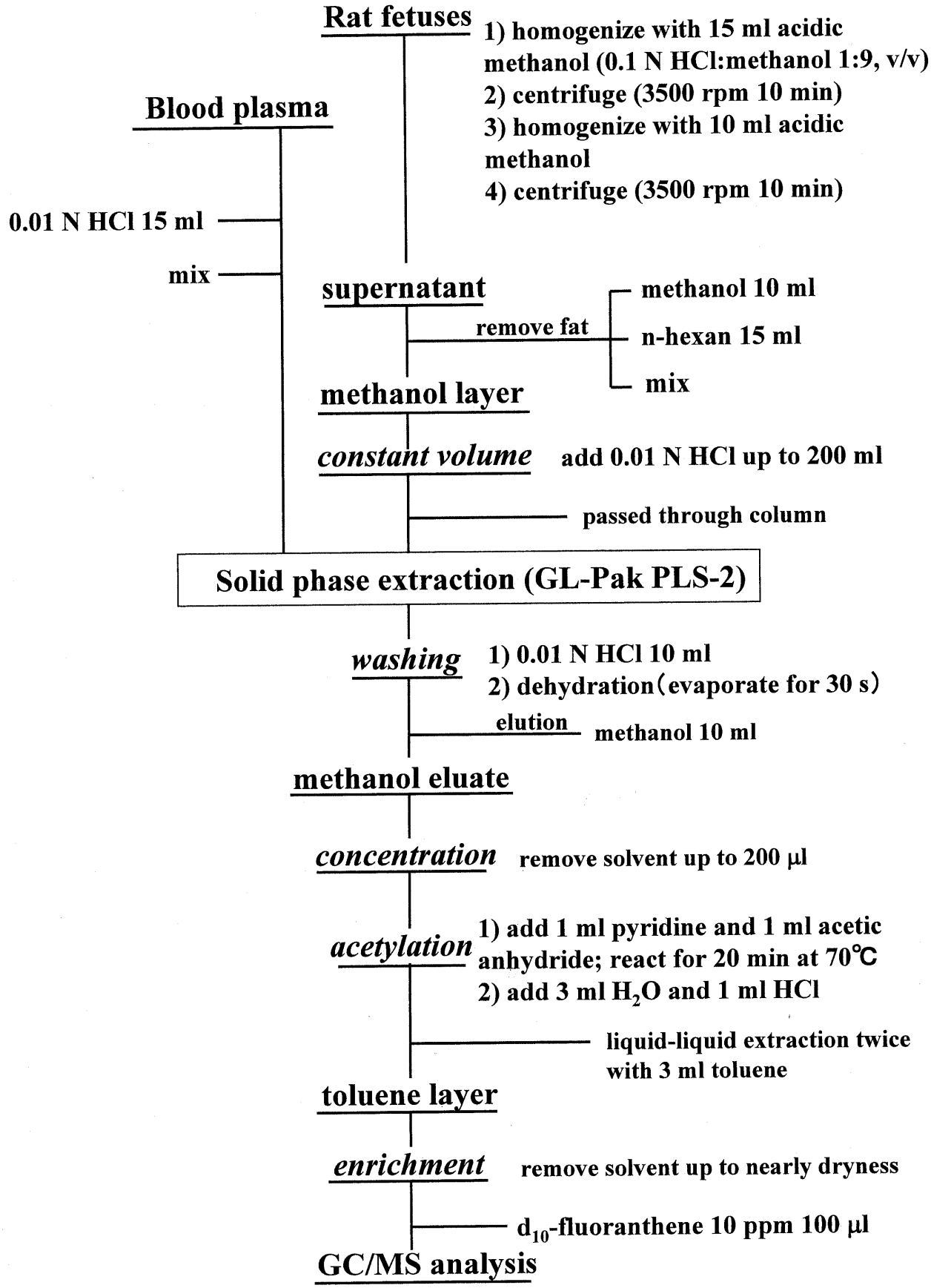

Fig. 1. Flow Sheet of the Method for Determining Bisphenol A Concentrations

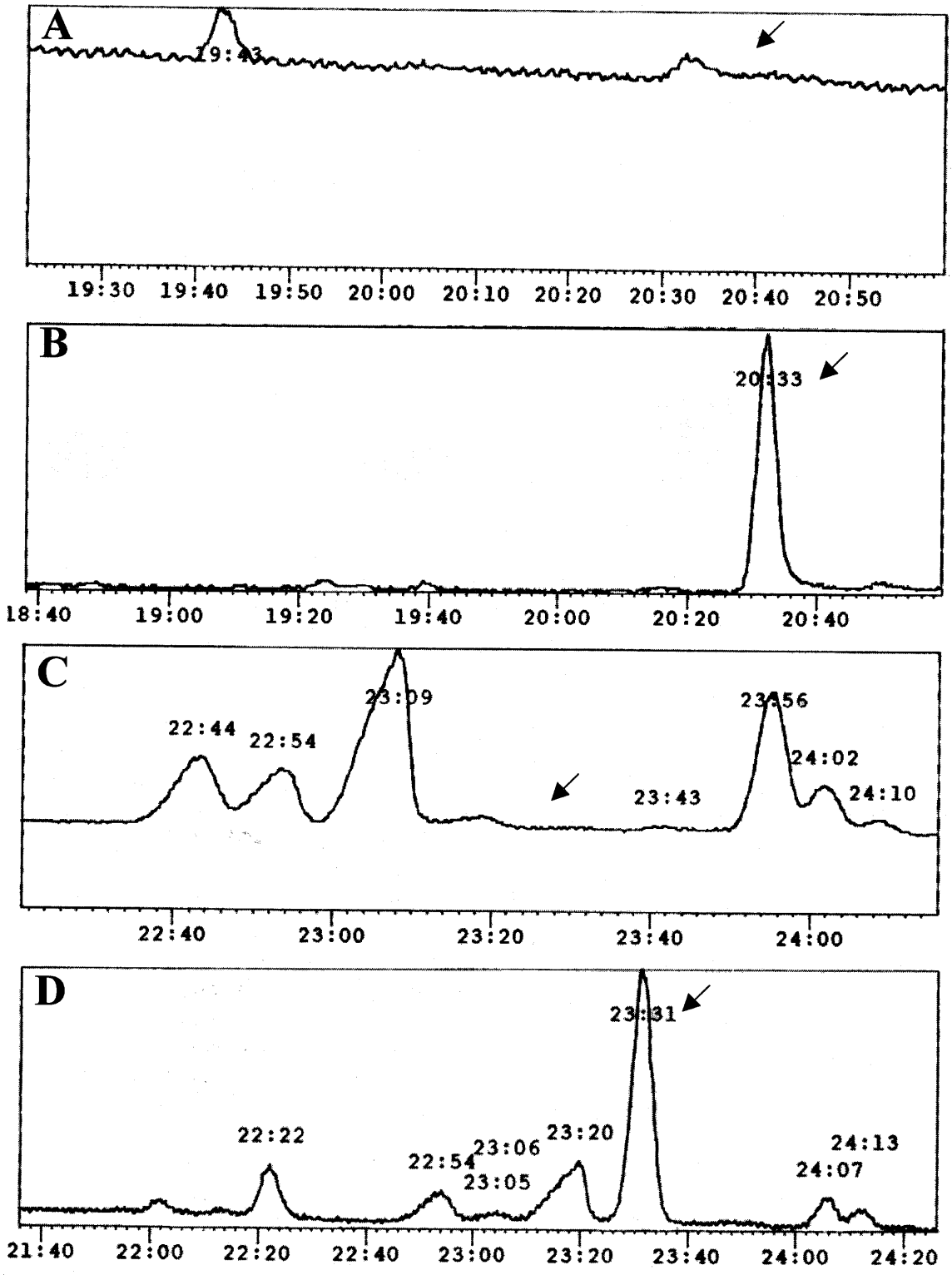

Fig. 2. Total Ion Chromatograms for Extracts from Biological Samples

One hour after administration, maternal blood plasma was separated, and fetuses were removed. A: Blood plasma of pregnant rats after oral administration of corn oil (control). B: Blood plasma of pregnant rats after oral administration of bisphenol A (one dose of 10 mg/kg bisphenol A). C: Fetuses removed from pregnant rats after oral administration of corn oil (control). D: Fetuses removed from pregnant rats after oral administration of bisphenol A (one dose of 10 mg/kg bisphenol A).

blood plasma and fetuses, and purified using this protocol and GC/MS conditions.

Quantitative data for bisphenol A in pregnant rats and their fetuses after oral administration (one dose of 10 mg/kg bisphenol A) are shown in Fig. 3. The concentration of bisphenol

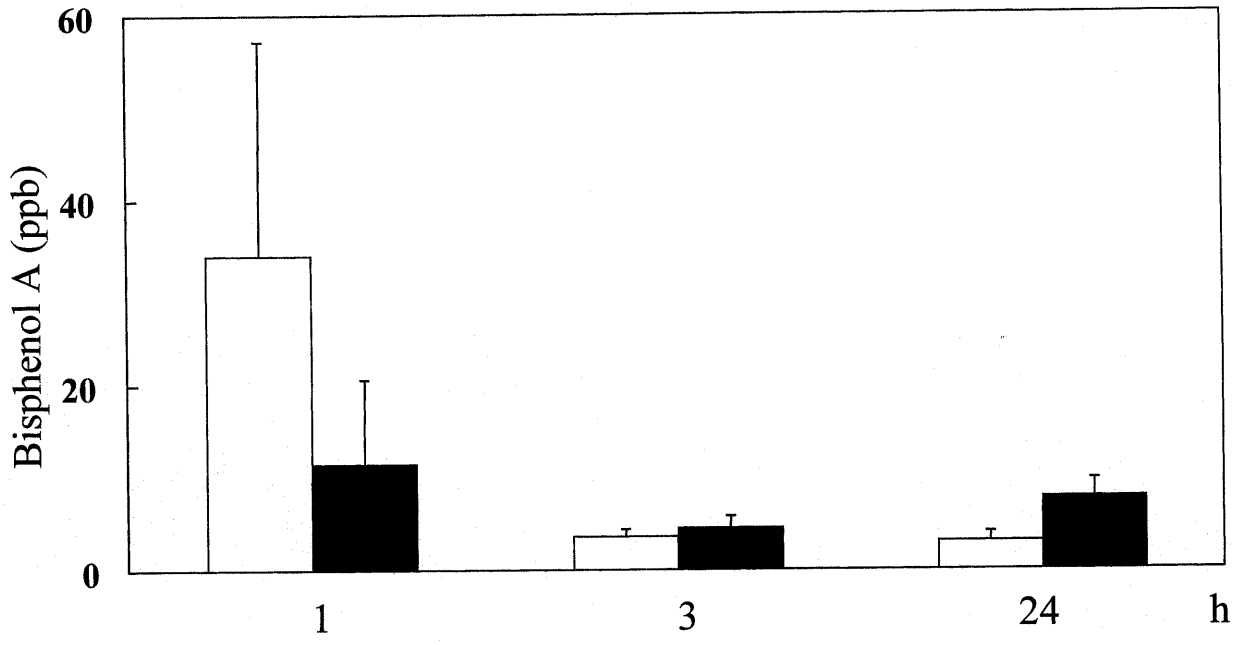

Fig. 3. The Concentrations of Bisphenol A in Maternal Blood Plasma and Fetuses after Oral Administration

Passage of bisphenol A to the fetuses from pregnant rats using the oral route of exposure (one dose of 10 mg/kg bisphenol A) was examined at gestation day 19. At 1, 3, and 24 h after exposure, the concentration of bisphenol A in maternal blood plasma and fetuses was measured as described in the Materials and Methods. □, blood plasma ; ■, fetuses. Values are mean ± S.D. of 5 or 7 separate experiments.

A in both maternal blood plasma and fetuses peaked within 1 h of oral administration, and the values were approximately 34 and 11 ppb respectively. At 3 h, the concentration of bisphenol A in maternal blood plasma had decreased to approximately 10% of the peak value. The 3-h reduction in fetuses was only about 40% of the peak, and by 24 h, the fetal concentration had increased again to the nearly 70% of the peak value.

DISCUSSION

The placental barrier is generally thought to be impervious to most sex hormones including estrogen, which are believed to bind to proteins such as sex hormone binding globulin in the placenta.[14] However, our present results show that orally administered bisphenol A easily crosses the placental barrier and enters the fetuse. The peak of bisphenol A in both maternal blood plasma and fetuses occurred within 1 h. The concentration of bisphenol A in maternal blood plasma was approximately 3-fold higher than that of fetuses at 1 h. However, 24 h after administration, the concentration in fetuses was more than twice that in maternal blood plasma. Therefore, it seems that the transfer of bisphenol A from pregnant rats to fetuses is rapid and that bisphenol A has a tendency to remain in the fetuses longer than in maternal blood plasma.

It has been suggested that the fetal toxicity of bisphenol A[8,9,15] is attributable to the low expression of UGT2B1 (an isoform of UDP-glucuronosyltransferase) during gestation. Bisphenol A is glucuronidated in rat liver microsomes and the isoform UGT2B1 is responsible for its glucuronidation.[16] Although the transitional ratio of bisphenol A is low, we cannot predict how much fetuses are affected by bisphenol A. As mentioned in the introduction, Vom Saal *et al.* reported that bisphenol A significantly reduced the efficiency of sperm production when pregnant rats were fed 20 ng/g bisphenol A body weight for gestation day 11—17.[8,9] Researchers have disagreed about the effect of low concentrations of bisphenol A on male reproductive systems[17] and further study of the toxicity of bisphenol A is required.

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