A Novel Mechanism for Polychlorinated Biphenyl-Induced Decrease in Serum Thyroxine Level in Rats

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ABSTRACT:

We have previously suggested that the decrease in the levels of serum total thyroxine (T₄) and free T₃ by a single administration to rats of Kanechlor-500 (KC500) at a dose of 100 mg/kg is not necessarily dependent on the increase in hepatic T₄-UDP-glucuronosyltransferase (UDP-GT). In the present study, we determined whether or not a consecutive treatment with KC500 at a relatively low dose (10 mg/kg i.p., once daily for 10 days) results in a decrease in the level of serum total T₄ and further investigated an exact mechanism for the KC500-induced decrease in the T₄. At 4 days after final treatment with KC500, the serum total T₄ and free T₃ levels were markedly decreased in both Wistar and UGT1A-deficient Wistar (Gunn) rats, whereas significant increases in hepatic T₄-UDP-GT activity were observed in Wistar rats but not in Gunn rats. The level of serum thyroid-stimulating hormone was not significantly changed in either Wistar or Gunn rats. Clearance from serum of the [¹²⁵I]T₄ administered to the KC500-pretreated Wistar and Gunn rats was faster than that to the corresponding control (KC500-untreated) rats. The accumulated level of [¹²⁵I]T₄ was increased in several tissues, especially the liver, in the KC500-pretreated rats. The present findings demonstrated that a consecutive treatment with KC500 resulted in a significant decrease in the level of serum total T₄ in both Wistar and Gunn rats and further indicated that the KC500-induced decrease would occur through increase in accumulation of T₄ in several tissues, especially the liver, rather than increase in hepatic T₄-UDP-GT activity.

Most polychlorinated biphenyls (PCBs) are known to decrease the level of serum thyroid hormone and to increase the activity of hepatic drug-metabolizing enzymes in rats (Van Birgelen et al., 1995; Craft et al., 2002). As possible mechanisms for the PCB-induced decrease in the level of serum thyroid hormone, enhancement of thyroid hormone metabolism by PCB and displacement of the hormone from serum transport proteins, including transthyretin (TTR), by PCB and its ring-hydroxylated metabolites are considered (Baxter and Klaassen, 1992a, 1994; Brouwer et al., 1998). In particular, the decrease in the level of serum thyroxine (T₄) by 3,3′,4,4′-pentachlorobiphenyl, Aroclor 1254, and 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats is believed to occur mainly through induction of the UDP-glucuronosyltransferases (UDP-GTs), especially UGT1A subfamily enzymes, responsible for glucuronidation of T₄ (Baxter and Klaassen, 1992a; Van Birgelen et al., 1995).

However, the magnitude of decrease in the level of serum total T₄ is not necessarily correlated with that of increase in T₄-UDP-GT activity (Craft et al., 2002; Hood et al., 2003). Furthermore, we have reported that in Kanechlor-500 (KC500)-treated mice, serum T₄ level decreased without an increase in T₄-UDP-GT activity (Kato et al., 2003) and that the decrease in serum total T₄ level by a single administration of either KC500 or 2,2′,4,4′,5,5′-pentachlorobiphenyl occurred even in UGT1A-deficient Wistar (Gunn) rats (Kato et al., 2004). Thus, an exact mechanism for the PCB-induced decrease in the level of serum thyroid hormone remains unclear. To date, most studies on biological effects of PCB have been performed using experimental animals treated once at a high dose (more than 100 mg/kg body weight), and the effect of the consecutive treatment at a low dose has been little reported. Humans and wild animals are exposed to a wide variety of environmental chemicals, including PCB, at a low level over a long period of time. Therefore, a study on biological effects by consecutive treatment with PCB at a low dose would be very important.

In the present study, therefore, we examined whether or not a consecutive treatment with KC500 at a relatively low dose (10 mg/kg i.p., once daily for 10 days) results in decrease in the level of serum total T₄ and further discussed a mechanism underlying the PCB-induced decrease in the T₄.

ABBREVIATIONS: PCB, polychlorinated biphenyl; KC500, Kanechlor-500; T₃, triiodothyronine; T₄, thyroxine; TTR, transthyretin; TSH, thyroid-stimulating hormone; UDP-GT, UDP-glucuronosyltransferase.
Materials and Methods

Chemicals. Panacete 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co. Ltd. (Tokyo, Japan). The [125I]T4, radiolabeled at the 5'-position of the outer ring, was obtained from PerkinElmer Life and Analytical Sciences (Waltham, MA). The KC500 used in the present experiments contains 2,2',5,5'-tetrachlorobiphenyl (5.6% of total PCBs), 2,2',3,3',4,6-hexachlorobiphenyl (6.5%), 2,2',4,5,5'-pentachlorobiphenyl (10%), 2,3,3',4,6-pentachlorobiphenyl (7.4%), 2,3',4,4',5-pentachlorobiphenyl (7.7%), 2,2',3,3',4,4',5-hexachlorobiphenyl (5.6%), and 2,2',4,4',5,5'-hexachlorobiphenyl (5.4%) as major PCB congeners (Haraguchi et al., 2005). All the other chemicals used herein were obtained commercially in appropriate grades of purity.

Animal Treatments. Male Wistar rats (160–200 g) and UGT1A-deficient Wistar rats (Gunn, 190–260 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Male Wistar and Gunn rats were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM to 8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%), and handled with human care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Rats received consecutive intraperitoneal injections of KC500 (10 mg/kg) dissolved in Panacete 810 (5 ml/kg) at 24-h intervals for 10 days. Control animals were treated with vehicle alone (5 mg/kg).

In Vivo Study. Rats were killed by decapitation 4 days after the final administration of KC500. The liver was removed, and hepatic microsomes were prepared according to the method of Kato et al. (1995) and stored at −85°C until use. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at −50°C until use.

Materials and Methods. Constitutive levels: T4-UDP-GT, 14.17 nmol/mg protein/min (Wistar) and 6.36 nmol/mg protein/min (Gunn).

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Wistar</th>
<th>Gunn</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Benzoylresorufin</td>
<td>0.07 ± 0.01</td>
<td>3.34 ± 0.33</td>
</tr>
<tr>
<td>7-Deoxyresorufin</td>
<td>0.03 ± 0.03</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>7-Ethoxyresorufin</td>
<td>0.14 ± 0.01</td>
<td>9.02 ± 0.09</td>
</tr>
</tbody>
</table>

* P < 0.05, significantly different from each control.

FIG. 2. Effects of KC500 on the activity of hepatic microsomal alkoxyresorufin O-dealkylases in Wistar and Gunn rats.

FIG. 3. Representative Western blot profiles for hepatic microsomal UGT isoforms in the KC500-treated Wistar and Gunn rats.
Analysis of serum hormones. Levels of total T<sub>4</sub>, free T<sub>4</sub>, total triiodothyronine (T<sub>3</sub>), and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay using Total T4 and Free T4 kits (Diagnostic Products Corporation, Los Angeles, CA), the Triiodothyronine kit GammaCoat T<sub>3</sub> II (DiaSorin Inc., Stillwater, MN), and the rTSH [125I] Biotrak assay system (GE Healthcare UK, Ltd., Little Chalfont, Buckinghamshire, UK), respectively.

Hepatic microsomal enzyme assays. Hepatic microsomal fraction was prepared according to the method described previously (Kato et al., 1995), and the amount of hepatic microsomal protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Microsomal O-dealkylase activities of 7-benzoyloxy-, 7-ethoxy-, and 7-pentoxyresorufins were determined by the method of Burke et al. (1985).

Hepatic T<sub>4</sub> metabolizing enzyme assay. The activity of microsomal UDP-GT toward T<sub>4</sub> (T<sub>4</sub>-UGT activity) was determined by the methods of Barter and Klaassen (1992b).

Western blot analysis. The polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and specific antibodies against UGT1A1, UGT1A6, and UGT2B1, which were established by Ishikuro et al. (1995, 1997), were used. Western blot analyses for microsomal UGT isoforms were performed by the method of Luquita et al. (2001). The bands corresponding to UGT1A1, UGT1A6, and UGT2B1 on a sheet were detected using chemical luminescence (ECL detection kit; GE Healthcare UK, Ltd.), and the level of each protein was determined densitometrically with LAS-1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan).

**Ex Vivo Study.** At 4 days after a consecutive 10-day treatment with KC500, the rats were anesthetized with a saline (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP31; Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml), and then the animal’s body was warmed barbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP31; Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml), and then the animal’s body was warmed.

**Clearance of [125I]T<sub>4</sub> from serum.** The study on the clearance of [125I]T<sub>4</sub> from serum was performed according to the method of Oppenheimer et al. (1968). In brief, after the administration of [125I]T<sub>4</sub>, a portion (0.3 ml) of blood was sampled from the artery at the indicated times, and serum was prepared and stored at −50°C until use. Two aliquots (15 μl each) were taken from each serum sample for determining [125I]T<sub>4</sub> level by a gamma counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences).

**Analysis of [125I]T<sub>4</sub> bound to serum proteins.** The levels of serum [125I]T<sub>4</sub>-albumin and [125I]T<sub>4</sub>-TTR complexes were determined according to the method of Davis et al. (1970). In brief, serum was diluted in 100 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol, and subjected to electrophoresis on 4 to 20% gradient native polyacrylamide gels PAG Mid “Daiichi” 4/20 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The electrophoresis was performed at 4°C for 11 h at 20 mA in the 0.025 M Tris buffer (pH 8.4) containing 0.192 M glycine. The human albumin and TTR, which were incubated with [125I]T<sub>4</sub>, were also applied on the gel as templates. After the electrophoresis, a gel was dried and radioautographed for 20 h at room temperature using Imaging Plate 2040 (Fuji Photo Film Co., Ltd.). The levels of [125I]T<sub>4</sub>-albumin and [125I]T<sub>4</sub>-TTR in serum were determined by counting the gel fractions identified from Bio Imaging Analyzer (BAS-2000II IP Reader; Fuji Photo Film Co., Ltd.).

**Tissue distribution of [125I]T<sub>4</sub>.** The study on the tissue distribution of [125I]T<sub>4</sub> was performed according to the modified method of Oppenheimer et al. (1968). In brief, at 60 min after administration of [125I]T<sub>4</sub>, blood sample was taken from abdominal aorta. Then, cerebellum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adrenal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, cecum, brown fat, skeletal muscle, bone marrow skin, spinal cord, and fat were removed and weighed. Radioactivities in serum and the tissues were determined by a gamma-counter (COBRA II AUTO-GAMMA 5002; PerkinElmer Life and Analytical Sciences), and amounts of [125I]T<sub>4</sub> in various tissues were shown as ratios of tissue to serum.

**Statistics.** The data obtained were statistically analyzed according to Stu-
dent's t test or Dunnett's test after analysis of variance. In addition, data of the clearance of $[^{125}\text{I}]T_4$ from serum and analysis of $[^{125}\text{I}]T_4$ bound to serum proteins were statistically analyzed according to the Newman-Keuls test after analysis of variance. The pharmacokinetic parameters of $[^{125}\text{I}]T_4$ were estimated with noncompartmental methods as described previously (Tabata et al., 1999).

Results

**Serum Hormone Levels.** Effects of KC500 on levels of serum thyroid hormones were examined in Wistar and Gunn rats (Fig. 1). In both Wistar and Gunn rats, KC500 treatment resulted in decreases of the serum total $T_4$ and free $T_4$, and the magnitude of the decrease in each serum thyroid hormone was almost the same in both strains of rats. On the other hand, a significant decrease in the level of serum total $T_3$ was observed in Gunn rats but not in Wistar rats. In addition, the level of serum total $T_4$ and free $T_4$, and the magnitude of the decrease in both Wistar and Gunn rats, KC500 treatment resulted in decreases of approximately 40% of the initial level within 5 min. In both Wistar and Gunn rats, these decreases were significantly increased by KC500 (Table 1), and the increase in each enzyme activity was much greater in Wistar rats than in Gunn rats.

**Hepatic Drug-Metabolizing Enzymes.** Effects of KC500 on hepatic microsomal activities of benzoxoxyresorufin $O$-dealkylase (CYP2B1/2 and CYP3A1/2), penoxysresorufin O-dealkylase (CYP2B1/2), and ethoxysresorufin O-dealkylase (CYP1A2) were examined in Wistar and Gunn rats. In both Wistar and Gunn rats, these enzyme activities were significantly increased by KC500 (Table 1), and the increase in each enzyme activity was much greater in Wistar rats than in Gunn rats.

**Western Blot Analysis for UGT1As.** Levels of the proteins responsible for UGT1A enzymes, UGT1A1 and UGT1A6, were increased by KC500 treatment in Wistar rats but not in Gunn rats (Figs. 3 and 4). In addition, no expression of the UGT1A enzymes was confirmed in Gunn rats. On the other hand, the level of UGT2B1 was significantly increased by KC500 in both Wistar and Gunn rats, and the magnitude of the increase in both strains of rats were almost the same (Figs. 3 and 4).

**Serum Proteins Bound to $[^{125}\text{I}]T_4$.** The effects of KC500 on the binding of $[^{125}\text{I}]T_4$ to serum proteins, TTR, and albumin were examined in Wistar and Gunn rats (Figs. 5 and 6). In both Wistar and Gunn rats, pretreatment with KC500 resulted in a significant decrease in the level of $[^{125}\text{I}]T_4$-TTR complex, whereas it resulted in a significant increase in the level of $[^{125}\text{I}]T_4$ bound to albumin (Figs. 5 and 6).

**Clearance of $[^{125}\text{I}]T_4$ from Serum.** After an i.v. administration of $[^{125}\text{I}]T_4$ to the KC500-pretreated Wistar and Gunn rats, concentrations of $[^{125}\text{I}]T_4$ in sera were measured at the indicated times (Fig. 7). In both Wistar and Gunn rats, pretreatment with KC500 promoted the clearance of $[^{125}\text{I}]T_4$ from serum, and serum $[^{125}\text{I}]T_4$ levels were decreased to approximately 40% of the initial level within 5 min. In the KC500-untreated Wistar and Gunn rats, serum $[^{125}\text{I}]T_4$ levels were gradually decreased to approximately 40% of the initial level at 120 min later. The serum pharmacokinetic parameters of the $[^{125}\text{I}]T_4$ estimated from these data (Fig. 7) were summarized in Table 2. The mean total body clearances (CL$_{tb}$) of $[^{125}\text{I}]T_4$ in the KC500-pretreated rats were 2.4 and 2.9 times, respectively, larger than those in the corresponding control rats. The steady-state volumes of distribution (Vss) in the KC500-pretreated rats were 1.6 and 2.4 times, respectively, larger than those in the corresponding control rats.
Tissue Distribution of $^{125}$I$^4$. The tissue-to-serum concentration ratio ($K_p$ value) and distribution level of $^{125}$I$^4$ in tissue after the administration of $^{125}$I$^4$ to the KC500-pretreated Wistar and Gunn rats are shown in Figs. 8 and 9, respectively. $K_p$ values of the thyroid gland and liver were the greatest among those of the tissues examined in either Wistar or Gunn rats (Fig. 8). In addition, $K_p$ values in all the tissues examined, with the exception of the testis and ileum, were greater in KC500-pretreated Wistar rats than those in the corresponding control (KC500-untreated) rats. $K_p$ values in the thyroid gland, liver, and jejunum in the KC500-pretreated Wistar and Gunn rats were 1.6 to 1.8, 3.3 to 3.8, and 4.7 to 11.5 times, respectively, higher than those in corresponding control rats (Fig. 8).

In the control Wistar and Gunn rats, the accumulation level of $^{125}$I$^4$ was highest in the liver, among the tissues examined (Fig. 9). In both Wistar and Gunn rats, pretreatment with KC500 resulted in an increase in the accumulation level in the liver, and the levels increased to more than 40% of the $^{125}$I$^4$ dosed (Fig. 9). Likewise, significant increase in accumulation of $^{125}$I$^4$ was observed in the jejunum (Fig. 9). In addition, significant increases in the liver weight and accumulation level (per g liver) of $^{125}$I$^4$ occurred in KC500-pretreated Wistar rats, but not in Gunn rats (Tables 3 and 4).

Discussion

In the present study, we found that consecutive treatment with KC500 (10 mg/kg i.p., once daily for 10 days; total dose, 100 mg/kg) promoted accumulation of T4 in several tissues, especially the liver, and resulted in a drastic decrease in the levels of serum total T4 and free T4 in both Wistar and Gunn (UGT1A-deficient) rats. Thus, a decrease in the level of serum total T4 is also observed in the Wistar and Gunn rats treated with KC500 (a single i.p. administration at a dose of 100 mg/kg) (Kato et al., 2004). In addition, constitutive levels of serum total T4 and T3 were higher in Gunn rats than in Wistar rats, and the results were identified with those as previously described by Benathan et al. (1983). The difference in constitutive level of serum thyroid hormone between Wistar and Gunn rats seems to be dependent on differences in the level and/or activity of T3/T4-UDP-GTs.

As a possible explanation for a chemical-induced decrease in serum thyroid hormones, a hepatic T4-UDP-GT-dependent mechanism is generally considered, because T4-UDP-GT inducers, including PCB, phenobarbital, 3-methylcholanthrene, pregnenolone-16α-carbonitrile, and clofibrate, show strong activities for decreasing the level of serum total thyroid hormones, including T4 and T3 (Bartei and Klaassen, 1994; Van Birgelen et al., 1995; Miyawaki et al., 2003). However, among the experimental animals treated with a T4-UDP-GT inducer, the difference in magnitude of decrease in the level of serum total T4 is not necessarily correlated with that of hepatic T4-UDP-GT activity (Craft et al., 2002; Hood et al., 2003; Kato et al., 2003). Our present and previous results (Kato et al., 2004, 2005) using Wistar and Gunn rats support a hypothesis that significant decrease in the level of serum total thyroid hormones by either PCB or phenobarbital occurs primarily in a hepatic T4-UDP-GT-independent pathway.

As a possible mechanism for the PCB-induced decrease in serum T4 level, an increase in hepatic drug-metabolizing enzymes might be considered. However, these are induced to a greater extent in the Wistar rats than in the Gunn rats, whereas magnitudes of decrease in serum T4 level in Wistar and Gunn rats were almost the same. Accordingly, the KC500-induced decrease in serum T4 level is thought to be independent of the KC500-induced drug-metabolizing enzymes, including UDT-GTs and cytochromes P450.

As the factors regulating the level of serum total T4, serum TSH, hepatic type I iodothyronine deiodinase, and TTR are known. However, no significant change in the level of serum TSH occurs in the PCB-treated rats (Liu et al., 1995; Hood et al., 1999; Hallgren et al., 2001; Kato et al., 2004). Hepatic type I iodothyronine deiodinase activity was significantly decreased in Wistar and Gunn rats by KC500 (Kato et al., 2004). On the other hand, a TTR-associated pathway might be considered as an explanation for the PCB-induced decrease in the level of serum total

![Graph showing tissue-to-serum concentration ratio ($K_p$ value) of $^{125}$I$^4$ in various tissues after administration of $^{125}$I$^4$ to the KC500-pretreated Wistar and Gunn rats. KC500 (10 mg/kg) was given i.p. to animals once daily for 10 days, and then, the animals were administered i.v. $^{125}$I$^4$. At 60 min after administration of $^{125}$I$^4$, the radioactivity in each tissue was measured. Each column represents the mean ± S.E. (vertical bars) for three to six animals. * $P < 0.05$, significantly different from each control. □ control; ■ KC500.](attachment://graph.png)
T₄, because PCB and its ring-hydroxylated metabolites act as T₄ antagonists to TTR (Lans et al., 1993; Brouwer et al., 1998; Meerts et al., 2002; Kato et al., 2004). Thus, competitive inhibition by PCB and/or its metabolites would promote a decrease in the level of serum total T₄. In the present study, significant decrease in the level of [¹²⁵I]T₄ bound to serum TTR and increase in the level of [¹²⁵I]T₄ bound to serum albumin occurred in both KC500-pretreated Wistar and Gunn rats, suggesting that PCB and/or its metabolite(s) inhibit the formation of serum T₄-TTR complex.

Thus, inhibition of the T₄-TTR formation might lead to change in the tissue distribution of T₄. Therefore, to clarify this, we administered [¹²⁵I]T₄ to KC500-pretreated Wistar and Gunn rats and, thereafter, determined the levels of [¹²⁵I]T₄ in their tissues. In addition, since [¹²⁵I]T₄ in either plasma or tissues is known to be stable during 48 h (Oppenheimer et al., 1968), the radioactivity detected in the serum and tissues would be attributed to [¹²⁵I]T₄ in each tissue. Marked increases in the mean total body clearance of [¹²⁵I]T₄ and in the steady-state distribution volume of [¹²⁵I]T₄ were observed in the KC500-pretreated rats. A tissue-to-serum concentration ratio (Kₑ value) was greater in several tissues, especially the liver, of the KC500-pretreated Wistar and Gunn rats than in the corresponding control (KC500-untreated) rat tissues. In addition, in both KC500-pretreated Wistar and Gunn rats, more than 40% of the [¹²⁵I]T₄ dosed was accumulated in the liver.

In conclusion, the present findings confirmed that PCB-induced decrease in serum T₄ occurs not only in Wistar rats but also in Gunn (UGT1A-deficient) rats and further led to a hypothesis that the PCB-induced decrease occurs through increase in accumulation (transportation from serum to liver) of T₄ in the liver, rather than through induction of hepatic T₄-UDP-GT. In addition, the increased accumulation in the liver might be attributed to the PCB- and its metabolite(s)-mediated inhibition of formation of serum T₄-TTR complex.

**References**
Barter RA and Klaassen CD (1992b) Rat liver microsomal UDP-glucuronosyltransferase activity

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**FIG. 9.** Tissue distribution of [¹²⁵I]T₄ after administration of [¹²⁵I]T₄ to the KC500-pretreated Wistar and Gunn rats. The experimental conditions were the same as those described in Fig. 8. Each column represents the mean ± S.E. (vertical bars) for four to six animals. *P < 0.05, significantly different from each control. ■, control; □, KC500.

**TABLE 3**
Liver weights after the administration of KC500 to Wistar and Gunn rats
Animals were killed at 4 days after the final administration of KC500 (10 mg/kg i.p., once daily for 10 days). The values shown are expressed as the mean ± S.E. for four to six animals.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>KC500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>3.07 ± 0.04</td>
<td>3.81 ± 0.17*</td>
</tr>
<tr>
<td>Gunn</td>
<td>3.25 ± 0.08</td>
<td>3.38 ± 0.10</td>
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</table>

*P < 0.01, significantly different from each control.

**TABLE 4**
Accumulation of [¹²⁵I]T₄ in the KC500-pretreated Wistar and Gunn rat livers
The experimental conditions were the same as those described in Fig. 8. The values shown are expressed as the mean ± S.E. for four to six animals.

<table>
<thead>
<tr>
<th>Animal</th>
<th>[¹²⁵I]T₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>KC500</td>
</tr>
<tr>
<td>Wistar</td>
<td>3.86 ± 0.18</td>
</tr>
<tr>
<td>Gunn</td>
<td>4.74 ± 0.43</td>
</tr>
</tbody>
</table>

*P < 0.001, significantly different from each control.


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