THE STUDY ON THE BIOLOGICAL FATE OF PARABEN AT THE DOSE OF PRACTICAL USAGE IN RAT. II.*¹ THE PHARMACOKINETIC STUDY ON THE BLOOD CONCENTRATION AFTER THE ADMINISTRATION OF ETHYL PARABEN OR *p*-HYDROXYBENZOIC ACID*²

HIROSHI KIWADA,*³ SHOJI AWAZU,*⁴ AND MANABU HANANO

Faculty of Pharmaceutical Science, University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo 113, Japan

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The biological fates of ethyl paraben and *p*-hydroxybenzoic acid in rat were investigated after intravenous and intraduodenal administrations at the dose of 2 mg/kg. The blood concentrations were measured in detail from 3 min after the administration at appropriate time intervals until 90 min. Areas under the blood concentration curves and clearances were calculated from these time course data.

Ethyl paraben was little detected in blood after intraduodenal administration. It is suggested that the intestinal metabolism and the first pass effect in liver greatly contribute to the hydrolysis of ethyl paraben.

Total radio activity after intraduodenal administration did not show the maximum peak and decreased rapidly. The maximum peak was not observed also in the time course of *p*-hydroxyhippuric acid after intravenous administration. It shows that not only the rate of hydrolysis but also absorption and conjugation are very rapid.

The differences of the areas under the blood concentration curves of *p*-hydroxybenzoic acid or *p*-hydroxyhippuric acid was found between the routes or chemical forms of administration. The complex kinetic mechanisms were assumed in the biological fates of these compounds as follows: Conjugation to *p*-hydroxyhippuric acid is excellent in ethyl paraben administration than *p*-hydroxybenzoic acid, and in intraduodenal administration than intravenous administration. These phenomenon can not be explained by the conventional kinetic model which is constructed with the connected blood compartments in series. The kinetic models including the assumed routes conjugating directly ethyl paraben in blood or intestine to *p*-hydroxyhippuric acid were presented, and the least square curve fitting analyses were carried out on these kinetic models.

Keywords—biological fate; metabolism; conjugation; paraben; ethyl *p*-hydroxybenzoate; *p*-hydroxybenzoic acid; pharmacokinetics; first pass effect

The excretion in urine and bile of rat after the administration of ethyl paraben (ethyl *p*-hydroxybenzoate, EP) or *p*-hydroxybenzoic acid (HB), hydrolyzed product of EP, at the dose of 2 mg/kg, that is in practical usage as preservatives, were reported in a previous paper.¹⁾ It was also showed

that the main metabolites of these compounds were *p*-hydroxyhippuric acid (glycine conjugate of HB, M1), *p*-hydroxybenzoil glucuronide (ester type glucuronide of HB, M3), and *p*-carboxyphenyl sulfate (ether sulfate of HB, M4).

The most conventional study^{2,3)} on the biolog-

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*³ Present address: *Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi Shinjuku-ku, Tokyo 162, Japan.*

*⁴ Present address: *Tokyo College of Pharmacy, Horinouchi Hachioji, Tokyo 192-03, Japan.*

ical fates of paraben were carried out not only at the nonpractical high dose but on the metabolites excreted in urine. A few studies were carried out on the blood concentration. Derache *et al.* studied the blood concentration of paraben at 30 or 60 min after oral administration at the dose of 500 mg/kg in rat.⁴⁾ The joint FAO/WHO expert committee on food additives reported⁵⁾ that EP was not detected in blood of man 1 hr after the oral administration at the dose of 10 or 20 mg/kg.

In this study, the blood concentrations of EP, HB, and their metabolites were traced in detail at 3 min after the intraduodenal or intravenous administration in rat at the dose of 2 mg/kg subsequently. These experiments made it clear that the absorption, metabolism, and excretion of EP or HB were rapid, especially the hydrolysis of EP was very rapid, and the unchanged EP was detected as little as 5% per total radio activity at 3 min after the intravenous administration and then decreased very rapidly. In the case of intraduodenal administration, the unchanged EP was little detected in the blood. Also in other concentration-time curves, the differences compared to the intravenous administration was observed for the intestinal metabolism or the first pass effect. These differences were also observed between the administered chemical forms (EP or HB). These facts suggest that the complex mechanisms take part in the biological fate, that is absorption, hydrolysis, and conjugation of EP at the low dose.

The time course data of blood concentration were analyzed by the nonlinear least square curve fitting method on the pharmacokinetic models suitable for the complex biological fate.

EXPERIMENTAL

Materials — The labeled compounds were obtained as described in previous paper.¹⁾ ¹⁴C-HB was purchased from Daiichi Pure Chemicals Co. and ¹⁴C-EP was synthesized from ¹⁴C-HB and EtOH with H₂SO₄ according to the method of Cavill *et al.*⁶⁾ as described previously.

Method of Determination — To a 0.1 ml of heparinized blood specimen, 0.5 ml of water was added to hemolyze it. An aliquot of 0.1 this

hemolyzed blood solution was used for determination of the total radio activity as follows, and the rest of the solution was used for a separative determination of EP, HB, and other metabolites. To the former, 0.5 ml of 20% ethanol solution of Protosol[®] (NEN) was added to decompose protein by standing for 24 hr at room temperature. It was decolorised by adding a drop of 30% hydrogen peroxide at room temperature. Then 15 ml of liquid scintillator, of which composition was described in the previous paper,¹⁾ was added and the total radio activity in the blood was counted with a liquid scintillation counter (Packard 3214). The rest of the hemolyzed blood specimen was treated in the same manner as described for the urine specimen in the previous paper.¹⁾

Experimental Procedure — The male albino rat of Wistar (210–240 g body weight) operated on the cannulations as described in the previous paper,¹⁾ and infused with the bile into the duodenum over night was used. The 0.05% solution of EP or HB was injected at the dose of 4.0 ml/kg through the polyethylene tubing set into the femoral vein for intravenous administration, and that placed into the bile duct for intraduodenal administration, respectively. The exact dose was determined in every experiment by counting the radio activity of the administered solution. The blood samples were collected in the heparinized micro tubes through the polyethylene tubing set into femoral artery. The first sampling time was 3 min after the administration and then the samplings were continued subsequently at appropriate time intervals until 90 min.

Data Analysis — A cpm (counts per minute) value obtained from each sample was converted to the dpm (disintegration per minute) value with the quenching curve obtained in each treatment of sample with the auto external standard method. The concentration per dose of each fraction was calculated from dpm value. Then the area under the blood concentration curve (AUC) was calculated. This numerical integration was carried out successively in approximation of the algebraic quadratic equation which was deter-

mined to pass through the neighboring three points.

The initial values of kinetic parameters for starting the nonlinear least square curve fitting of blood concentrations were determined as follows: The initial values for the distribution processes of EP and HB were obtained by the analysis on the time course data of EP and on that of HB after the intravenous administration using the program reported by Awazu *et al.*⁷⁾ for the least square analysis of 2-compartment model, respectively. And those of excretion process of the conjugates were determined with the deconvolution method reported previously by the authors.⁸⁾ With these initial values of distribution and excretion processes, the time course data of HB and its conjugates after intravenous administration of HB were again analyzed by the steepest decent method in nonlinear least square curve fitting.⁹⁾ Then converged kinetic parameters were used as the initial

values for the non-linear curve fitting analysis of further experiments.

These calculations were carried out by HITAC 8800/8700 digital computer in the Computer Center of University of Tokyo. The routine programs for these calculations were written in Fortran by the authors.

RESULTS

The Blood Concentration-Time Courses after HB Administration

The total radio activity in blood after intravenous administration of HB decreased rapidly, and the activity of HB itself also decreased rapidly as shown in Fig. 1-a. This suggests that the rate of elimination, that is excretion and conjugation of HB as reported previously,¹⁾ was very rapid. The blood concentration of M1 shows the highest concentration at the first sampling time (3 min). This suggests that the con-

FIG. 1. The Time Courses of Blood Concentration per Dose after Intravenous Administration of *p*-Hydroxybenzoic Acid (HB) at the Dose of 2 mg/kg

Points are mean values of two rats and standard deviations are expressed with vertical bars. Broken line is drawn by visual approximation and full lines are drawn by nonlinear least square curve fitting analysis. Figure (a) shows the courses of total radio activity (O) and HB (Δ), and figure (b) shows the courses of its conjugates (O: *p*-hydroxyhippuric acid (M1), ◇: *p*-hydroxybenzoil glucuronide (M3), and Δ: *p*-carboxyphenyl sulfate (M4)).

FIG. 2. The Time Courses of Blood Concentration per Dose after Intraduodenal Administration of *p*-Hydroxybenzoic Acid at the Dose of 2 mg/kg
All expressions are the same as in Fig. 1.

FIG. 3. The Time Courses of Blood Concentration per Dose after Intravenous Administration of Ethyl Paraben (EP) at the Dose of 2 mg/kg
All expressions are the same as in Fig. 1 except for EP (◇) in figure (a).

jugation rate to M1 is particularly rapid. These time courses of the blood concentration per dose of total activity, HB and its conjugates (M1, M3, and M4) are shown in Fig. 1.

The time courses after intraduodenal administration are shown in Fig. 2. As shown in Fig. 2-a, the time course of total radio activity had a max-

imum peak at the early stage after the administration. The time course of HB, which was the compound administered, did not show a peak, and M1 had it.

The Blood Concentration Time Courses after EP Administration

The total radio activity in the blood after

FIG. 4. The Time Courses of Blood Concentration per Dose after Intraduodenal Administration of Ethyl Paraben at the Dose of 2 mg/kg
All expressions are the same as in Fig. 3.

TABLE I. The Area under the Blood Concentration Curves (AUC)

	AUC (hr mg/ml)			
	HB-IV	HB-ID	EP-IV	EP-ID
Tl	70.5 ± 6.3 ^{a)}	84.7 ± 6.8	114.4 ± 2.5	113.1 ± 16.3
EP	—	—	13.5 ± 3.8	0.9 ± 0.4 ^{a)}
HB	25.4 ± 3.2	29.7 ± 1.2	20.7 ± 3.6	10.0 ± 3.0 ^{a)}
M1	18.7 ± 2.3 ^{a)}	31.7 ± 3.5 ^{b)}	51.5 ± 4.0	54.8 ± 4.9 ^{c)}
M3	9.2 ± 0.6	12.2 ± 4.9	10.8 ± 0.9	11.4 ± 5.9
M4	7.9 ± 2.5	9.7 ± 1.8	7.1 ± 0.4	18.4 ± 3.5 ^{a)}

Tl: total radio activity, EP: ethyl paraben, HB: *p*-hydroxybenzoic acid, M1: *p*-hydroxyhippuric acid, M3: *p*-hydroxybenzoyl glucuronide, M4: *p*-carboxyphenyl sulfate, HB-IV: intravenous administration of HB, HB-ID: intraduodenal administration of HB, EP-IV: intravenous administration of EP, EP-ID: intraduodenal administration of EP. Significance; $p < 0.05$, a) vs. EP-IV, b) vs. HB-IV, c) vs. HB-ID.

intravenous administration of EP also decreased rapidly. The activity of EP was detected as low as 5% of the total activity in the blood at 3 min, then decreased very rapidly, and was below the detectable level at 45 min after the administration. The detectable limit of this experiment is 5 ng/ml blood. Also the fact that the time course of HB did not show a peak suggests a very rapid hydrolysis of EP, as shown in Fig. 3-a. The elimination of HB was very rapid, and again a peak of M1 was not observed. These time courses are shown in Fig. 3.

After intraduodenal administration, the time course of total activity did not show a peak as that observed in HB administration and the time course decreased slowly at an early stage and then rapidly as shown in Fig. 4-a. EP was detected at a detectable limit in all samples.

The concentration of HB after intraduodenal administration of EP was lower than that after intravenous administration. At 3 min after administration, it appeared only about 12% of the total activity in the blood as shown in Fig. 4-a.

The Area under the Blood Concentration Curve (AUC)

The area under the time courses shown in Fig. 1,2,3, and 4 was calculated by the numerical integration and shown in Table I after the correction of dose.

On the AUC values of total radio activities, no significant differences were observed between

intravenous and intraduodenal administrations, and a complete absorption of both HB and EP were proved. Comparing between HB and EP administrations, the value after EP administration was larger after the correction of molecular weight.

In the administration of HB, M1 had a large AUC value after intraduodenal administration than intravenous administration, because of the increase of conjugation for the intestinal metabolism and first pass effect. In the intraduodenal administration of EP, EP was little detected in the blood and its AUC was 0.9 hr·mg/ml for the first pass effect as mentioned previously. The AUC of HB was 10.0 hr·mg/ml, which was smaller than intravenous administration for the same effect, also. Comparing the AUC ratio of M1 per HB between HB and EP administrations, the ratio is larger in EP administration, and the conjugation to M1 is suggested to be greater from EP than HB.

FIG. 5. *The Semilogarithmic Time Courses of Blood Concentration per Dose of p-Hydroxybenzoic Acid (HB) and Ethyl Paraben (EP) after Intravenous Administration*

● : HB after HB administration.

○ : EP after EP administration.

Lines are drawn by visual approximation.

TABLE II. *The Body Clearances calculated from AUC*

	Clearances (ml/hr)			
	HB-IV	HB-ID	EP-IV	EP-ID
Tl	478.2	432.7	386.4	383.3
EP	—	—	3268.0	49500.0
HB	1324.5	1235.7	2137.0	4323.3
M1	938.7	626.0	368.8	351.7
M3	677.7	738.0	1216.3	1242.3
M4	373.4	365.8	434.4	206.2

All symbols are same as Table I.

On the basis of AUC, the body clearances were calculated on the assumption that the clearance was independent of time and dose. These values are shown in Table II.

Kinetic Parameters

The curves of the logarithmic blood concentration of EP and HB per dose against time after intravenous administration of EP and HB are

CHART. 1. The Pharmacokinetic Models on the Fates of *p*-Hydroxybenzoic Acid (HB) and Ethyl Paraben in Rat

Compartment (1): EP in intestine, (1'): HB in intestine, (2): EP in blood, (12): distribution of EP, (3): HB in blood, (13): distribution of HB, (41): *p*-hydroxyhippuric acid (M1) in blood, (43): *p*-hydroxybenzoyl glucuronide (M3) in blood, (44): *p*-carboxyphenyl sulfate (M4) in blood.

The arrows and k_{ij} show the first order processes and rate constants from compartment (i) to (j), respectively.

Model 1: for intravenous administration of HB.

Model 2: for intraduodenal administration of HB.

Model 3: for intravenous administration of EP.

Model 4: for intraduodenal administration of EP.

TABLE III. The Pharmacokinetic Parameters of the Fate of *p*-Hydroxybenzoic Acid after Intravenous Administration on the Model 1 in Chart 1

Rate constant (hr^{-1})		Distribution volume (ml)
$k_{313} = 16.4$	$k_{343} = 2.38$	$V_3 = 112.2$
$k_{133} = 15.7$	$k_{43} = 14.3$	$V_{41} = 21.1$
$k_{341} = 5.22$	$k_{344} = 1.24$	$V_{43} = 58.2$
$k_{41} = 39.6$	$k_{44} = 5.08$	$V_{44} = 92.0$

shown in Fig. 5. These curves were used for determination of the initial values of distribution processes of EP and HB, respectively.

The curve fitting analysis of the time course data after intravenous administration of HB was

carried out on the model 1 shown in Chart 1. The parameters obtained with the correlation coefficient (c.c.) of 0.993 were shown in Table III.

By using the parameters in Table III as the initial values, the data of intraduodenal adminis-

TABLE IV. *The Pharmacokinetic Parameters of the Fate of p-Hydroxybenzoic Acid after Intraduodenal Administration*

Rate constant (hr ⁻¹)		Distribution volume (ml)
$k_{1'3} = 23.2$	$k_{1'41} = 0.69$	$V_3 = 143.5$
$k_{313} = 11.4$	$k_{341} = 8.38$	$V_{41} = 43.4$
$k_{133} = 11.6$	$k_{41} = 15.5$	$V_{43} = 57.5$
$k_{343} = 2.88$	$k_{344} = 1.60$	$V_{44} = 84.0$
$k_{43} = 13.0$	$k_{44} = 5.51$	

TABLE V. *The Pharmacokinetic Parameters of the Fate of Ethyl Paraben after Intravenous Administration on the Model 3 in Chart 1*

Rate constant (hr ⁻¹)		Distribution volume (ml)
$k_{212} = 16.3$	$k_{241} = 0.61$	$V_2 = 247.4$
$k_{122} = 3.47$	$k_{341} = 10.1$	$V_3 = 116.6$
	$k_{41} = 32.6$	$V_{41} = 14.0$
$k_{23} = 19.8$	$k_{243} = 0.24$	$V_{43} = 45.5$
$k_{313} = 9.91$	$k_{343} = 3.33$	$V_{44} = 91.6$
$k_{133} = 13.2$	$k_{43} = 12.4$	
	$k_{344} = 1.33$	
	$k_{44} = 4.89$	

TABLE VI. *The Pharmacokinetic Parameters of the Fate of Ethyl Paraben after Intravenous Administration on the Model 4 in Chart 1*

Rate constant (hr ⁻¹)		Distribution volume (ml)
$k_{12} = 5.66$	$k_{141} = 2.85$	$V_2 = 288.7$
$k_{212} = 20.6$	$k_{241} = 0.56$	$V_3 = 98.7$
$k_{122} = 3.85$	$k_{341} = 10.8$	$V_{41} = 10.7$
$k_{13} = 1.50$	$k_{41} = 40.4$	$V_{43} = 16.1$
$k_{23} = 30.1$	$k_{143} = 1.41$	$V_{44} = 83.9$
$k_{313} = 13.8$	$k_{243} = 0.20$	
$k_{133} = 12.2$	$k_{343} = 2.68$	
	$k_{43} = 16.1$	
	$k_{144} = 1.55$	
	$k_{344} = 1.47$	
	$k_{44} = 4.43$	

tration of HB were analyzed on the model 2 adding the first order processes of absorption ($k_{1'3}$) and direct conjugation from intestinal HB to M1 ($k_{1'41}$) on the basis of the information from blood concentration curves. The parameters obtained in this analysis were shown in Table IV (c.c.=0.995).

Similarly, the analysis of the data of intravenous administration of EP was tried on the model with the first order rate process of hydrolysis (k_{23}), but the reasonable parameters were not obtained with a good correlation coefficient. Therefore, the analysis on the model 3 with the direct conjugation processes (k_{241} and k_{243}) was carried out. Because the direct conjugation processes from EP to M1 *etc.* without *via* HB in the circulating blood compartment were suggested by the observations on the blood concentrations and the excretion data reported in the previous paper.¹⁾ The parameters obtained were shown in Table V (c.c.=0.994).

The data of intraduodenal administration of EP were analyzed on the model 4 similarly to the first order rate process of absorption (k_{12}) and the direct conjugation processes (k_{141} , k_{143} , and k_{144}). The parameters of this experiment were shown in Table VI (c.c.=0.997). The calculated concentration curves with the parameters shown in these tables are presented as full lines in Fig. 1, 2, 3, and 4.

DISCUSSION

EP was little detected in the blood of rat after intraduodenal administration at the dose of 2 mg/kg, while it was detected slightly and then disappeared rapidly after intravenous administration. These results agree with the report of FAD⁵⁾ in which it was not detected in the serum 1 hr after the oral administration at the dose of 10 or 20 mg/kg in man. Derache *et al.* have detected EP in the plasma of rat after the oral administration at the dose of 100 mg/head.⁴⁾ This appearance shows that the hydrolysis of EP depends on dose levels.

It has been described that the ester bond of paraben is stable *in vitro*, and does not degrade

by heating at 100° for 2 hr or at 120° for 30 min.¹⁰⁾ Therefore, it seems that the rapid hydrolysis *in vivo* presented in this experiments due to the carboxyesterase existing in blood, liver, and other tissues¹¹⁾. The ester form was less detected after intraduodenal than intravenous administration as mentioned above. The time course of each metabolite shows that the absorption rate is very rapid. Therefore it is assumed that not only the faster hydrolysis rate than the absorption but also the intestinal hydrolysis and the first pass effect in liver contribute to this phenomenon.

The AUC of M1 after intravenous administration of EP was larger than that after HB administration. When EP absorbed into circulating blood is hydrolyzed to HB, HB liberated into the blood is accepted in the metabolic site and conjugated. The AUC of a conjugate, that indicates the amount to pass the blood compartment, depends on the AUC of HB and the ratio between that of HB and its conjugate is constant in spite of the HB administered and transformed from the ester form. The differences in the AUC values are observed in this experiment between the origins of HB, that is directly injected HB and produced HB by the hydrolysis of administered EP. From this result, the direct metabolic route from EP to M1, instead of liberation as HB into blood for very rapid hydrolysis and conjugation rate, added to the generally thought metabolic routes as mentioned above was assumed in order to elucidate the observed data in blood compartment.

Similar phenomenon is observed between intravenous administration and intraduodenal administration. It is considered that this is due to the intestinal metabolism and the first pass effect in which the rate of conjugation was the same as that of hydrolysis as mentioned previously.

Two rats were used in each experiment. Although the number of subjects in these experiments are too small to discuss the differences in detail, it is considered that the experimental number (2+2 rats) was enough to find the statistically significant differences.

The intestinal conjugation was reported on salicylamide in detail by Riegelman *et al.*^{12,13)}

Although the conjugation with glycine in such a large proportion has not been reported, it is assumed that a fair part of paraben, also found in the present experiment was conjugated with glycine in intestine at a low dose level like that in salicylamide, or the first pass effect may play a large role in the direct conjugation of EP.

The direct conjugation mechanism from EP assumed in this study has been little known as mentioned in the previous paper.¹⁾ Only one example, Shomura *et al.* studied the macrolide antibiotics and its metabolites and presented the differences in bile excretion between the administered chemical forms.¹⁴⁾

The kinetic parameters obtained by the least square analysis are discussed as follows. The absorption rate constant of HB, k_{13} in Table IV, is larger than that of EP, k_{12} in Table VI, but it is not necessarily concluded that the absorption of HB is faster than EP, because the absorption of EP contains the route of direct metabolized absorption. The rate constant k_{13} in Table VI and model 4 in which HB appeared in circulating blood hydrolyzed directly from intestinal EP after intraduodenal administration of EP is appreciable comparing with k_{12} . Therefore, it suggests that about 27% per the amount absorbed as unchanged EP appeared in circulating blood as hydrolyzed HB.

The apparent rate constants of hydrolysis in circulating blood, k_{23} in Table V and VI, are 19.8 hr⁻¹ and 30.1 hr⁻¹ in intravenous administration and intraduodenal administration, respectively. Therefore, the kinetic mechanism assumed above, that is the direct route of conjugation is partially supported. The larger k_{23} in intraduodenal administration comparing with that in intravenous administration suggests the presence of an unknown factor which is accelerating the rate of hydrolysis of EP.

The mean rate constants of the direct conjugation mechanism, k_{241} *etc.* in model 3 and 4, show small values in all cases, while the mean rate constants of the direct conjugation from intestine, $k_{141}=2.85$ hr⁻¹, $k_{143}=1.41$ hr⁻¹, and $k_{144}=1.55$ hr⁻¹ in model 4, show the appreciable values, and

it is considered that about 50%, 25%, and 27% per the amount of unchanged EP absorbed into circulating blood were conjugated directly to M1, M3, and M4, respectively.

The excretion rate constants, k_{41} *etc.*, show fairly large values, and the clearances of the conjugates do not show any distinguishable differences among all the conjugates or experiments.

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