BIOPHARMACEUTICAL STUDIES ON HYDANTOIN DERIVATIVES. I. PHYSICO-CHEMICAL PROPERTIES OF HYDANTOIN DERIVATIVES AND THEIR INTESTINAL ABSORPTION

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The physico-chemical properties of a series of hydantoin derivatives and their intestinal absorption from solution were studied.

The introduction of the benzensulfonyl group at the 1-position of the hydantoin ring greatly affects the physico-chemical properties: the acid dissociation constants were increased 1000-fold and the partition coefficients were increased 100- to 1000-fold in the chloroform/water system and 10- to 100-fold in the n-octanol/water system. The solubilities of 1-benzenesulfonylhydantoin derivatives increased with increasing pH of the solution at pH more than 5, but the solubilities of the 1-unsubstituted hydantoin derivatives were scarcely dependent on the pH of the solution in the pH 1 to 8 region.

The intestinal absorption from solution was found to be caused by the passive transport according to the first-order kinetics. The rate constants of absorption of 1-benzenesulfonylhydantoin derivatives were rather large even under the condition where they were 99% ionized in the solution than that of the corresponding 1-unsubstituted hydantoin derivatives which exist mainly as the unionized form under the same condition.

The intestinal absorption from a solution and the partitioning to chloroform produced linear free energy relationships to each other for the 1-benzenesulfonylhydantoin derivatives and for the 1-unsubstituted hydantoin derivatives independently. However when the partition coefficients in the n-octanol/water system were applied, the hydroxyl derivatives were found to deviate from linear relationships.

On the basis of the results, a suggestion was made on the in vivo behavior and the bioavailability.

Keywords—hydantoin derivatives; phenytoin; 1-benzenesulfonyl-5,5-diphenylhydantoin; physico-chemical property; solubility; pKa; partition ratio; partition coefficient; in situ intestinal absorption in rat; pharmacokinetics.

Phenytoin is one of the most frequently used drugs in the therapy of epilepsy. Among a large number of hydantoin derivatives synthesized in the search for new anti-epileptic drugs that may be more effective and less toxic, the derivative in which the benzenesulfonyl group is introduced at the 1-position of phenytoin was found not to exert any effect on the central nervous system in contrast to phenytoin, but to have peripherally active anti-inflammatory effects in the pharmacological screening tests.

The changes in the pharmacological properties due to the introduction of the benzenesulfonyl group into the hydantoin ring are particularly interesting in connection with the structure-activity relationship and therefore it has been attempted to explore the benzenesulfonylhydantoin derivatives in comparison with the derivatives having no benzenesulfonyl group, on the standpoint of biopharmaceutics.

The purpose of the present study is to reveal the effects of the introduction of the benzenesulfonyl group into the hydantoin ring on the physico-chemical properties and the rate of absorption from the small intestine of rats. Furthermore, the aim is to find the factors deter-
mining the bioavailability of hydantoin derivatives, based on the results obtained.

EXPERIMENTAL

Chemical Structures of Hydantoin Derivatives and Conditions under which Measurements were carried out — The hydantoin derivatives studied were synthesized in our research laboratory. The chemical structures are listed in Table I, along with wavelengths at which some physical properties were measured, and analytical conditions of gas chromatography.

**Apparatus** — Spectrophotometric measurements were made on Hitachi EPS-2U Spectrophotometer and Shimadzu Double-40 Multi-convertible Spectrometer. The pHs were measured with a TOA HM-5A pH meter. A JEOL JGC-750 Gas Chromatograph equipped with a flame ionization detector was used for the quantitative analysis. A 0.75 m, 3 mmø stainless steel tubing packed with Shimalite 80/100 mesh coated with 3% OV-17 was used as the column.

**Stability to the Gastro-intestinal Contents** — The experiments were carried out by incubating each

<table>
<thead>
<tr>
<th>TABLE I. Structures of Hydantoin Derivatives used in This Study, Gas Chromatographic Conditions for the Quantitative Analysis, and Wavelengths at which Absorbance was measured for the Determination of Solubility, pKₐ Value, and Partition Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical Structures" /></td>
</tr>
<tr>
<td>Wavelength (nm)</td>
</tr>
<tr>
<td>Gas chromatography&lt;sup&gt;a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \text{No.} ) ( \text{R}_1 ) ( \text{R}_2 ) ( \text{R}_3 ) ( \text{Solubility} ) ( \text{pK}_a ) ( \text{3-NH} ) ( \text{OH} ) ( \text{Partition} ) ( \text{Column temp.} ) ( \text{Retention time (min)} ) ( \text{Internal standard} )</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>VI</td>
</tr>
<tr>
<td>VII</td>
</tr>
<tr>
<td>VIII</td>
</tr>
<tr>
<td>IX</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>XI</td>
</tr>
<tr>
<td>XII</td>
</tr>
<tr>
<td>XIII</td>
</tr>
<tr>
<td>XIV</td>
</tr>
</tbody>
</table>

<sup>a</sup>) Gas chromatographic condition is as follows: helium flow, 60 ml/min (2 kg/cm²); temperature of detector and injection port is 300°C.

<sup>b</sup>) Internal standard: \( \text{N-(3,3-diphenylpropyl)-N-trifluoroacetylaminopropyl-3,4,5-trimethoxybenzoate} \);

<sup>c</sup>) Cholesterol;

<sup>d</sup>) Benzophenone.
derivative with the gut contents of rats, according to the procedure previously reported.  

Measurement of Solubility in Water — An excess amount of each derivative was added to 50 ml of buffers of various pHs which were kept at 37°C under vigorous stirring. After an equilibrium was reached, an aliquot of the solution was withdrawn by using a pipet equipped with a paper filter at its tip and assayed spectrophotometrically and/or gas chromatographically. Isotonic sodium phosphate buffers and ammonium-ammonium chloride buffers were used for 1-benzenesulfonylhydantoin derivatives and for 1-unsubstituted derivatives respectively. The solubility of unionized form of each derivative was determined in hydrogen chloride-sodium acetate buffers.

Measurement of Partition Ratio between Organic Solvent and Water — Prior to the partitioning, a mutually saturated organic phase and an aqueous phase were prepared. Each derivative was dissolved in an aqueous phase. The partition ratio was determined by measuring the concentration in the aqueous phase before and after the partitioning, spectrophotometrically. The partitioning was carried out by shaking mechanically the mixture of the organic phase (4—20 ml) and aqueous phase (10—50 ml) at three different volume ratios in a glass-stoppered flask for 30 minutes, followed by centrifugation.

Measurement of Acid Dissociation Constant  

— Acid dissociation constants were determined by the solubility method and the spectrophotometrical method.

Intestinal Absorption from Aqueous Solution — The recirculation experiment was carried out by using the small intestine of rats in situ. Male Wistar rats, weighing 180 g, were fasted for 15 hours prior to experiment except for drinking water. After anesthesia with pentobarbital, their small intestines were cannulated. Forty milliliters of the isotonic sodium phosphate buffer solution of each derivative was circulated continuously from the pylorus to the end of the ileum at the rate of 7 ml/min. At a certain interval of time, the residual amount in the circulating solution was determined. Two different assay procedures were adopted according to the type of chemical structures; For the derivatives (I, II, V, VIII, IX, X, and XIII), 5 ml of 0.1 N HCl and 5 ml of chloroform were added to 0.5 ml of the circulating solution in a 40 ml glass-stoppered centrifuge tube. The tube was mechanically shaken for 20 minutes and then centrifuged. Three milliliters of the chloroform phase was transferred to a 10 ml centrifuge tube and evaporated to dryness. The residue was analyzed by gas chromatography after methylation with diazomethane. For the derivatives (XI, XII, and XIV), 2 ml of methanol was added to 0.5 ml of the circulating solution in a 10 ml centrifuge tube to cause the precipitation of inorganic salts. After centrifugation, 2 ml of the supernatant was transferred to a second 10 ml centrifuge tube and evaporated to dryness. The residue was likewise analyzed as above.

The concentration changes of each derivative with water transfer were corrected by determining phenol red added to the circulating solutions.

Determination of Plasma Concentration of I — To 1 ml of plasma in a 40 ml centrifuge tube, 10 ml of 0.1 N NaOH and 10 ml of chloroform were added. The tube was mechanically shaken for 15 minutes and centrifuged. Eight milliliters of the aqueous phase were transferred to a second 40 ml centrifuge tube containing 2 ml of 1 N HCl and 8 ml of chloroform. The tube was shaken and centrifuged. The aqueous phase was pipetted and discarded. Five milliliters of chloroform phase were transferred to a 10 ml centrifuge tube and evaporated to dryness. The residue was analyzed by gas chromatography after methylation with diazomethane.

Determination of Tissue Concentration of I — The tissues were homogenized with the isotonic phosphate buffer of pH 7.0 to obtain the homogenate three times the weight of the tissues. After the transfer of 5 g of the homogenate to a 40 ml centrifuge tube containing 2 ml of 2 N HCl and 25 ml of chloroform, the tube was mechanically shaken for 20 minutes and
centrifuged. A 20 ml aliquot of the chloroform phase was transferred to a 40 ml centrifuge tube containing 10 ml of 0.1 N NaOH. After the tube was shaken and centrifuged, 8 ml of the aqueous phase was transferred to another centrifuge tube containing 2 ml of 1 N HCl and 8 ml of chloroform. The tube was shaken and centrifuged as above-mentioned. Five milliliters of the chloroform phase were transferred to a 10 ml centrifuge tube and evaporated to dryness. The residue was analyzed by gas chromatography after methylation with diazomethane.

RESULTS AND DISCUSSIONS

Stability of the Hydantoin Derivatives in the Digestive Tract

The hydantoin derivatives used in this study were neither hydrolyzed in the physiological range of pH 1 to 8 in the gastrointestinal tract nor subjected to enzymatically catalyzed decomposition by the gastro-intestinal contents. These results suggest that the hydantoin derivatives are stable in the process of gastro-intestinal absorption.

Solubility in Water and Acid Dissociation Constant

Hydantoin derivatives dissociate in water, as shown in Chart 1. The pH dependency of the solubility was studied. Fig. 1 illustrates the results on the derivatives (I) and (XIII) as the representative of 1-benzenesulfonylhydantoin and of 1-unsubstituted hydantoin, respectively.

It was found that the 1-benzenesulfonyl-5,5-diphenylhydantoin are practically insoluble in water at pH not more than 5, but at pH more than 5 the solubility increases with an increasing pH of the solution. On the other hand, the solubility of the 1-unsubstituted hydantoin derivatives increases with an increasing pH of the solution at pH more than 8—9.

The solubility S of an acidic compound may be represented by Eq. 1:

\[ S = [A] + [A^-] + [A] \cdot 10^{(pH - pK_a)} = [A] + [A] \cdot K_a \cdot [H^+]^{-1} \]  \hspace{1cm} (Eq. 1)

where [A] and [A\(^-\)] are the concentrations of unionized and ionized forms, respectively; \( K_a \) is the acid dissociation constant.

In accordance with Eq. 1, a plot of solubility, S, against the reciprocal of hydrogen ion concentration, \([H^+]^{-1}\), proved to be linear in the pH 5 to 7 region for the 1-benzenesulfonylhydantoin derivatives, and also in the pH 8 to 9.5 region for the 1-unsubstituted hydantoin derivatives, as shown in Fig. 2 and Fig. 3, respectively.

In Table II are listed p\( K_a \) values and solubilities of the unionized forms, together with calculated solubilities at pH 7 evaluated from Eq. 1. The p\( K_a \) values of the derivatives (XI), (XII), and (XIII) which were reported by Edsall, Butler, and Agarwal are in agreement with our results.

![Chart 1: Dissociation Equilibrium of Hydantoin Derivatives in Water](image)
1-Benzene sulfonylhydantoin derivatives have pK_a values approximately 3–4 units lower than that of the 1-unsubstituted hydantoin derivatives, the large acid-strengthening effect of the benzenesulfonyl group being attributed to the formal dipolar bond of the sulfonyl group which strongly attracts the electron by the inductive mechanism, as shown in Chart 2.

On 1-substituted benzenesulfonyl-5,5-diphenylhydantoins, the effect of the substituent on the pK_a was studied. The relationship between logarithms of the acid dissociation constant, log K_a, and the substituent constant, σ^9, is analyzed, as shown in Fig. 4. The plot of log K_a against σ proved that the data follow a straight line which can be well represented by Eq. 2.

\[ \log K_a = 0.439 \sigma - 4.88 \]  
(Eq. 2)  
\( r = 0.989, n = 8 \)

The positive coefficient of 0.439 demonstrated that the dissociation constant increases with an increasing electron withdrawing power of the substituent introduced into the benzenesulfonyl group at the 1-position of the hydantoin ring, but the magnitude of the effect is relatively small.  

**Partition Coefficient between Organic Solvent and Water**

**FIG. 1. pH Dependency of Solubility in Water for I (○) and XIII (●).**  
Refer to Table I for the numbers noted in this figure.

**FIG. 2. Relationship between Solubility and 1/[H^+] for I (○) and II (●).**  
Refer to Table I for the numbers noted in this figure.

**FIG. 3. Relationship between Solubility and 1/[H^+] for XIII.**  
Refer to Table I for the number noted in this figure.
TABLE II. Solubilities in Water and pK<sub>a</sub> Values of Hydantoin Derivatives

<table>
<thead>
<tr>
<th>Derivatives No.</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt; 3-NH</th>
<th>OH</th>
<th>Solubility, µg/ml at pH 7.0</th>
<th>S&lt;sub&gt;o&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.89 a)</td>
<td>4.90 b)</td>
<td>1.80</td>
<td>234</td>
</tr>
<tr>
<td>II</td>
<td>5.00</td>
<td>5.12</td>
<td>0.76</td>
<td>67.0</td>
</tr>
<tr>
<td>III</td>
<td>4.76</td>
<td>4.77</td>
<td>0.34</td>
<td>59.2</td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>4.95</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V</td>
<td>4.46</td>
<td>4.60</td>
<td>0.65</td>
<td>192</td>
</tr>
<tr>
<td>VI</td>
<td>5.03</td>
<td>—</td>
<td>0.51</td>
<td>51.5</td>
</tr>
<tr>
<td>VII</td>
<td>—</td>
<td>4.58</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VIII</td>
<td>5.01</td>
<td>5.20</td>
<td>7.81</td>
<td>3.30</td>
</tr>
<tr>
<td>IX</td>
<td>5.11</td>
<td>5.13</td>
<td>1.40</td>
<td>108</td>
</tr>
<tr>
<td>X</td>
<td>—</td>
<td>5.15</td>
<td>—</td>
<td>337</td>
</tr>
<tr>
<td>XI</td>
<td>—</td>
<td>9.20</td>
<td>150000</td>
<td>150000</td>
</tr>
<tr>
<td>XII</td>
<td>—</td>
<td>8.51</td>
<td>805</td>
<td>834</td>
</tr>
<tr>
<td>XIII</td>
<td>8.31</td>
<td>8.30</td>
<td>38.5</td>
<td>40.4</td>
</tr>
<tr>
<td>XIV</td>
<td>—</td>
<td>7.90</td>
<td>36.0</td>
<td>40.6</td>
</tr>
</tbody>
</table>

S<sub>o</sub>: solubility of unionized form.

a) determined by solubility method.
b) determined by spectrophotometry.

Refer to Table I for the numbers noted in this table.

At partition equilibrium, where a compound dissociates only in the water phase, the apparent partition ratio (APR) is given by Eq. 3:

\[
APR = \frac{[A_0]}{[A_w] + [A^-]} = \frac{[A_0]}{[A_w] \cdot [1 + 10^{pH - pK_a}]} = [PC] \cdot [1 + 10^{pH - pK_a}]^{-1}
\]

where \([A_w]\) and \([A_0]\) are the concentrations of the unionized form in the water phase and in the organic phase, respectively; \([A^-]\) is the concentration of the ionized form in water; hence, the partition coefficient (PC) of the unionized form is represented by \([A_0]/[A_w]\).

In Table III are listed apparent partition ratios at pH 7 and partition coefficients of unionized forms calculated from Eq. 3.

A linear relationship was observed between the logarithms of partition coefficients of a series of derivatives in two different sets of solvent

CHART 2
Intestinal Absorption of Hydantoins

When the data in Table III are plotted with the logarithms of partition coefficients in the chloroform/water system as the abscissa and with those in the n-octanol/water system as the ordinate in Fig. 5, a straight line which can be adequately approximated by Eq. 4 is obtained:

$$\log PC_{oct} = 0.644 \log PC_{chlor} + 1.27 \quad (\text{Eq. 4})$$

where $PC_{oct}$ and $PC_{chlor}$ are partition coefficients in the n-octanol/water system and the chloroform/water system, respectively.

The plots of the hydroxyl derivatives (VIII) and (XIV) deviate from the linear relationship, their partitioning to n-octanol being much larger than predicted on the relationship. This may be attributed to a strong interaction, hydrogen bonding between the hydroxyl groups of the derivatives and of n-octanol, which cannot be regarded as significant in the chloroform/water system.

**Intestinal Absorption from Aqueous Solution**

The absorption rates of the derivatives from an aqueous solution were examined by a recirculation method, using the small intestine of rats in situ.

As can be seen from Fig. 6 and Fig. 7, plotting with the lapse of time on the logarithm of the residual amount in the recirculating solution showed the linearity for each derivative, and the slope of the line is constant irrespective of the initial concentration. Hence, it appears that the absorption of the hydantoin derivatives through the epithelial cell membrane of the small intestine is caused by the passive transport depending upon the concentration gradient. The first order rate

![Graph showing Hammett equation plot for the ionization of 1-substituted benzenesulfonyl-5,5-diphenylhydantoins](image)

**FIG. 4. Hammett — Equation Plot for the Ionization of 1-Substituted benzenesulfonyl-5,5-diphenylhydantoins**

Refer to Table I for the numbers noted in this figure.

---

**TABLE III. Apparent Partition Ratios at pH 7.0 (APR) and Partition Coefficients of Unionized Form (PC) in Chloroform/water and n-Octanol/Water Systems**

<table>
<thead>
<tr>
<th>Derivatives No.</th>
<th>Chloroform/Water system APR</th>
<th>Chloroform/Water system PC</th>
<th>n-Octanol/Water system APR</th>
<th>n-Octanol/Water system PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>322</td>
<td>41900</td>
<td>84.6</td>
<td>11000</td>
</tr>
<tr>
<td>II</td>
<td>325</td>
<td>28600</td>
<td>113</td>
<td>9960</td>
</tr>
<tr>
<td>III</td>
<td>433</td>
<td>74100</td>
<td>178</td>
<td>30400</td>
</tr>
<tr>
<td>V</td>
<td>64.6</td>
<td>19200</td>
<td>37.2</td>
<td>11000</td>
</tr>
<tr>
<td>VIII</td>
<td>1.19</td>
<td>110</td>
<td>41.4</td>
<td>3840</td>
</tr>
<tr>
<td>IX</td>
<td>17.0</td>
<td>1310</td>
<td>24.1</td>
<td>1850</td>
</tr>
<tr>
<td>X</td>
<td>2.72</td>
<td>195</td>
<td>3.81</td>
<td>273</td>
</tr>
<tr>
<td>XI</td>
<td>0.039</td>
<td>0.039</td>
<td>0.333</td>
<td>0.333</td>
</tr>
<tr>
<td>XII</td>
<td>2.80</td>
<td>290</td>
<td>21.3</td>
<td>22.1</td>
</tr>
<tr>
<td>XIII</td>
<td>36.2</td>
<td>380</td>
<td>90.6</td>
<td>95.0</td>
</tr>
<tr>
<td>XIV</td>
<td>0.153</td>
<td>0.172</td>
<td>47.0</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Refer to Table I for the numbers noted in this table.
FIG. 5. Relationship between Logarithms of Partition Coefficients in n-Octanol/Water System (PCoct.) and Those in Chloroform/Water System (PCchlor.)

Refer to Table I for the numbers noted in this figure.

FIG. 6. Semilog Plot of Remaining Amount in Recirculating Solution Versus Time

Each point represents the mean of three experiments and vertical bracketed lines are the standard error of the mean. Refer to Table I for the number noted in this figure.

FIG. 7. Semilog Plot of Average Concentration of X in Recirculating Solution versus Time

Each point represents the mean of three experiments and vertical bracketed lines are the standard error of the mean. Refer to Table I for the number noted in this figure.

constants of the absorption were calculated from the slopes of the straight lines and listed in Table IV, along with the proportions of the ionized and the unionized forms in the aqueous solution of pH 7 at which the experiment was performed.

Noteworthy among the data in Table IV is the effect of the benzenesulfonyl group upon absorption. As can be seen in comparison with 1-benzenesulfonylhydantoin derivatives (I) and (X) and the corresponding 1-unsubstituted derivatives (XIII) and (XII) respectively, the rate constants of absorption of the former are rather greater, even under the condition where each is about 99% ionized in the solution. For the latter, about 95% of its molecules exist as the unionized species under these conditions. The partition coefficients of the former, much greater than those of the latter, may explain the above results. The fact suggests that even drugs, most of whose
TABLE IV. In Situ Intestinal Absorption of Hydantoin Derivatives from Aqueous Solution of pH 7.0 in Rat

<table>
<thead>
<tr>
<th>Derivatives No.</th>
<th>First order rate constant of absorption, (h(^{-1})) mean ± s.e.</th>
<th>% ionized at pH 7.0</th>
<th>% unionized at pH 7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.10 ± 0.015 (3)</td>
<td>99.23</td>
<td>0.77</td>
</tr>
<tr>
<td>II</td>
<td>2.11 ± 0.179 (3)</td>
<td>98.86</td>
<td>1.14</td>
</tr>
<tr>
<td>V</td>
<td>1.92 ± 0.110 (3)</td>
<td>99.66</td>
<td>0.34</td>
</tr>
<tr>
<td>VIII</td>
<td>0.889 ± 0.079 (3)</td>
<td>98.92</td>
<td>1.08</td>
</tr>
<tr>
<td>IX</td>
<td>1.32 ± 0.125 (3)</td>
<td>98.70</td>
<td>1.30</td>
</tr>
<tr>
<td>X</td>
<td>1.16 ± 0.031 (3)</td>
<td>98.61</td>
<td>1.39</td>
</tr>
<tr>
<td>XI</td>
<td>0.473 ± 0.029 (3)</td>
<td>0.63</td>
<td>99.37</td>
</tr>
<tr>
<td>XII</td>
<td>0.957 ± 0.088 (3)</td>
<td>3.47</td>
<td>96.53</td>
</tr>
<tr>
<td>XIII</td>
<td>1.44 ± 0.085 (3)</td>
<td>4.67</td>
<td>95.33</td>
</tr>
<tr>
<td>XIV</td>
<td>0.650 ± 0.022 (3)</td>
<td>11.22</td>
<td>88.78</td>
</tr>
</tbody>
</table>

Refer to Table I for the numbers noted in this table.
a) Numbers in parentheses indicate the number of experiment.

molecules exist as the ionized form in the digestive tract, are well absorbed if their partition coefficients are sufficiently large.

In order to study that a compound eliminated from the recirculating solution is actually transferred into blood, plasma and intestine concentrations also were simultaneously determined along with the residual amount in the recirculating solution, in the experiment using the derivative (I). Prior to analysis of the data, pharmacokinetic constants of I in the rat were determined. Fig. 8 shows the semi-logarithmic plot of plasma concentration against time after injection of I into femoral vein of rats, suggesting that the elimination of I from body obeys the first-order kinetics. The rate constant of elimination, \( K_d \), and the biological half-life, \( T_{1/2} \), calculated from the straight line was found to be 0.462 h\(^{-1}\) and 1.50 h, respectively. The volume of distribution \( V_d \) was proved to be 0.32 l/kg, which corresponds approximately to the amount of extra-cellular fluid. The pharmacokinetic constants determined after injection into the portal vein and after injection into the femoral vein were nearly equivalent, suggesting that the first-pass effect in the liver is negligible. Fig. 9 shows the plot of the residual amount of I in

FIG. 8. Semilog Plot of Plasma Concentration of I versus Time after Intravenous Administration of Following Solution (2.2 ml/kg) to Rats

- The sterile solution of I for injection, 10 mg/ml.
- Sodium salt of I 105.6 mg.
- Ethanol 1.0 ml.
- Propylene glycol 4.0 ml.
- Water for injection to make 10.0 ml.

Refer to Table I for the number noted in this figure.
the recirculating solution, the amount in the body, \( Cp \cdot Vd \), calculated from the plasma concentration \( Cp \), and the amount in the tissue of the small intestine against time. In the figure, points at each time represent the experimental values in a rat and the lines are the theoretical curves representing respectively the amount (A) of I in the recirculating solution and the amount (B) of I in the body, calculated from Eq. 5 and Eq. 6 based on the pharmacokinetics model shown in Chart 3.

\[
A = A_o \cdot e^{-K_{abs} \cdot t} \tag{Eq. 5}
\]

\[
B = A_o \cdot \frac{K_{abs}}{K_{el} - K_{abs}} (e^{-K_{abs} \cdot t} - e^{-K_{el} \cdot t}) \tag{Eq. 6}
\]

Where \( K_{abs} \) and \( K_{el} \) are the first-order rate constants of absorption and of elimination; \( A_o \) is the initial amount of I in the recirculating solution (3.8 mg). The theoretical curves approximately follow each point, suggesting that the model shown in Chart 3 is reasonable on representing the transfer of I in the body. These results confirm that even the derivative (I) which is eliminated rapidly from the recirculating solution does not remain in the tissue of the intestine, but is quickly transferred into the blood.

The correlation of the rate constant of absorption with the partition coefficient was examined, by using the logarithms of these constants, which correspond respectively to the free energy changes of partitioning to organic solvent and of absorption. As seen from Fig. 10, the plot of logarithms of the rate constant of absorption \( \log K_{abs} \) against the logarithm of the partition coefficient \( \log PC_{chlor} \) in the chloroform/water system revealed the applicability of two separate linear relationships which are respectively represented by Eq. 7 for the 1-benzenesulfonylhydantoin derivatives and by Eq. 8 for the 1-unsubstituted hydantoin derivatives.

\[
\log K_{abs} = 0.134 \log PC_{chlor} - 0.289 \quad \text{(Eq. 7)}
\]

\[
\log K_{abs} = 0.157 \log PC_{chlor} - 0.088 \quad \text{(Eq. 8)}
\]

The difference between two linear relationships may be due to such a difference in their tendencies to ionize that under the experimental condition the former is about 99% ionized whereas the latter exist mostly as the unionized form. The same type of plot on the partition coefficients in the \( n \)-octanol/water system is given in Fig. 11. Although two separate linear relationships are also applicable to each series of the derivatives in this case as well, attention should be given to the

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**FIG. 9. Relationship between Amount of I and Time**

- ○: amount of I in body, \( Cp \cdot Vd \).
- ●: amount of I in recirculating solution.
- △: amount of I in tissue of small intestine.

Refer to Table 1 for the number noted in this figure.

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**CHART 3. Pharmacokinetic Model on Transfer of I in Body**

- \( K_{abs} \cdot A \): amount of I in recirculating solution.
- \( K_{el} \cdot B \): amount of I in body, \( Cp \cdot Vd \).
- \( K_{abs} \): first order rate constant of absorption.
- \( K_{el} \): first order rate constant of elimination.

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fact that the plots for hydroxyl derivatives (VIII) and (XIV) deviate from the lines. This result suggests that such a strong hydrogen bonding as is expected in n-octanol is not concerned in the absorption process and that n-octanol is not necessarily an adequate solvent for the prediction of absorbability of a homologous series containing the hydroxyl derivatives. The above way of thinking may be inconsistent with the view proposed by Kakemi et al.\textsuperscript{11)} that the absorption of barbituric acids from the rat intestine is mediated by the process favoring hydrogen bonding, and the view by Hansch\textsuperscript{12)} that in selecting a reference system close to the biophase, the hydroxyl function of octanol would seem to be a reasonable model of macromolecules. To clarify this point, further study is necessary because the numbers and kinds of the derivatives used in this study are limited.

From the results of the studies on the physicochemical properties of hydantoin derivatives and their intestinal absorption from solution, the following steps of assumption may be made on the \textit{in vivo} behavior and the bioavailability.

On the \textit{in vivo} behavior; There may be differences in the distribution and the metabolism between the benzenesulfonylhydantoin derivatives and the derivatives having no benzenesulfonyl group, because the former exists almost in the ionized form in the plasma, whereas the lat-
ter exists almost in the unionized form.

On the bioavailability; As the solubility of the unionized form of 1-benzensulfonyl-5,5-di-phenylhydantoin derivatives having a $pK_a$ value of about 5 is extremely low, the derivatives are practically insoluble in the stomach, but begin to dissolve in the ionized form as it descends from the pylorus to the lower intestine. On the other hand, the intestinal absorption from solution is very good even if they are ionized. Hence, the dissolution into the gastro-intestinal fluids may be the rate-determining step in the bioavailability.

Each 1-unsubstituted hydantoin derivatives having a $pK_a$ value of 8 to 9 will dissolve at a certain concentration equal to the solubility of the unionized form over the whole range of the gastro-intestinal tract. For example, it is considered that 5,5-diphenylhydantoin dissolves as the unionized form at a concentration of about 40 $\mu$g/ml in both the stomach and the intestine. As the solubility of this compound is not so large, but the absorption from solution is fairly good, the bioavailability will depend mainly on the dissolution rate.

Irrespective of the presence of the benzene-sulfonyl group at the 1-position of hydantoin ring, the solubilities of the 5-methyl or 5-ethylhydantoin derivatives are sufficiently large. Hence, the dissolution rate seems not to be a determining factor in the bioavailability.

To verify the assumption mentioned above, in vivo behavior of the derivatives will be studied and reported successively.

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