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Pharmacokinetic Aspects of Elimination from Plasma and Distribution to Brain and Liver of Barbiturates in Rat[1]

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Elimination from plasma and distribution to brain and liver of eight barbituric acid derivatives in J. P. VIII were studied pharmacokinetically after intravenously administration to rat. Plasma concentration time courses of the barbituric acid derivatives were analyzed well with two compartment model. Distribution to brain of the lipid soluble barbituric acid derivatives was so rapid that brain was assigned to compartment I which includes plasma, while the distribution of the acids which have low lipid solubility was slow and the brain was found to be assigned to compartment II, especially for barbital, allobarbital and phenobarbital. On the other hand, liver was assigned to compartment I.

As for pharmacokinetic constants, elimination rate constant was found to have a significant regression against lipid solubility (measured as a partition coefficient between CCl_4 and pH 7.4 phosphate buffer) except thiopental which belongs to the different series from the other seven barbituric acid derivatives in this paper. On the other hand, distribution rate constants, k_{12} and k_{21} were found to have no regression against lipid solubility. From these results, a clue was given to anticipate the plasma concentration and brain level time courses of barbituric acid derivatives from their lipid solubility.

In order to clarify the pharmacological action and behavior of drugs, pharmacokinetic study of time course on target organ and metabolic organ is one of the important informations to be studied. Although many studies on the pharmacokinetic analysis for drug elimination and metabolism have been reported recently,[3] pharmacokinetics about tissue or organ distribution are few.[4] As for barbituric acid derivatives, it has been known that lipid soluble derivatives, such as thiopental[5,6] and hexobarbital,[7] distribute to brain very rapidly and the concentration in brain attains the equilibrium with the plasma concentration within few minutes after intravenous administration. On the other hand, it has been also known that barbital and phenobarbital which have low lipid solubility distribute to brain slowly and 30 min or more are necessary to attain the equilibrium.[6] It is not clear, however, how such a slow distribution can be described from the compartment analysis stand point. Slow distribution to brain means only that brain belongs to a different compartment from the compartment which includes plasma. Plasma concentration data of all barbituric acid derivatives studied here were found to be analyzed with two compartment model in Chart 1. There-

1) This work was presented to the 92th Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972, and abstracted in part from the thesis presented by Yi-Jong Lin to the Graduate School, the University of Tokyo, in partial fulfillment of Master of Pharmaceutical Science degree requirement.

2) Location: *Hongo, Bunkyo-ku, Tokyo.*

3) As examples, *a*) J. Shibasaki, T. Koizumi, and T. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1661 (1968); *b*) W.T. Jusko and G. Levy, *J. Pharm. Sci.*, **59**, 765 (1970); *c*) J.B. Nagwekar and A. Lunikrishnan, *ibid.*, **60**, 375 (1971); *d*) S.A. Kaplan, M. Lewis, M.A. Schwartz, E. Postma, S. Colter, C.W. Abruzzo, T.L. Lee, and R.E. Weinfeld, *ibid.*, **59**, 1569 (1970).

4) J.G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1 st ed., Drug Intelligence Publications, Hamilton, Ill., 1971, p. 260, for qualitative discussion.

5) L.C. Mark, J.J. Burns, L. Brand, C.I. Campomanes, N. Trousof, E.M. Papper, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **123**, 70 (1958).

6) S. Mayer, R.P. Maickel, and B.B. Brodie, *J. Pharmacol. Explt. Therap.*, **127**, 205 (1959).

7) J. Noordhoek, *Europ. J. Pharmacol.*, **3**, 242 (1968).

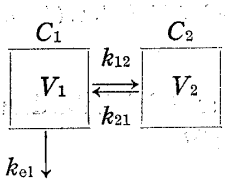

Chart 1. Two Compartment Model

C: concentration in compartment
V: distribution volume of compartment
k: rate constant
Equal concentration is supposed at equilibrium for compartment I and II to calculate V_2, with the Eq. $V_2=k_{12}/k_{21}\,V_1$.

fore, if brain can be assigned to compartment II, the time course of distribution to brain will be predictable from the important and easily obtainable plasma data.

In the present paper, eight barbituric acid derivatives in J. P. VIII are used, and plasma, brain and liver concentration time courses of drugs administered intravenously in rat are analyzed with compartment model, and the compartmental characters of the above two important organs are studied. And further, pharmacokinetic constants are compared with their lipid solubility to try to predict the pharmacokinetic behavior and to obtain the clue for the meaning of the pharmacokinetic constants.

Experimental

Drug Administration and Samplings——Male albino rats (Donryu), weighing 250—260 g, were used. Femoral artery cannulation and bladder fistula were operated to study the elimination of the drug from plasma, and the drug excretion in urine, respectively. Operations were given under ether anesthesia. All the barbiturates were administered through femoral vein in dose of 60 mg/kg, except in thiopental of 12 mg/kg. Barbiturate solutions which contained 500 mg sodium salts in 10 ml H_2O were prepared shortly before the experiment started. When sodium salts were commercially available, the salts were used directly, or otherwise the equivalent acids were solved in equimolar NaOH solution.

About 0.3 ml blood were taken at given times, and the plasma was obtained with centrifugation. For tissue samples, animals were cut on the carotid and killed by bleeding at an appropriate time, the brain and the liver were removed as rapidly as possible, and homogenized immediately in pH 7.0 phosphate buffer.

Materials——Barbital (BaB), Allobarbital (AlB), and Phenobarbital-Na (PhB) were purchased from Sanko Seiyaku Kogyo Co., Ltd., and Thiopental-Na (ThP) and Pentobarbital-Na (PeB) from Tanabe Seiyaku Co., Ltd., and Cyclobarbital-Ca (CyB)[8] and Hexobarbital-Na (HeB) from Shionogi Co., Ltd., and Amobarbital-Na (AmB) from Nihon Shinyaku Co., Ltd. They were used without further purification. All other reagents were commercially available and of special grade.

Analytical Methods——1) Hexobarbital Determination: The determination of hexobarbital was carried out according to the method of Noordhoek[7] with slight modification as the following. a) Plasma: Each plasma sample of 0.1 ml was put into a 10 ml glass stoppered centrifuge tube, 0.5 ml pH 5.5 phosphate buffer and 5 ml petroleum ether containing 1.5% isoamyl alcohol were successively added and then shaken for 45 min. After centrifugation 4 ml of organic layer was pipetted off and shaken with 4 ml of pH 11.0 phosphate buffer for 15 min. The concentration of the buffer layer was determined spectrophotometrically at 245 mμ by Hitachi 124 spectrophotometer. b) Brain: The brain sample was weighed and homogenized in 7 ml of pH 7.0 phosphate buffer to which 200 g NaCl was added per liter, and shaken for 45 min with 3 ml 1/10 N HCl and 20 ml petroleum ether containing 1.5% isoamyl alcohol. After centrifugation 15 ml of the organic phase was shaken for 15 min with 5 ml of pH 11.0 phosphate buffer. The HeB concentration of buffer layer was determined similarly as above. c) Liver: The liver sample was homogenized with the equal volume of pH 7.0 phosphate buffer. Two grams of the homogenate was taken for the determination as described for the brain.

2) The other Seven Barbiturates Determination: The spectrophotometrical method reported by Goldbaum[9] and Bjerre[10] was used with the slight modification as the following. a) Plasma: To each the 0.1 ml of plasma sample, 1 ml 1/10 N HCl and 3 ml $CHCl_3$ were added and shaken for 30 min. After centrifugation 2 ml organic layer was shaken with 5 ml pH 12.0 borate buffer for 15 min. The concentration of buffer layer was determined spectorophotometrically. b) Brain and Liver: Brain and two grams liver homogenate which was described in the determination of HeB were shaken with 20 ml CH_2ClCH_2Cl and 3 ml 1/10 N HCl for 30 min. After centrifugation 15 ml organic layer was shaken with 5 ml of pH 12.0 borate buffer for 15 min. The concentration of buffer layer was determined spectrophotometrically. c) Urine: The same method with that for plasma was adopted except that 1 ml urine was used.

8) After extraction with ether from acidified solution, ether was evaporated, and the residue was recrystallized from EtOH to obtain cyclobarbital.
9) L.R. Goldbaum, *J. Pharmacol. Exptl. Therap.*, **94**, 68 (1948).
10) S. Bjerre and C.J. Porter, *Clin. Chem.*, **11**, 137 (1964).

Determination of Partition Coefficient——Fifteen ml of pH 7.4 phosphate buffered barbiturates solution, 200 μg/ml except 40 μg/ml for thiopental, was added to an equal volume of CCl_4 which was previously saturated with the same buffer. After shaking and equilibrated at 37°, the separated buffer layer was analyzed. The partition coefficient of the barbiturates was calculated from the decrease of concentration in the buffer layer.

Data Analysis——Non-linear least square method was applied for data analysis. For plasma data analysis with two compartment model, the program PHART for which no initial approximate parameter values are necessary was used. For simultaneous analysis of plasma and brain data, the program PHAR2 which integrates the rate equations numerically with Runge-Kutta-Gill method was used. PHART and PHAR2 were written in the authors' laboratory for HITAC 5020E in the computer center of the University of Tokyo.

Result and Discussion

Elimination from Plasma

Three rats or more were used in each barbiturate experiment. The average data of the barbiturates of plasma concentration were analysed with the compartment model as shown in Chart 1, assuming the weight at each point as the reciprocal value of the square of the standard error. Good fitness of data to calculated lines was obtained as shown in Fig. 1. Although Sharma[11] analysed ThP plasma data from 0 to 360 min with three exponents equation, the present data of ThP from 0 to 150 min were analysed enough with two compartment model. The calculated pharmacokinetic parameters were listed in Table I.

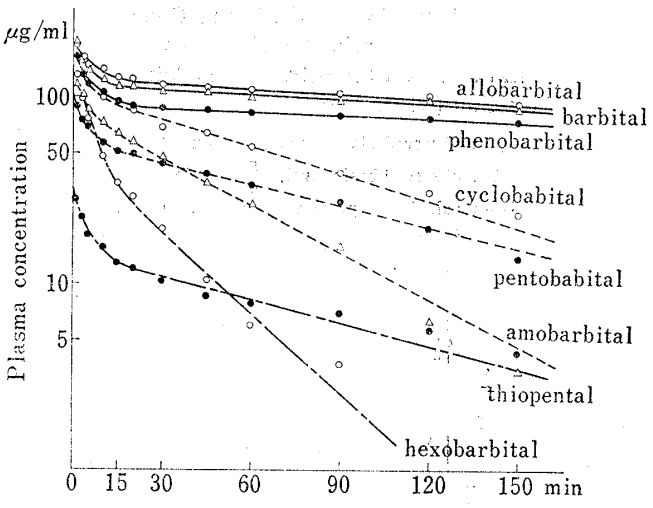

Fig. 1. Time Courses of Plasma Concentration of Barbiturates in Rat after Intravenous Administration (60 mg/kg; Except Thiopental 12 mg/kg)

lines: calculated with pharmacokinetic parameters in Table I

Distribution to Brain

The results were shown in Fig. 2—9. The observed brain level time courses were shown in solid circles. AmB (Fig. 2), PeB (Fig. 3), HeB (Fig. 4), and ThP (Fig. 5) which have high

Table I. The Calculated Pharmacokinetic Parameters of Barbiturates[a)]

Compounds	k_{12}[b)]	k_{21}[b)]	k_{el}[b)]	V_1[c)]	V_2[c)]
Barbital	0.0809±0.0151	0.1227±0.0139	0.00285±0.00029	79.73±3.30	52.56±11.64
Allobarbital	0.0464±0.0101	0.0892±0.0164	0.00298±0.00031	80.53±3.19	41.87±12.07
Phenobarbital	0.0742±0.0097	0.0868±0.0098	0.00204±0.00065	91.69±3.45	78.44±13.81
Cyclobarbital	0.0939±0.0186	0.1353±0.0247	0.01747±0.00066	84.06±2.00	58.30±15.76
Amobarbital	0.1067±0.0362	0.2479±0.0470	0.03182±0.00195	111.50±6.99	47.99±18.90
Pentobarbital	0.0838±0.0132	0.1466±0.0186	0.01471±0.00066	156.10±4.28	89.25±18.23
Hexobarbital	0.0842±0.0186	0.1102±0.0169	0.07784±0.00448	101.30±5.76	77.35±21.24
Thiopental	0.0489±0.0100	0.0563±0.0113	0.01308±0.00140	118.40±7.95	102.90±30.28

a) values represent pharmacokinetic parameters±standard error
b) pharmacokinetic rate constant: min^{-1}
c) distribution volume: ml

11) R.P. Sharma, C.M. Stowe, and A.L. Good, *J. Pharmacol. Exptl. Therap.*, 172, 128 (1970).

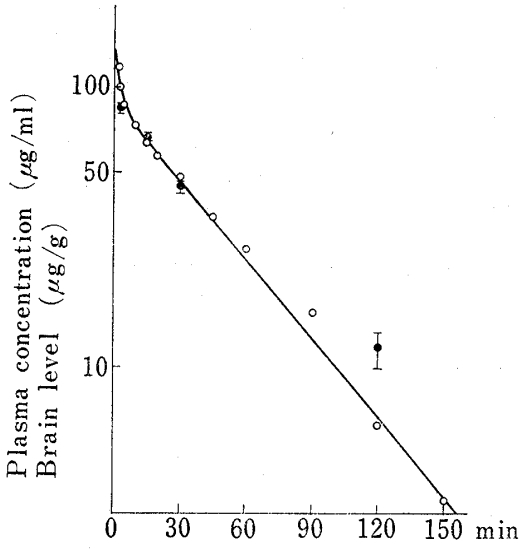

Fig. 2. Time Course of Plasma Concentration and Brain Level of Amobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course

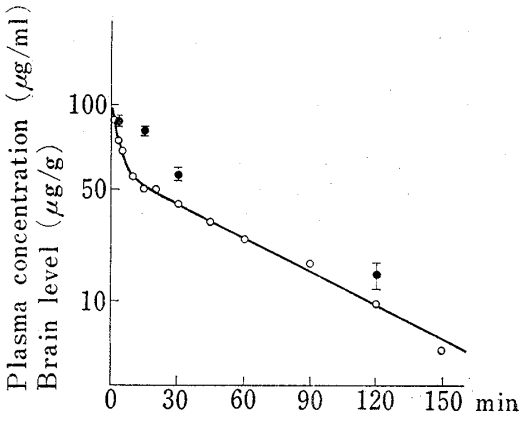

Fig. 3. Time Course of Plasma Concentration and Brain Level of Pentobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course

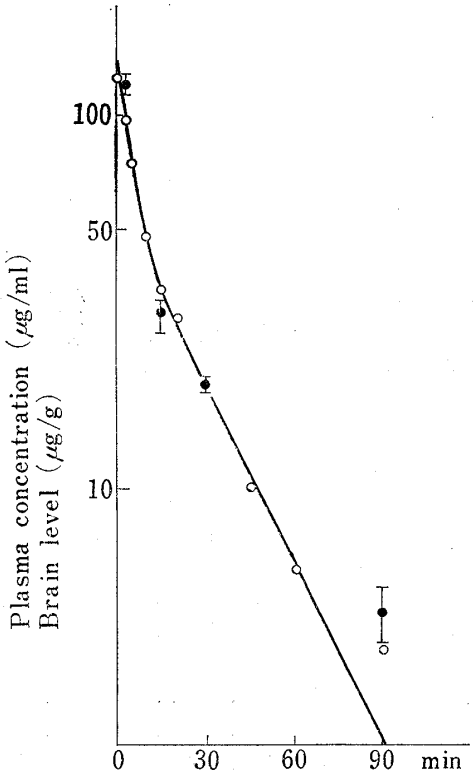

Fig. 4. Time Course of Plasma Concentration and Brain Level of Hexobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course

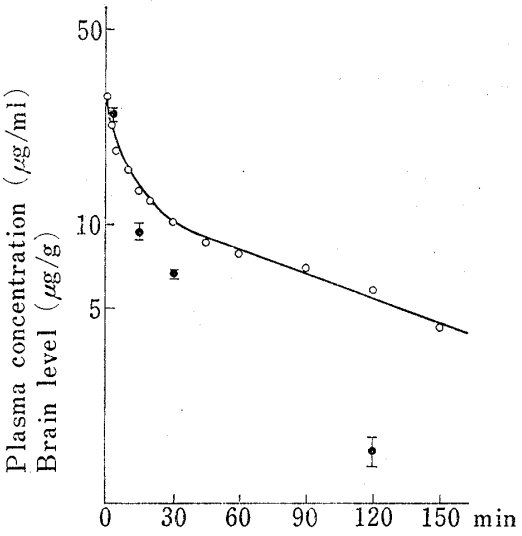

Fig. 5. Time Course of Plasma Concentration and Brain Level of Thiopental in Rat after Intravenous Administration (12 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course

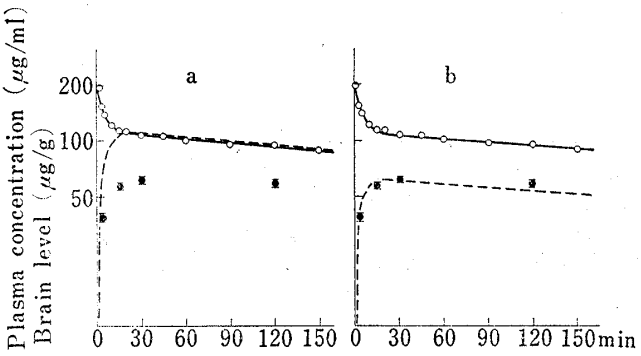

Fig. 6. Time Courses of Plasma Concentration and Brain Level of Barbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course
------: calculated concentration time course of compartment II

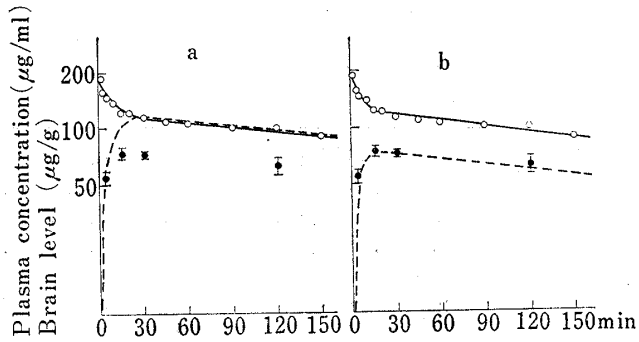

Fig. 7. Time Courses of Plasma Concentration and Brain Level of Allobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course
------: calculated concentration time course of compartment II

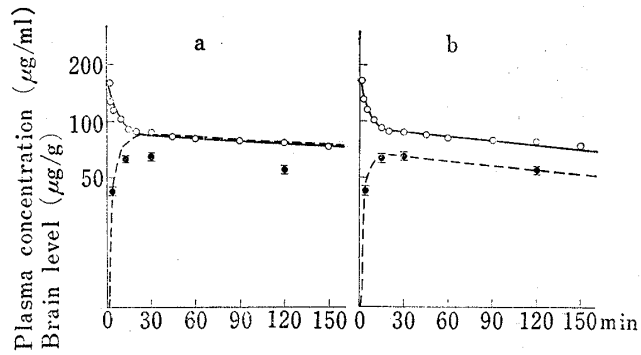

Fig. 8. Time Courses of Plasma Concentration and Brain Level of Phenobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course
------: calculated concentration time course of compartment II

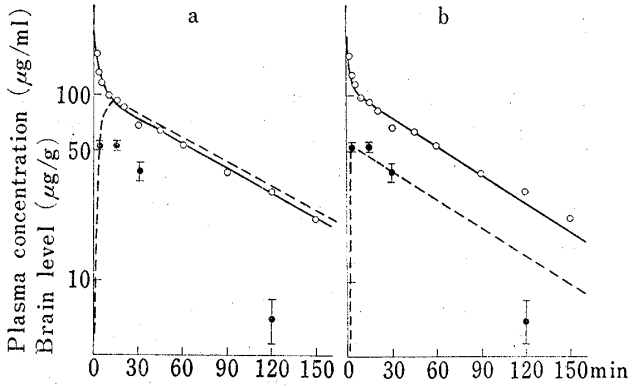

Fig. 9. Time Courses of Plasma Concentration and Brain Level of Cyclobarbital in Rat after Intravenous Administration (60 mg/kg)

○ : observed plasma concentration
● : observed brain level, ⊥: standard error
——: calculated plasma concentration time course
------: calculated concentration time course of compartment II

lipid solubility distributed so rapidly to brain that the brain level time courses were almost in parallel with the corresponding plasma concentration time courses. These were consistent with those of Brodie[5,6] for ThP and PeB, and of Noordhoek[7] for HeB. From these results, it can be said that brain can be assigned to compartment I for highly lipid soluble barbiturates. On the other hand, BaB (Fig. 6a), AlB (Fig. 7a), PhB (Fig. 8a) and CyB (Fig. 9a) appeared slowly in brain. To study this slow distribution process with compartment analysis, the concentration time course of compartment II which was calculated with parameters in Table I was represented with dotted line for each barbiturate (Fig. 6a—9a). It was found that the brain level time courses were almost in parallel with the time courses of compartment II. Although the time courses were parallel, the levels were different, and the following model was considered. In the prevailing two compartment model, to calculate the distribution volume of compartment II (V_2), the equilibrium between compartment I and II is assumed when the concentrations are equal in both compartments, and the equation $V_2=k_{12}/k_{21}\cdot V_1$ is given. But such an equilibrium condition is just for the convenience to calculate V_2 only from plasma concentration data. As the more general form, the partition coefficient (f) between

Table II. The Calculated Pharmacokinetic Parameters[a] of Barbiturates adjusted with Equilibrium Factor

Compounds	k_{12}[b]	k_{21}[b]	k_{el}[b]	f[c]	V_1[d]	V_2[d]
Barbital	0.1125±0.0259	0.1357±0.0154	0.00253±0.00050	0.565±0.017	73.42± 5.61	60.87±16.32
Allobarbital	0.0787±0.0235	0.1382±0.0290	0.00350±0.00033	0.611±0.006	75.76± 4.19	43.18±15.93
Phenobarbital	0.1134±0.0199	0.1264±0.0146	0.00343±0.00070	0.732±0.028	84.70± 5.06	75.99±16.60
Cyclobarbital	0.4698±0.3909	0.4196±0.1333	0.02535±0.00720	0.505±0.047	62.50±17.78	69.98±65.40

a) values represent pharmacokinetic parameters ± standard error
b) pharmacokinetic rate constant: min^{-1}
c) partition coefficient between compartment I and II
d) distribution volume: ml

the two compartments was assumed and the equations in Appendix was proposed in the present study. To examine if brain can be assigned to compartment II more definitely, brain and plasma data were analysed simultaneously with the proposed model (see Appendix for equations). The results were shown in Fig. 6b—9b. Good fitnesses were obtained for BaB, AlB and PhB, and the parameters were listed in Table II. The pharmacokinetic parameters in Table II are not significantly different from those in Table I. From these results it was ascertained that the brain is assigned to compartment II for BaB, AlB and PhB which have low lipid solubility. There was found ambiguity to assign brain to compartment II in the case of CyB where some discrepancy was found between brain level and calculated compartment II concentration (Fig. 9). The problem if the ambiguity can be overcome with more experimental runs or if metabolism in brain can not be neglected or if some other reason contributes, remained unsolved in the present study.

Distribution to Liver

PhB, AmB and HeB were chosen for the distribution study, referring to their lipid solubility. As shown in Fig. 10, the liver level time courses showed the very similar patterns of the corresponding plasma concentration time courses written in the former section of the present study. It was probable that the liver level reflected just the plasma level which remained liver. But this was ruled out with the following reason that an intact liver has about 20% blood,[12] and that a rat was cut on the carotid and then killed by bleeding in the present

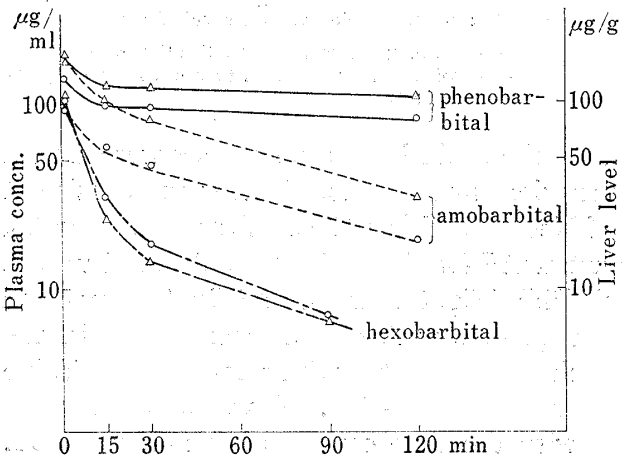

Fig. 10. The Plasma Concentration and Liver Level of Barbiturates in Rat after Intravenous Administration (60 mg/kg)

○: liver level △: plasma concn.

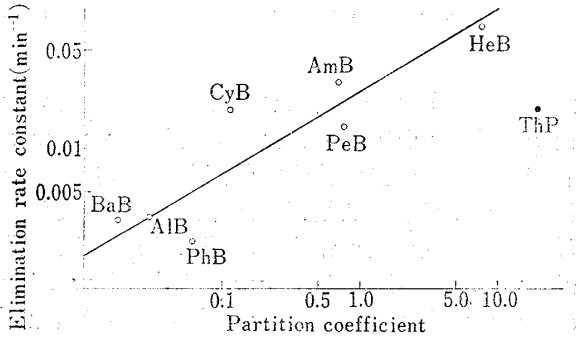

Fig. 11. The Relationship Between Elimination Rate Constant of Two Compartment Model and Partition Coefficient (CCl_4) of Barbiturates

$r=0.911$

12) A.E. Lewis, *J. Lab. Clin. Med.*, 39, 704 (1952).

study, and moreover, that the liver level was similar to or more than the plasma concentration. Accordingly, it was considered that liver could be assigned to compartment I of two compartment model system for barbiturates.

Properties of Rate Constants

Since biological membrane is usually considered as lipid barrier, rate constants which are assumed to be related to transport or affinity in the biological system were examined on the lipid solubility. Rate constants calculated from plasma data were plotted against the partition coefficients (CCl_4/pH 7.4 phosphate buffer) in logarithmic scales. As shown in Fig. 11, the elimination constants, k_{el} had a significant regression for partition coefficients (r=0.911), except ThP which belongs to the different series from the other seven barbiturates in this paper. These barbiturates except BaB are mostly excreted in the metabolised form. Therefore, the elimination process can be regarded to correspond to metabolic process. This is supported by the fact that the metabolism of PeB and HeB are stimulated and their sleeping time decrease after enzyme induction of PhB.[13] Microsome where drug is metabolized has lipid membrane and it is said that the more lipid soluble drug is, the more easily metabolized.[14] Accordingly, it may be reasonable that k_{el} had a significant regression for lipid solubility.

But the above discussion is inadequate for BaB which is excreted for the most part in the unmetabolized form. If the elimination process of BaB corresponds dierctly to the excretion process, low lipid solubility will favour the excretion, *i.e.*, elimination. To elucidate the elimination process of BaB, the excretion rate of BaB in urine was studied. The rate constant for excretion was calculated from the slope of sigma-minus plots, and was 0.00078 min^{-1} as the average three runs, about a quater of k_{el} of BaB. (Fig. 12, 13) Therefore the

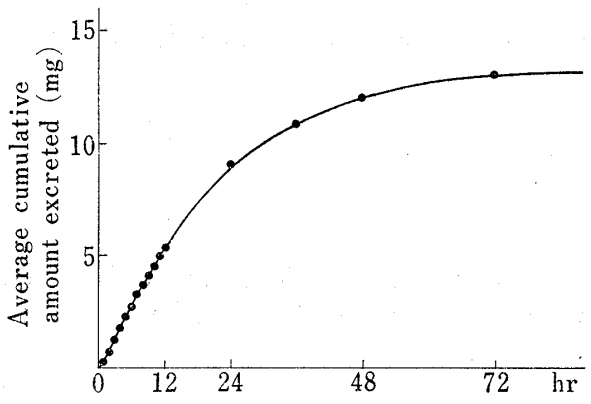

Fig. 12. Plot of Average Cumulative Amounts of Barbital excreted in the Urine after Intravenous Administration (60 mg/kg)

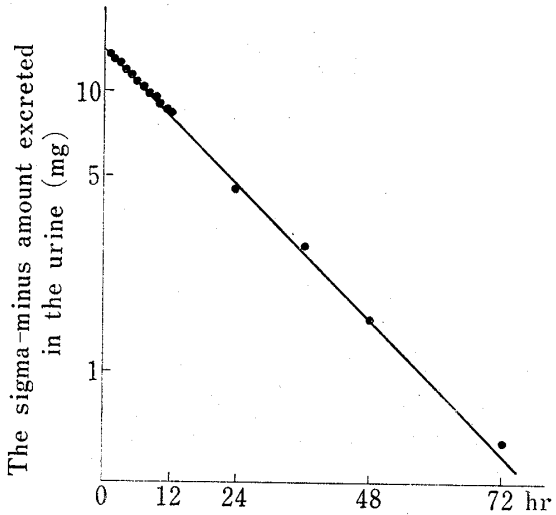

Fig. 13. The Sigma-minus Plot of Barbital excreted in the Urine after Intravenous Administration (60 mg/kg)

elimination process could not be regarded as the excretion process, and it was considered that the elimination was the elimination from plasma to a so-called deep compartment. If the distribution to the deep compartment is assumed to be favoured with hydrophobicity, it may be explainable that k_{el} of BaB had some regression for lipid solubility in Fig. 11. There was a still objectable point, however that even if k_{el} of BaB was influenced with lipid solubility there was no reason that k_{el} of BaB had the same regression with those of the other

13) A.H. Conney, I.A. Michaelson, and J.J. Burns, *J. Pharmacol. Exptl. Therap.*, **132**, 202 (1961).
14) R.E. McMahon, *J. Med. Pharm. Chem.*, **4**, 67 (1961).

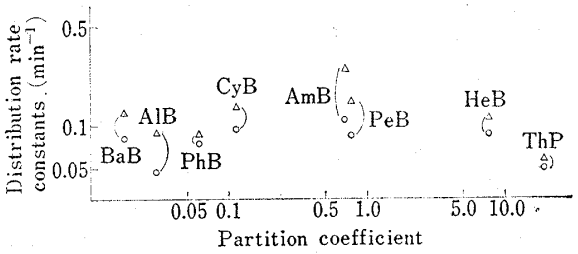

Fig. 14. The Relationship between Distribution Rate Constants of Two Compartment Model and Partition Coefficient (CCl_4) of Barbiturates

○: k_{12} △: k_{21}

six barbiturates, because the elimination process was different each other. This was not elucidated in the present study.

The distribution rate constants, k_{12} and k_{21} showed similar degree and had no relationship for lipid solubility as shown in Fig. 14.

If the process of k_{12} and k_{21} is the distribution to lipoidal tissue, lipid solubility will favour the distribution. But the results did not coincide with it. It suggested that the distribution corresponds to flow, diffusion or some other process which are not related to hydrophobicity.

Summarizing the present paper, a clue was given to anticipate the plasma concentration and brain level time courses of barbiturates from their lipid solubility, though the mechanism was elucidated not exactly.

Appendix

At the equilibrium Eq. (1) is given,

$$C_2 = \mathrm{f} C_1 \tag{1}$$

Where f is the partition coefficient between compartment I and II. Then, the distribution volume of compartment II (V_2) is expressed by Eq. (2),

$$V_2 = \frac{k_{12}}{\mathrm{f} k_{21}} V_1 \tag{2}$$

In this case the following differential equations are given for the two compartment model in Chart 1.

$$\frac{dC_1}{dt} = \frac{k_{12}}{\mathrm{f}} C_2 - (k_{12} + k_{e1}) C_1 \tag{3}$$

$$\frac{dC_2}{dt} = \mathrm{f} k_{21} C_1 - k_{21} C_2 \tag{4}$$

The definitions of the letters in the above equations are the same in Chart 1. The model in Chart 1 is the special case where f is fixed as 1 in the above equations as the more general form of two compartment model.