all regards with an authentic sample[13] and 15 mg of (+)-β-slinene (with slight β-muurolene contamination), $[\alpha]_D^{20}$ +25.0°, retention time 22 min at 150°, identical in all respects with an authentic specimen.[16]

Distillation was used as an alternate mode of separation of the "hydrocarbon fraction." A 9.30 g portion was distilled at 30—77°/0.2 mmHg and the pot residue, 1.20 g, chromatographed on 40 g of 100—200 mesh silica gel impregnated with 10% silver nitrate. Elution with petroleum ether led to 196 mg of a long-chain hydrocarbon mixture, 549 mg of ar-abietatriene, 169 mg of isopimaradiene, $[\alpha]_D^{20}$ −30.4° (c=1.7, $CHCl_3$), identical in all particulars with an authentic specimen[17] and a hydrocarbon of unknown constitution. Preparative thin-layer chromatography of the latter on 750 mμ layers of silica gel G impregnated with 10% silver nitrate and elution with chloroform afforded 22 mg of colorless prisms, mp 54—55°, mass m/e 256.2192+0.0005 (Calcd for $C_{16}H_{28}$: 256.2191), λ_{max} (hexane) 233 mμ (log ε 2.43), ν_{max} (KBr) 3115, 2940, 2859, 1641, 1440, 1372, 1180, 1139, 999, 909, 889, 860, 825 and 734 cm^{-1}, δ ($CDCl_3$) 0.70 (s, 3H), 0.89 (s, 3H), 1.95 (broad s, 5H), 4.65 (d, J=13Hz, 2H), 5.35 (broad m, 1H), 12-line signal 4.7—6.1 (3H, vinyl group), ord (c=0.08, hexane), plain positive curve, $[\alpha]_{700}$ 0°, $[\alpha]_{600}$ +12°, $[\alpha]_{589}$ +15°, $[\alpha]_{500}$ +75°, $[\alpha]_{450}$ +100°, $[\alpha]_{400}$ +150°, $[\alpha]_{350}$ +220°, $[\alpha]_{300}$ +300°, $[\alpha]_{250}$ +720°.

Acknowledgement M.K., A.Y. and Y.K. thank Dr. Akira Tahara (The Institute of Physical and Chemical Research) for a gift of dehydroabietic acid and the Ogawa Perfumery Company for a supply of the essential leaf oil of Hiba. J.P.C., J.D.M., D.J.W. and E.W. are indebted to the U.S. National Science Foundation for partial support of their work, to the New Zealand D.S.I.R. for a supply of *P. ferrugineus* bark, to Drs J.K. Crandall, S. Dev, V. Herout, L.K. Montgomery, G. Ourisson and L. Westfelt for gifts of natural hydrocarbons and to Drs. Z. Barneis and R. Bates for copies of spectra of some natural hydrocarbons.

[Chem. Pharm. Bull. 18(2) 405—411 (1970)]

UDC 615.213.015

Kinetics of Diphenylhydantoin Disposition in Man

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(Received October 30, 1969)

Diphenylhydantoin (DPH) is one of the most useful drugs for the treatment of convulsive disorders. It has been, however, proved that there was only a narrow range between the optimum therapeutic and minimally toxic plasma level of DPH.[2] It was demonstrated that no DPH or only small amounts, approximately 1%, of DPH in unmetabolized form could be recovered in the urine of human subjects[3] or rats.[4] Most ingested DPH is hydroxylated in the liver and the product, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH) is finally eliminated through the kidney after being conjugated mainly with glucuronic acid. In order to use DPH effectively, it is necessary to know the rate of its metabolism and excretion and the variable factors which influence the rate.

The rate of drop of plasma DPH levels in human subjects received a single 400 mg oral dose of diphenylhydantoin sodium (DPH-Na) was 50% in 18 to 24 hours.[4] Solomon and Schrogie[5] reported that healthy female volunteers were orally given 100 mg of DPH 3 times

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2) *a*) F. Buchthal and O. Svensmark, *Epilepsia*, **1**, 373 (1959/60); *b*) F. Buchthal, O. Svensmark, and P.J. Schiller, *Arch. Neurol.*, **2**, 624 (1960); *c*) H. Kutt, W. Winters, R. Kokenge, and F. McDowell, *Arch. Neurol.*, **11**, 642 (1964); *d*) E. Frantzen, J.M. Hansen, O. E. Hansen, and M. Kristensen, *Acta Neurol. Scandinav.*, **43**, 440 (1967).
3) T.C. Butler, *J. Pharmacol. Exptl. Therap.*, **119**, 1 (1957).
4) W.A. Dill, A. Kazenko, L.M. Wolf, and A.J. Glazko, *J. Pharmacol. Exptl. Therap.*, **118**, 270 (1956).
5) H.M. Solomon and J.J. Schrogie, *Clin. Pharmacol. Therap.*, **8**, 554 (1967).

daily for 5 days, and the biological half-life of DPH as calculated from the plasma DPH levels after 12 hours from the last dose was 22 to 34 hours. Svensmark, *et al.*[6] observed that plasma DPH concentrations decreased exponentially at a rate of 10 to 15% per hour (biological half-life, 4.3—6.0 hr) in six patients after intravenous administration of 300 to 700 mg of DPH-Na, and also the rate of fall of plasma DPH levels in three patients received DPH orally for two weeks was a exponential decrease of 35 to 55% in 24 hours (biological half-life, 15.8—27.8 hr) after cessation of the administration. The biological half-life after a single intravenous administration of 100 mg of DPH-Na was 7.3 to 12 hours in human subjects.[7]

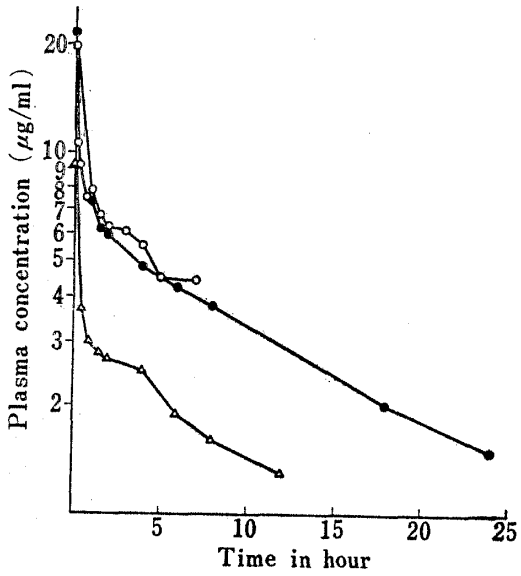

Fig. 1. DPH Plasma Levels after Intravenous Administration of DPH-Na

—○—○— : subject Y (250 mg)
—●—●—
—△—△— : subject S (125 mg)

It may be considered from the literatures cited above that the rates of fall after intravenous administration had a tendency to be definitly faster than the rates of fall from almost similar plasma DPH levels after oral administration or withdrawal of the drug from patients who had been on long-term oral doses. Bigger, *et al.*[8] reported that the decrease in plasma DPH concentration after a single 300 mg intravenous injection of DPH-Na was not shown to be exponential, and there was a rapid decline to level of 4 to 8 μg/ml at 20 to 40 minutes after injection.

In the present investigation, the elimination of DPH and excretion of total HPPH at several dose levels in man have been studied.

Two male subjects were received intravenously a single dose of 125 or 250 mg of DPH-Na at the rate of 50 mg per minute. Fig. 1 shows plasma levels of DPH as a function of time after the end of intravenous administration. There was a rapid decline to plasma levels of 4 to 8 μg/ml in 30 minutes after injection. The decrease in plasma DPH concentration of Subject Y shown in Fig. 1 may be described approximately by a biexponential equation of the following form, if minor unsmoothness of the curve was ignored.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad (1)$$

The result of the calculating by a graphical analysis technique, backward projection method,[9] is summarized in Table I together with the half-lives previously reported using 4-[^{14}C]-labeled DPH-Na by Hansen, *et al.*[7]

Wallis, *et al.*[10] mentioned that intravenous dose more than 1000 mg of DPH-Na was at least necessary for achieving plasma DPH level of 10 μg/ml or greater, and this would probably be explained by the rapid distribution of the drug into tissues. Bigger, *et al.*[8] reported that the initial fall of plasma DPH after a single 300 mg intravenous dose was more rapid than the exponential decrease observed with larger single intravenous doses (300—700 mg) by Svensmark, *et al.*[6] and followed much slower elimination process, and that the overall time-course of plasma DPH concentration was not exponential. This apparent contradiction of these informations

6) O. Svensmark, P.J. Schiller, and F. Buchthal, *Acta Pharmacol. Toxicol.*, **16**, 331 (1960).
7) *a*) J.M. Hansen, M. Kristensen, L. Skovsted, and L.K. Christensen, *Lancet*, 265 (1966); *b*) J.M. Hansen, M. Kristensen, and L. Skovsted, *Epilepsia*, **9**, 17 (1968).
8) J.T. Bigger, Jr. D.H. Schmidt, and H. Kutt, *Circulation*, **38**, 363 (1968).
9) D.S. Riggs, "The Mathematical Approach the Physiological Problems," Baltimore, The Williams & Wilkins Company,1963, pp. 146—161.
10) W. Wallis, H. Kutt and F. McDowell, *Neurology*, **18**, 513 (1968).

Table I. Constants of the Elimination of DPH from the Plasma after Intravenous Injection

Dose (mg, DPH–Na)	250		125	Hansen, *et al.* 100	
Subject [Age, Weight (kg)]	Y[37,57]		S[35,63]	3 subjects[7a]	4 subjects[7b]
A (μg/ml)	12.2	—	—		
B (μg/ml)	7.5	6.1	3.1		
α (hr^{-1})	6.0	—	—		
$0.693/\alpha$	0.1	—	—		
β (hr^{-1})	0.076	0.062	0.073		
$0.693/\beta$	9.1	11.2	9.5	9.0	8
				9.8	8
				7.3	15
					12

will be reconcile, if a central and at least one peripheral compartment[11] are assumed to be necessary to describe adequately the disposition of DPH in the body, and if the compartments are assumed to be connected mathematically with first–order rate constants of transfer and metabolism of DPH. The faster rate constant of the biexponential expression was expressed about 80 times as much as the slower rate constant (Table I). The initial rapid decreases during first 30 minutes are considered to be due not only to metabolism of DPH, but also to its uptake into tissues having a higher affinity than plasma. The half–lives reported by Hansen, *et al.*[7] (Table I) were determined during a period beginning at 2 hours after the end of injection of DPH–Na, and demonstrated to be 7.3 to 15 hours ($0.693/\beta$). The half–lives ($0.693/\beta$) determined in Subject Y and S were in satisfactory agreement with the result by Hansen, *et al.*[7]

It was shown that DPH and its metabolites after intravenous administration of radioactive DPH to rats appeared in the gastro–intestinal tract and were involved in an excretion–absorption cycle *via* the bile duct or other routes. Further, DPH and its metabolites excreted into the intestinal tract were reabsorbed almost completely, since hardly any DPH or radioactivity was found in the feces and 95% of the administered radioactivity could be recovered in the urine.[12,13] If an excretion–absorption circulation of a drug administered intravenously is prominent, it may cause secondary peak, shoulder, or up–and–down variation in plasma concentration, and the true rate constant for elimination can not be estimated from drug level decline in plasma.[14]

Noach, *et al.*[12] reported that second peaks of DPH concentration in all organs were shown 90 minutes after intravenous administration of DPH–Na to rats and might resulted from the excretion–absorption cycle. However, the maximum gastro–intestinal content of DPH was considerably lower than HPPH which reached more than 50% of administered radioactivity, and the amount of DPH recovered was only a small percentage of the dose.[12,13] The time–courses of DPH plasma level in Fig. 1 were expressed by almost smooth curves, and it might be assumed that the excretion-absorption circulation of DPH would not interfere with measurement of disappearence rate in order to estimate plasma levels of DPH.

The open circles in Fig. 2 show the plasma DPH concentrations as a function of cumulative dosage taken from the result of the study of Bigger, Schmidt and Kutt.[15] Twelve patients received DPH–Na by repeating 100 mg of intravenous doses every five minutes in the treatment of cardiac arrhythmia in order to avoid undesirable effects of the drug. The plasma

11) S. Riegelman, J.C.K. Loo, and M. Rowland, *J. Pharm. Sci.*, **57**, 117 (1968).
12) E.L. Noach, D.M. Woodbury, and L.S. Goodman, *J. Pharmacol. Exptl. Therap.*, **122**, 301 (1958).
13) E.L. Noach and H.V. Rees, *Arch. Int. Pharmacodyn.*, **150**, 52 (1964).
14) J.G. Wagner, *Ann. Rev. Pharmacol.*, **8**, 67 (1968).
15) T.T. Bigger, D.H. Schmidt, and H. Kutt, *Am. Heart J.*, **77**, 572 (1969)

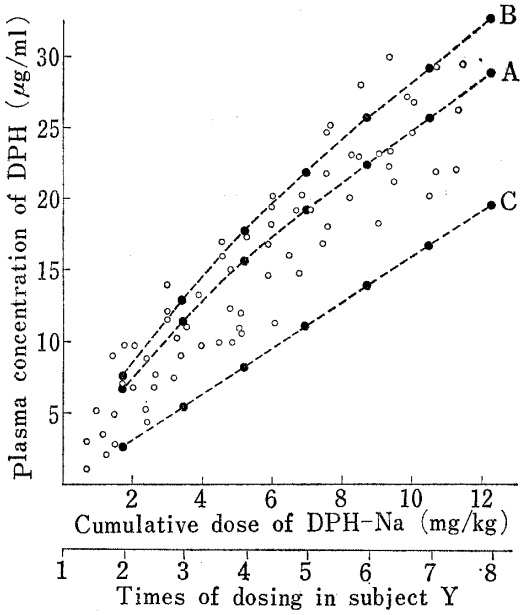

Fig. 2. Plasma DPH Levels after Repeated DPH-Na Injection

Open circles show the plasma DPH concentrations just prior to each dose after repeating 100 mg of intravenous doses (DPH-Na) every 5 min as a function of cumulative dosage taken from the result of the study of Bigger, *et al.*[15] Solid circles on dotted Curve A, B and C show the predicted plasma DPH concentration in Subject Y after repeated injection (100 mg) of DPH-Na at a constant rate of 50 mg/min. Solid circles on Curve A and B were calculated under the condition of repeating the following injections every 5 min after the end and the start of the preceding injection, respectively. The both calculations were based on schematic diagram in Fig. 3. Solid circles on Curve C were based on the one-compartmental open model with the elimination rate constant (0.076 hr^{-1}) and the volume of distribution (33.3 liters).

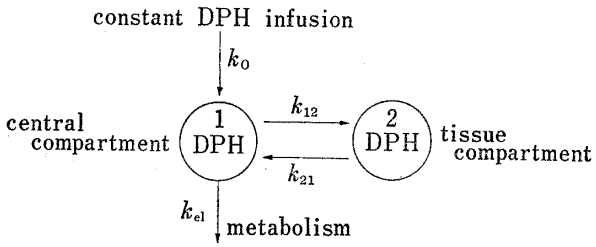

Fig. 3. Schematic Diagram Showing the Disposition of DPH after Constant-Rate Intravenous Infusion in Subject Y

k_0 (3×10^6 μg/hr) is constant intravenous rate, k_{12} (3.55 hr^{-1}) is the transfer rate constant from the central compartment to the tissue compartment, k_{21} (2.33 hr^{-1}) is the transfer rate constant from the tissue compartment to the central compartment, k_{el}(0.196 hr^{-1}) is the elimination rate constant, and the volume of distribution of the central compartment is 10.3 liters. DPH is assumed to eliminate exclusively from the central compartment. All the rate constants are assumed to be first- order rate constants.

samples for determination of DPH concentration were obtained just prior to each doses.[16] The solid circles on dotted Curve A and B in Fig. 2 indicate the calculated concentrations *versus* the cumulative dosage on administration of the same intermittent intravenous doses, using the weight and volume of distribution (V_d) in Subject Y, constant infusion rate (50 mg/min) and pharmacokinetic parameters[11] of a two-compartmental open model (Fig. 3) derived from Eq. (1). It was assumed that the rate constants (α and β) and V_d in Subject Y were independent of DPH dose. The V_d of the central compartment in Subject Y was evaluated to be 10.3 liters, as divided the amount of DPH calculated from the equation[17] based on the constant-rate intravenous infusion in the two-compartmental open model[18] by the measured plasma level (19.7 μg/ml) immediately after the end of intravenous injection (Fig. 1). The circles on Curve A and B indicate the calculated plasma levels resulted from repeating 100 mg intravenous doses every 5 minutes after the end and the start of injection, respectively. The solid circles on dotted Curve C show the calculated plasma concentrations based on the assumption that the body was represented as a one-compartmental open model with the slower elimination rate constant (β).[19] All the predicted plasma DPH levels were calculated with the aid of an analog computer (Hitachi ALM-502T analog computer).[20] The predicted concentrations based on the biexponential expression obtained in Subject Y may be a better approximation of the

16) The intravenous infusion rate of DPH was not mentioned, and whether the patients received successive infusion at 5 min after the start or the end of the preceding infusion was not clear in this report.[15] Kutt recommended that infusion rate of 50 mg per min or less be used in the treatment of cardiac arrhythmia, because side effects of intravenous DPH depend upon the dose and the rapidity of administration.[10]

17) $X_c=k_0\left(\frac{k_{21}}{\alpha\beta}-\frac{C_1}{\alpha}e^{-\alpha t}-\frac{C_2}{\beta}e^{-\beta t}\right)$. Where, X_c is the amount of the drug in the central compartment, C_1 is $(k_{21}-\alpha)/(\beta-\alpha)$, and C_2 is $(k_{21}-\beta)/(\alpha-\beta)$. X_c is obtained from substituting 2 min for t.

18) M. Gibaldi, *J. Pharm. Sci.*, **58**, 327 (1969).

19) V_d was calculated to be 33.3 liters from dividing the extraporated value (7.5 μg/ml) of the terminal exponential portion at zero time in Fig. 1 by the dose (250 mg).

20) H. Ropke and J. Riemann, "Analogcomputer in Chemie und Biologie," Springer-Verlag Berlin, Heidelberg, 1969, pp. 125—126.

plasma DPH levels shown in Fig. 2 than based on the slower rate constant alone obtained or based on half-life of 24 hr and relative volume distribution of 60% reported by Svensmark, *et al.*[2b] (almost similar as Curve C), although the only rate constants in Subject Y were too limited to assure that the fitting would always hold.

Intersubject variations in metabolic rate constant of DPH may exist in some degree.[21] The investigation of Kutt, *et al.*[23] indicated that smaller amounts of the metabolites appeared in the urine of patients who had been having DPH in a conventional dose and had have signs of DPH toxicity. It was suggested that defficient enzymatic factor in these individuals resulted in accumulation of unmetabolized DPH.

The substance excreted in the urine after administration of DPH is mainly a conjugate of HPPH with glucuronic acid. The urinary output of HPPH may account for 50—70% of the ingested DPH[23] and the amount of free HPPH excreted as such in urine is small or undetectable. The amounts of HPPH excreted in the urine after various intravenous or oral doses of DPH (50—250 mg) are given in Table II and Fig. 4. The excretion is expressed in terms

Table II. Cumulative Urinary Recoveries of DPH and Total HPPH to Dose after Single Oral or Intravenous Administration

Administration route	Subject	DPH dose (mg)	Urinary recovery of DPH (%)	Cumulative urinary recovery of total HPPH (%) hr after administration 0—24	0—48	0—72	0—96	0—120	0—144
i.v.[a]	Y	250	0.7	54.0 (65.5)[b]	77.8 (94.4)	82.4 (100)	82.4	—	—
	Y	250	0.4	58.0 (62.4)	85.5 (91.9)	90.8 (97.6)	93.0 (100)	93.0	—
	S	125	0.3	53.9 (57.8)	81.8 (87.7)	88.3 (94.6)	93.3 (100)	93.3	—
	Y	50	traces	61.3 (67.1)	79.9 (87.5)	91.3 (100)	91.3	—	—
p.o.	Y	250	0.1	26.8 (35.8)	60.9 (81.4)	70.5 (94.3)	73.9 (98.8)	74.8 (100)	74.8
	Y	250	0.1	28.2 (46.8)	49.3 (81.8)	58.6 (97.2)	60.3 (100)	60.3	—
	Y	200	0.2	34.0 (48.4)	57.9 (82.4)	67.6 (96.2)	70.3 (100)	70.3	—
	Y	150	0.1	49.8 (66.8)	67.1 (90.1)	73.8 (99.1)	74.5 (100)	74.5	—
	Y	100	—	50.5 (59.3)	74.9 (88.0)	85.1 (100)	85.1	—	—

a) dose as DPH–Na *b*) % excretion of ultimate HPPH recovery

of the percentage of the administered dose appearing in the urine as total HPPH after acid hydrolysis. The total amount of HPPH excreted in the first 24 hours after the single intravenous administration in doses of 50—250 mg and oral administration in doses of 100 and 150 mg was 59.3—67.1% of HPPH excreted ultimately (49.8—61.3% of the dose) in Subject Y. However, it was 35.8—48.4% (26.8—34.0% of the dose) after the single oral administration

21) After completion of this study, it was found that Glazko, *et al.*[22] have carried out the study of the metabolic disposition of DPH in six normal human subjects following intravenous administration, and the half-lives obtained in 8 to 72 hours were 10.5, 20.3, 12.2, 10.5, 28.7, 12.7 and 12.4 hr and the average half-life was 15.4 hr. They indicated that two of the subjects showed half-lives of 20 hr or more even after intravenous administration, although the reason for this was not clarified.

22) A.J. Glazko, T. Chang, J. Baukema, W.A. Dill, J.R. Goulet and R.A. Buchanam, *Clin. Pharmacol. Therap.*, **10**, 468 (1969).

23) H. Kutt, M. Wolk, R. Scherman and F. McDowell, *Neurology*, **14**, 542 (1964).

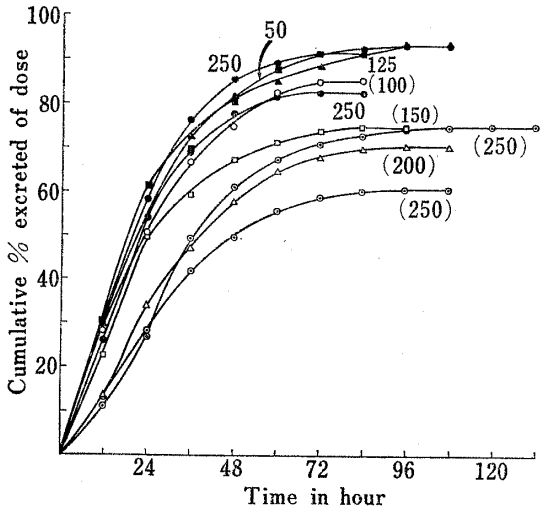

Fig. 4. Cumulative Urinary Excreted Total HPPH *versus* Time after Oral or Intravenous Administration

Open symbols show % excretion after oral administration of DPH, and solid symbols show % excretion after intravenous administration of DPH–Na in Subject Y. The intravenous 125 mg DPH dose was given Subject S. Each curve is labeled as to the number of mg of DPH dose (oral) or DPH–Na dose (intravenous), and oral doses were indicated in parentheses.

in doses over 150 mg. The total output of DPH was less than 1% in each dose. Panalaks[24] reported that HPPH recovered in the urine by a human subject after single oral doses of 100, 300 and 500 mg of DPH–Na were 85, 65, and 50%, respectively. The amount of HPPH excreted decreased with the increasing doses of DPH administered, which suggested dose–dependence for the metabolism of DPH.[25] Dayton, *et al.*[25] showed that a marked decrease (1/2—1/3) occurred in the case of higher dose (50 mg/kg) compared to lower dose (20 mg/kg) of intravenous DPH–Na in the study of effect of DPH dosage on rate of plasma level decay in dogs.

It was observed that the total amount of HPPH excreted did not always decreased as doses increased in our study with Subject Y given different doses ranging from 100 to 250 mg. It appears likely that the metabolism of DPH in conventional doses (100—250 mg) was not dose–dependent, as evidenced by the fact that the disappearance rate of intravenously administered DPH ranging from 100 to 700 mg followed approximately first–order kinetics after the initial distributive phase.[6,7]

Considerably long half–lives of DPH was observed after oral administration of DPH in doses more than 250 mg and estimated to be more than 24 hours.[4] The time required for half of plasma level in Subject Y following oral 250 mg DPH was about 24 hr over the 40 hr period after 10 hr from dosing.[26] DPH is poorly soluble in water except in strong alkaline solutions and the solubility of DPH in aqueous solution at 26° was 1.4 mg% over the pH range 1 to 7 and 16.5 mg% at pH 9.1.[4] Oral administration of DPH–Na which is more soluble than DPH resulted in higher plasma level with a faster rate of drop in plasma level.[4] DPH absorption depends very likely on the size of DPH particle. Maynert[27] explained that low urinary elimination after oral dose of isotopic DPH to dog might be attributed to poor absorption of the very insoluble property due to the larger size of the crystal of the isotopic preparation. It was shown that large oral doses of DPH was not completely absorbed by the rat,[4,27] and that the absorption occurred from all parts of the gastro–intestinal tract of rats.[4] Therefore, the considerable long half–lives after oral DPH administration are probably due to the continued absorption over a considerable longer of time.[28]

Experimental

Two healthy subjects were given a single intravenous dose of 250 mg or 125 mg DPH–Na (Aleviatin sodium for injection, Dainippon Pharmaceutical Co., Ltd. Japan) dissolved in 5 ml or 2.5 ml of a vehicle consisting of 40% propylene glycol and 10.5% ethanol in water, respectively. The drug was injected into

24) T. Panalaks, *Clin. Chim. Acta*, **8**, 968 (1963).
25) P.G. Dayton, S.A. Cucinell, M. Weiss and J.M. Perel, *J. Pharmacol. Exptl. Therap.*, **158**, 305 (1967).
26) K. Takagi, T. Suzuki, Y. Saitoh, S. Kusano and K. Nishihara, The 85th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
27) E.W. Maynert, *J. Pharmacol. Exptl. Therap.*, 130, 275 (1960).
28) J.G. Wagner, *J. Pharm. Sci.*, **52**, 1097 (1963).

the antecubital vein at the rate of 50 mg DPH–Na per min. Ten milliliters of heparinized blood samples from the opposite arm were taken at 1, 10, 20, 40 min, 1, 1.5, 2, 3, 4, 5, 7 hr after dosing of 250 mg DPH–Na in Subject Y, and after about one month interval, at 1 min, 1, 1.5, 2, 4, 6, 8, 12 and 24 hr after dosing of 250 mg DPH–Na in Subject Y. The same samples were taken at 2, 30 min, 1, 1.5, 2, 4, 6, 8, 12, 18 and 24 hr after dosing of 125 mg DPH–Na in Subject S. Blood samples were taken before dosing as blank. The plasma were spectrophotometrically assayed for DPH by the method of Wallace[29] with a slight modification. On oral administration, 100, 150, 200 and 250 mg of DPH (J.P. VII) powder were given with about 100 ml water. The drug was given Subject Y at 2 hr after light breakfast and food was withheld for at least 2 hr after drug administration. Urine specimens were collected every 12 hr until no total HPPH was found, and the samples were stored in a refrigerator until assayed. Urine pH was determined immediately after voiding except in the case of urines collected at night which were stored in the refrigerator and pH determined the following morning. The pH range of urine samples was about 5.0—6.5. The urine was assayed for DPH and total HPPH by the method of Wallace mentioned above and by a slight modified TLC method by Olesen,[30] respectively.

Acknowledgement The authors are grateful to Dr. Y. Honda of the Department of Psychiatry, Tokyo University Hospital for his cooperation in the intravenous administration of DPH, and to Miss S. Kusano and Mr. S. Isozaki for their excellent technical assistance.

29) J.E. Wallace, *Anal. Chem.*, **40**, 978 (1968).
30) O.V. Olesen, *Acta Pharmacol. Toxicol.*, **26**, 222 (1968).