[Chem. Pharm. Bull. 27(1) 129—138 (1979)]

UDC 615.212.3.015.45.033.1.076.9

Relationship between Blood Levels and Analgesic Effects of Acetaminophen in Mice[1)]

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(Received July 24, 1978)

The relationship between time course of blood level of acetaminophen (NAPA) and time–analgesic effect course was examined in mice after intravenous and subcutaneous administration of NAPA in doses of 149, 200, 268, and 360 mg/kg. The results obtained were as follows. (1) When the effect and the corresponding blood level obtained from time course studies of 4 kinds of doses were examined together, their relationship seemed to be rather poor. Namely, like blood level due to different doses produces no like effect. (2) When only the data after the peak effect were examined for each dose, respectively, higher relationship was found both in intravenous and subcutaneous studies. It was also found that with increasing of doses, the rate at which effect decreased from the peak became faster and higher blood level came to be necessary for obtaining a certain level of effect. (3) Comparing the time course data at the same doses, the effects after subcutaneous administration were always lower than those after intravenous administration even when NAPA blood level after subcutaneous administration were higher, suggesting that effect of NAPA might not depend on NAPA level in the blood or in the site of action at the time when effect was measured but rather on the peak NAPA level there and the elapse of time. (4) In conclusion, the relationship observed under a variety of doses and administration routes of NAPA is so complicated that even the possibility that analgesic effect elicited by this drug may be inherently beyond the limits of kinetic explanation cannot be ruled out.

Keywords——acetaminophen; acetaminophen glucuronide; drug level and pharmacological activity; pain threshold; pharmacokinetic model; administration route

It has long been recognized that the pharmacological effect of drug approximated linear relationship with logarithm of its dose. Recently intense interest has been shown towards the relationship between time course, after drug administration, of drug content in the body and the time–intensity course of the pharmacological elicited by the drug. Further, it becomes possible to assess not only the relationship between drug level in the blood and the intensity of pharmacological effect, but also the relationship between pharmacological effect and relative drug level in other apparent compartments of multicompartment models and some of such interpretations have been proved hepful in obtaining a better understanding of the relationship of drug level in the body and the intensity of pharmacological effect. In many of the previous works, however, interpretations were made on the experiments in which only one kind of dosage level was used. It is the purpose of this study to consider the relationship between time course of drug content in the body and that of the pharmacological effect on the basis of the experiments in which several kinds of dosage levels are involved. The example to be used is analgesic effect of acetaminophen (NAPA) in mice, the intensity of this effect having been determined at frequent intervals after intravenous and subcutaneous administration of NAPA, concurrently with determination of NAPA levels in the blood.

1) This work was presented in part at the 91th Annual Meeting of Pharmaceutical Society of Japan, April 1971.

2) Location: *a) 1-14, Bunkyo-machi, Nagasaki, 852, Japan; b) 3910, Gofuku, Toyama, 930, Japan.*

Experimental

Animal Experiments——Male mice weighing 18 to 22 g were used. For 12 hr before the test, the mice were allowed free access to water but not food. NAPA was dissolved in distilled water to make 111, 149, 200, 268, and 360 mg/10 ml solutions and each 10 ml/kg was administered *via* the tail vein or subcutaneously, the doses corresponding to 111, 149, 200, 268, and 360 mg/kg, respectively. These doses were set as the differences of logarithms of successive doses became equal. NAPA glucuronide was dissolved in distilled water to make 433 mg/10 ml solution (equivalent to 200 mg/10 ml NAPA solution), 10 ml/kg of which was given subcutaneously, the dose being equivalent to 200 mg/kg NAPA.

Blood and Urine Collection——Blood specimens were taken with a syringe containing 3.8% sodium citrate solution from the heart of intact animal by putting an injection needle into the heart through the skin or from the exposed heart of animal by performing heart incision after keeping the animal in a vessel filled with ether for about 45 sec. The volume of blood to be taken from a mouse was 0.5—1.0 ml, which was all applied to the determination of NAPA. The blood samples at each time point were collected from 4—7 animals.

Twenty four hr urine after NAPA administration was collected using a device which was capable of separating urine from feces and applied to thin-layer chromatography.

Materials——NAPA was of pharmaceutical grade. NAPA glucuronide[3] and sulfate[4] were prepared by synthetic methods.

Determination of NAPA in Blood——NAPA in blood was determined by spectrophotometric method reported by Routh *et al.*[5] with some modifications. The procedure was as follows. Blood specimen (0.5—1.0 ml) was added to the mixture of 3 g of sodium chloride and 4.0 ml of purified water in a 50 ml glass-stoppered centrifuge tube. Next, this mixture was shaken with 20.0 ml of refined ether[6] for 20 min. After centrifuging, 15.0 ml of organic phase was shaken with 6.0 ml of 1/100 N NaOH for 15 min. Absorbance of the alkaline layer was measured at 255 nm against the blood blank run through the above procedure. For preparing the calibration curve, 1.0 ml of citrated blank blood was added with 1.0 ml of standard solutions of NAPA (2, 4, 6, 8, and 10 mg%), 3.0 ml of purified water and 3 g of sodium chloride and the mixture was treated as above. The absorbance of blood blank was about 0.02 and that of 10.0 mg% standard solution was about 0.65.

Thin-layer Chromatography of Urine——Urine specimen was spotted on Silica gel HF_{254} (E. Merck, Darmstadt, West Germany) plate. After developing with the solvent system, ethyl acetate–methanol–water–acetic acid (60: 30: 9: 1),[4] the spots were detected under ultraviolet light. The *Rf* values of authentic NAPA, NAPA glucuronide, and NAPA sulfate were around 0.78, 0.18, and 0.43, respectively.

Assaying Analgesic Activity——A KN-205B apparatus (Natsume Seisakusho, Tokyo, Japan) giving pressure pain to mouse tail devised by Takagi *et al.*[7] was used. Pain threshold for pressure was estimated by the manometer reading taken when mouse responded by biting the compressed part of the tail. Only mice responding at 50—70 mmHg before the administration of the drug were used for the experiments. Pain thresholds after the administration of NAPA were followed every 7.5 min for 60 min. Since it was found that at least 15 min interval was necessary in order to read the pressure free from the influence of preceding measurement, two groups of mice, each consisting of 10 animals, were used and the pressures were read at 7.5, 22.5, 37.5, and 52.5 min in one group and at 15.0, 30.0, 45.0, and 60.0 min in other group. Pain thresholds after subcutaneous administration of NAPA glucuronide were followed every 15 min for 60 min using a single group of 5 mice.

Results

NAPA Metabolism in Mice

Thin-layer chromatographic investigation on 24 hr urine of mouse receiving NAPA intravenously showed that NAPA was completely transformed to the glucuronide and the sulfate, the former being predominant. These metabolic patterns are similar to those of man[4] and rabbits.[8]

3) J. Shibasaki, E. Sadakane, R. Konishi, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2340 (1970).
4) A.J. Cummings, M.L. King, and B.K. Martin, *Brit. J. Pharmacol. Chemother.*, **29**, 150 (1967).
5) J.I. Routh, N.A. Shane, E.G. Arrendondo, and W.D. Paul, *Clin. Chem.* **14**, 882 (1968).
6) Commercially available ether was shaken with 10% sodium bisulfite solution repeatedly, till the solution became colorless, further with purified water twice, 10% NaOH solution once, and finally with purified water once and then distilled after drying over sodium sulfate. Without these treatment, the determination was disturbed by high blank value.
7) K. Takagi, T. Kameyama, and K. Yano, *Yakugaku Zasshi*, **78**, 553 (1958).
8) H.G. Bray, W.V. Thorpe, and K. White, *Biochem. J.*, **52**, 423 (1952).

Analgesic Effect after Subcutaneous Administration of NAPA Glucuronide

Analgesic effect of NAPA glucuronide, the major metabolite of NAPA, was followed every 15 min for 60 min after subcutaneous administration of the glucuronide. As can be seen in Table I, NAPA glucuronide shows no significant effect. This study, therefore, is based on a viewpoint that the analgesic effect after administration of NAPA is resulted only from NAPA.

Table I. Pain Threshold for Pressure after Subcutaneous Administration of NAPA Glucuronide[a]

	Pain threshold for pressure (mmHg)[b]				
	Control[c]	15	30	45	60 (min after administration)
Mean	59.4	56.6[d]	61.0[d]	60.0[d]	58.2[d]
S.E.	2.0	4.2	4.1	4.9	1.8

a) Dose: 433 mg/kg (equivalent to 200 mg/kg NAPA).
b) Values for 5 mice.
c) The pressures measured twice at 15 min interval before administration for each animal were averaged.
d) Not significantly different from the control ($p=0.05$).

Time Course of Blood Levels of NAPA after Intravenous and Subcutaneous Administration of NAPA

The blood levels of NAPA were followed every 15 min for 90 min after administration of NAPA in doses of 111, 149, 200, 268, and 360 mg/kg. The mean blood levels and standard errors obtained from 4—7 mice are listed in Table II and III and the mean blood levels are plotted against time on a logarithmic scale (Figs. 1 and 2). Through all the dosage levels, the maximum blood levels are revealed at 15 min after intravenous administration and 30 min after subcutaneous administration. In all the dosage levels of NAPA administered intravenously and subcutaneously, the blood levels of NAPA decline linearly and both slopes at the same dosage levels are nearly same indicating that the elimination of NAPA from the blood obeys first-order kinetics and the absorption step after subcutaneous administration is not rate-limiting. Comparing NAPA blood levels after intravenous administration with

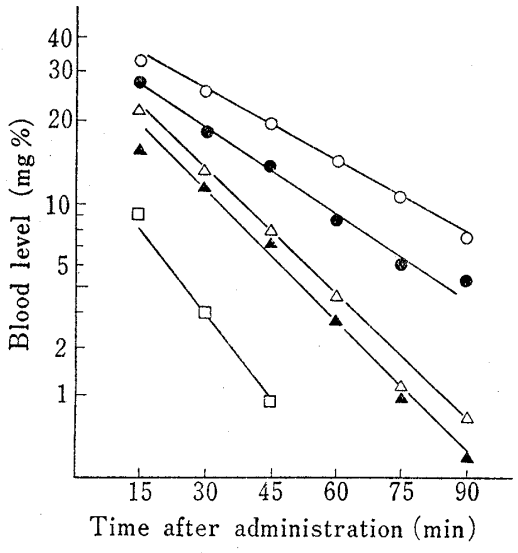

Fig. 1. Mean Blood Levels of NAPA after Intravenous Administration of NAPA

—□—, 111 mg/kg; —▲—, 149 mg/kg; —△—, 200 mg/kg; —●—, 268 mg/kg; —○—, 360 mg/kg.

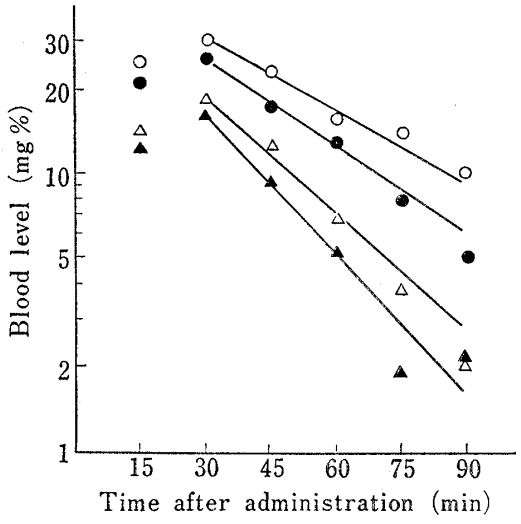

Fig. 2. Mean Blood Levels of NAPA after Subcutaneous Administration of NAPA

—▲—, 149 mg/kg; —△—, 200 mg/kg; —●—, 268 mg/kg; —○—, 360 mg/kg.

Table II. Blood Levels of NAPA after Intravenous Administration of NAPA

Time (min)	Blood levels (mg%, mean±S.E.)[a] after dose (mg/kg) of				
	360	268	200	149	111
15	33.2±5.4	27.3±3.1	21.6±4.8	15.8±1.5	9.4±0.8
30	25.5±3.7	18.2±2.2	13.1±3.8	11.5±1.9	4.0±0.6
45	19.1±2.8	13.4±3.5	8.1±2.1	7.3±1.6	1.9±0.5
60	14.2±2.4	8.8±0.8	4.7±1.4	3.8±0.4	1.1±0.1
75	10.8±3.0	6.1±1.1	2.2±1.2	2.0±0.5	
90	7.8±3.4	5.4±0.9	1.7±0.3	1.2±0.5	

a) Values for 4—7 mice.

Table III. Blood Levels of NAPA after Subcutaneous Administration of NAPA

Time (min)	Blood levels (mg%, mean±S.E.)[a] after dose (mg/kg) of			
	360	268	200	149
15	25.2±2.8	21.2±2.7	14.4±0.9	12.3±2.0
30	31.5±2.9	26.3±2.2	18.9±1.0	16.1±1.5
45	23.5±3.1	17.1±1.4	12.8±0.5	9.5±2.1
60	15.9±4.5	13.1±1.5	7.0±0.7	5.3±2.0
75	14.2±2.6	8.3±2.0	3.9±0.8	2.0±0.2
90	10.1±0.8	5.0±1.4	2.1±0.7	2.2±0.2

a) Values for 4—7 mice.

those after subcutaneous administration at each time point following administration of the same doses of NAPA, the latters are all higher than the formers later than 30 min after administration, which is an evidence for the excellent absorption of the drug after subcutaneous administration. Further, the declining slopes seem to decrease slightly with increasing doses suggesting that saturable process may be concerned in the elimination kinetic of NAPA from the blood.

Time Course of Effects of NAPA after Intravenous and Subcutaneous Administration of NAPA

Pain thresholds were followed every 7.5 min for 60 min after administration of NAPA in doses of 111, 149, 200, 268, and 360 mg/kg. The mean threshold values and standard errors at each time point from 10 animals are shown in Tables IV and V, in which 'maximum'[9] and 'area'[9] values are listed together. Here, it must be emphasized that almost all of the threshold values obtained from two groups of animals are aligned orderly according to the time, which provides a strong evidence for the validity of the assay. Upon the values listed in Tables IV and V, student's *t*-test was made against the control values. At the doses of 149, 200, 268, and 360 mg/kg, all of 'maximum' and 'area' values and most of the time point values except some at later time of lower doses are significantly higher than the control values. At the intravenous dose of 111 mg/kg, on the other hand, 'area' value and most of the time point values are not significantly different from the control value indicating that there is little effect below this dosage level, if any. Accordingly, the relationship between analgesic effect and blood level of NAPA was examined on four kinds of doses from 149 to 360 mg/kg,

9) See the legend in Table IV.

in this study. The elevation percentage of pain threshold after drug administration against the control value (E)[10] was used as a parameter for analgesic effect. E's obtained from the data in Tables IV and V are plotted against time (Figs. 3 and 4).

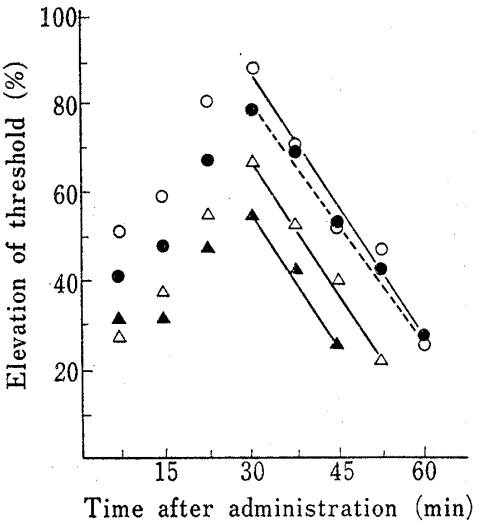

Fig. 3. Mean Elevation of Pain Threshold after Intravenous Administration of NAPA

—▲—, 149 mg/kg; —△—, 200 mg/kg;
---●---, 268 mg/kg; —○—, 360 mg/kg.

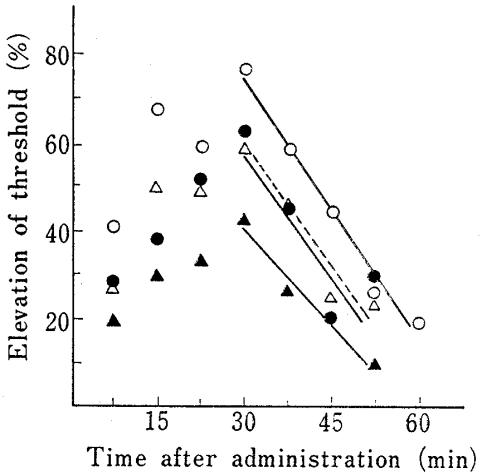

Fig. 4. Mean Elevation of Pain Threshold after Subcutaneous Administration of NAPA

—▲—, 149 mg/kg; —△—, 200 mg/kg;
---●---, 268 mg/kg; —○—, 360 mg/kg.

TABLE IV. Pain Threshold for Pressure after Intravenous Administration of NAPA

Time (min)	Pain threshold for pressure (mmHg, mean±S.E.)[a] after dose (mg/kg) of				
	360	268	200	149	111
Control[b]	58.7±1.5	56.6±1.0	59.3±1.4	57.9±1.4	60.4±3.7
15	93.3±7.2	84.0±5.2	82.0±4.7	76.5±1.4	67.0±2.8[f]
30	111.0±5.3	101.7±5.7	99.6±5.5	90.0±3.5	72.4±2.3[e]
45	90.2±4.4	87.2±4.9	83.9±4.6	73.1±3.8	59.4±6.0[f]
60	74.3±3.5[e]	72.9±3.9[e]	65.8±3.3[f]	58.3±3.6[f]	56.8±3.5[f]
Maximum[c]	114.7±6.3	109.3±3.6	105.4±4.7	93.2±2.9	75.4±2.4
Area[d]	90.2±3.7	84.4±1.9	82.0±1.7	74.4±1.7	64.8±1.8[f]
Control[b]	57.9±1.2	56.2±1.5	58.7±1.2	58.4±0.8	
7.5	87.9±3.4	79.2±4.7	75.1±3.7	77.1±5.6	
22.5	104.8±3.4	94.0±3.9	91.2±4.3	86.5±4.2	
37.5	99.3±3.4	95.4±3.6	90.6±2.8	83.8±2.5	
52.5	85.8±3.6	80.7±2.9	72.0±3.2[e]	65.0±2.7[f]	
Maximum[c]	108.1±3.1	101.1±2.2	99.2±2.0	95.1±2.8	
Area[d]	92.3±2.1	86.1±2.4	82.5±1.4	78.7±1.8	

a) Values for 10 mice. The values without alphabet in parentheses are significantly different from the control ($p<0.01$).
b) The pressures measured twice at 15 min interval before administration of the drug for each animal were averaged.
c) The maximum value in the time course of the measurement.
d) The area under threshold value *versus* time (0—60 or 52.5 min) curve, which is formed by joining the adjacent points by straight lines, divided by the terminal time of the measurement (the control value is used as zero time value).
e) Significantly different from the control ($p<0.05$).
f) Not significantly different from the control ($p=0.05$).

10) $E=100(P_0-P)/P_0$, where P_0 is the control pressure and P the pressure after drug administration.

Table V. Pain Threshold for Pressure after Subcutaneous Administration of NAPA

Time (min)	Pain threshold for pressure (mmHg, mean±S.E.)[a] after dose (mg/kg) of			
	360	268	200	149
Control[b]	56.4±1.1	60.7±2.2	56.2±1.2	58.8±1.7
15	94.4±8.6	83.8±4.5	84.4±4.2	76.2±3.0
30	100.2±7.7	98.8±6.9	89.2±8.9	84.0±5.5
45	81.6±7.1	73.0±5.7[e]	70.4±4.2[e]	65.5±4.3[f]
60	67.4±6.2[e]	69.8±5.3[f]	59.4±3.5[f]	60.0±5.9[f]
Maximum[c]	107.2±7.7	102.4±5.9	100.0±7.0	91.0±2.6
Area[d]	84.5±5.1	80.2±3.7	74.3±2.4	71.4±2.2
Control[b]	58.9±1.9	59.3±1.1	56.9±1.5	58.9±1.1
7.5	83.0±4.2	75.9±3.6	72.2±2.9	70.4±2.8
22.5	93.6±4.0	89.7±2.2	85.0±3.9	78.5±4.0
37.5	93.4±2.9	85.8±2.7	83.6±4.9	74.5±3.0
52.5	74.3±3.1	76.8±2.9	70.4±3.2[e]	64.9±2.4[e]
Maximum[c]	100.2±3.4	92.2±2.4	91.9±3.9	84.3±2.7
Area[d]	86.0±1.7	81.6±1.1	77.8±2.3	72.0±1.8

a—f) See the legend in Table IV.

Usually, the time course of pharmacological effect of drug has been described in term of first- or zero-order kinetics. As for NAPA, Nelson and Morioka[11] interpreted the data for man receiving NAPA orally, which had been reported by Flinn and Brodie,[12] to assign first-order kinetics to the decay of threshold elevation caused by the drug. Their interpretation, however, appears questionable because of the lack of sufficient time course data. As shown in Figs. 3 and 4, E first increases after the administration to attain the maximum at 30 min and then, decreases rapidly to return the control value through all intravenous and subcutaneous experiments. The decline of E is linear indicating that it obeys zero-order kinetics though it appears to be simulated also by first-order kinetics as well.[13] In addition it is observed from Figs. 3 and 4 that E's after intravenous administration are somewhat higher than those after subcutaneous administration at almost all time points when comparing at the same dosage level.

Discussion

Relationship between Doses of NAPA and Analgesic Effects

As demonstrated in Fig. 5, the intensities of pharmacological effects of NAPA, which are represented by Es calculated from 30 min values in Tables IV and V, are related linearly to the logarithms of the doses of NAPA over a range from 149 to 360 mg/kg indicating that the general relationship between dose and effect is also observed in this drug. It is, further, noticed that at the same dosage level, the effect after intravenous administration is highr than that after subcutaneous administration.

Relationship between Blood Levels of NAPA and the Corresponding Analgesic Effects after Administration of Various Doses of NAPA

In Figs. 6 and 7, E at each time point after administration of 149, 200, 268, and 360 mg/kg NAPA is plotted together against logarithm of the corresponding blood level of NAPA[14] for

11) E. Nelson and T. Morioka, *J. Pharm. Sci.*, **52**, 868 (1963).
12) F.B. Flinn and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **94**, 76 (1948).
13) An example of the fitting can be seen in Fig. 10.
14) The blood levels of NAPA were estimated every 15 min, while the pain thresholds were measured every 7.5 min as described above. The lacking blood levels were partly supplemented by reading from the linear parts of the plots in Figs. 1 and 2. Further details are shown in the legends of Figs. 6, 7, 8, and 9.

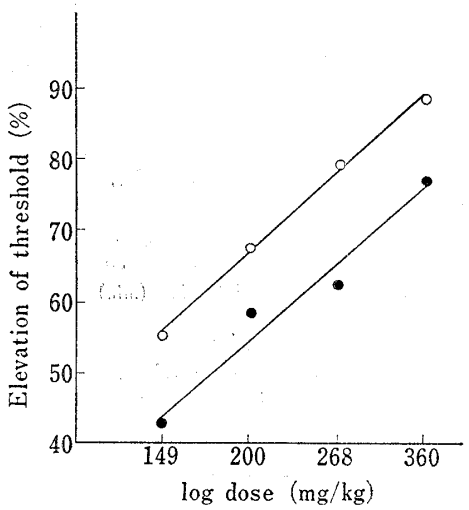

Fig. 5. log Dose-Response Relationship after Intravenous and Subcutaneous Administration of NAPA

Elevation of threshold (%) due to 30 min value in Tables IV and V is used.
—○—: intravenous
$y=86.8x-132.2$ ($r=1.00$).
—●—: subcutaneous
$y=82.8x-135.3$ ($r=0.98$).

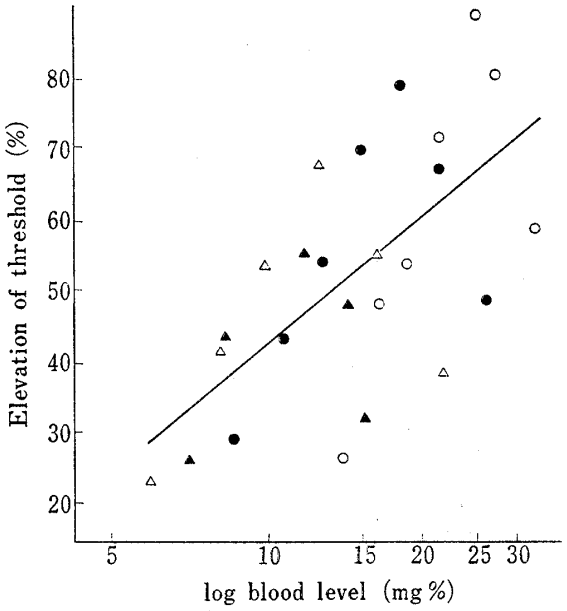

Fig. 6. Effect of NAPA as a Function of Blood Level of NAPA following 149, 200, 268, and 360 mg/kg Dose of NAPA administered intravenously

The blood levels at 22.5, 37.5, and 52.5 min after administration are read from the linear parts of the plots in Fig. 1. ▲, 149 mg/kg; △, 200 mg/kg; ●, 268 mg/kg; ○, 360 mg/kg.
$y=57.9x-15.5$ ($r=0.63$).

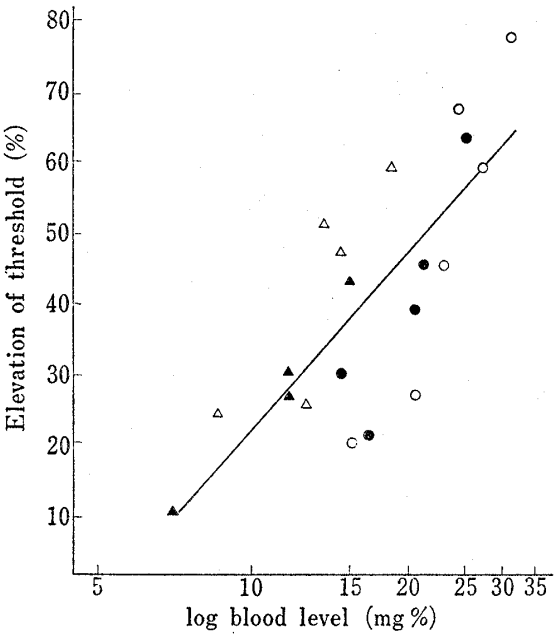

Fig. 7. Effect of NAPA as a Function of Blood Level of NAPA following 149, 200, 268, and 360 mg/kg Dose of NAPA administered subcutaneously

The blood levels at 37.5 and 52.5 min after administration are read from the linear parts of the plots in Fig. 2. ▲, 149 mg/kg; △, 200 mg/kg; ●, 268 mg/kg; ○, 360 mg/kg.
$y=84.8x-64.1$ ($r=0.77$).

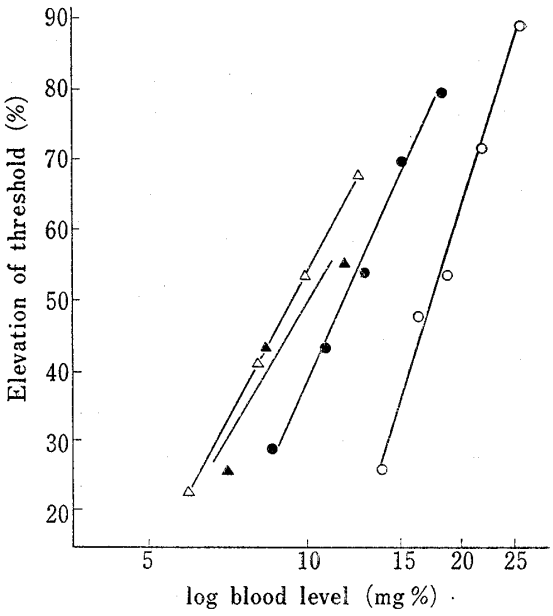

Fig. 8. Relationship between Effect of NAPA and Blood Level of NAPA after the Peak Effect for Each Dosage Level of NAPA administered intravenously in the Doses of 149, 200, 268, and 360 mg/kg

The blood levels at 37.5 and 52.5 min after administration are read from the linear parts of the plots in Fig. 1.
—▲—: 149 mg/kg $y=120.3x-73.0$ ($r=0.94$).
—△—: 200 mg/kg $y=137.8x-84.3$ ($r=1.00$).
—●—: 268 mg/kg $y=164.5x-127.0$ ($r=1.00$).
—○—: 360 mg/kg $y=232.3x-239.5$ ($r=0.99$).

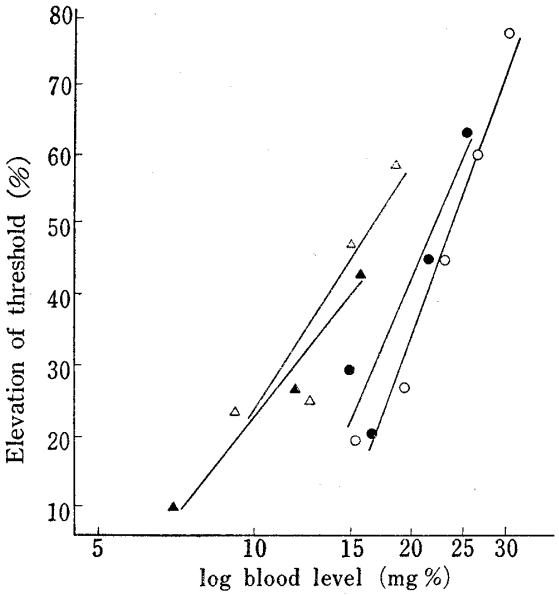

Fig. 9. Relationship between Effect of NAPA and Blood Level of NAPA after the Peak Effect for Each Dosage Level of NAPA administered subcutaneously in the Doses of 149, 200, 268, and 360 mg/kg

The blood levels at 37.5 and 52.5 min after administration are read from the linear parts of the plots in Fig. 2.
—▲—: 149 mg/kg $y=87.6x-65.4 (r=0.98)$.
—△—: 200 mg/kg $y=118.8x-96.5 (r=0.92)$.
—●—: 268 mg/kg $y=165.5x-175.0 (r=0.93)$.
—○—: 360 mg/kg $y=194.8x-219.6 (r=0.98)$.

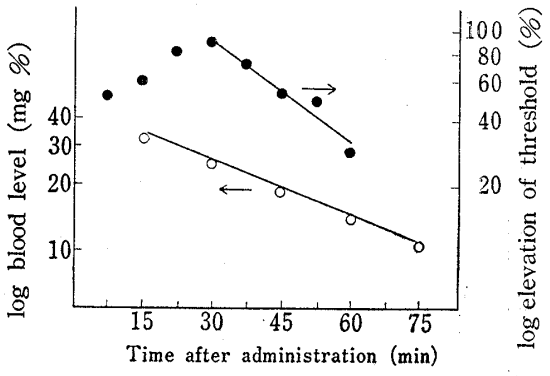

Fig. 10. Blood Level of NAPA and Effect of NAPA as a Function of Time after Intravenous Administration of 360 mg/kg NAPA

—○—, blood level; —●—, elevation of threshold.

the intravenous and subcutaneous experiments, respectively. The overall correlation observed there seems to be rather poor as understood also by the correlation coefficient for the regression line inserted in Figs. 6 and 7. This is not improved when E is plotted against the intact value of NAPA blood level instead of the logarithmic value. Like blood level of NAPA resulted from different dosage levels produces no like intensity of the effect even when the intravenous and subcutaneous data are examined individually. In other words, intensity of the effect at a certain blood level of NAPA scatters over a considerable range according to dosage level and route of administration.

Meanwhile, limiting the observation of Figs. 6 and 7 only to the data after the peak E in each dose, higher linear correlation is recognized as shown in Figs. 8 and 9, equations of the regression lines and correlation coefficients being inserted. With increasing of doses, the slope becomes steeper and the absolute value of the intercept becomes greater. These facts mean that the rate at which the effect decreases from its peak becomes faster and higher blood level comes to be necessary for obtaining a certain level of the effect as the dosage level becomes higher. In the highest dose, the minimum limit of the blood level that NAPA exhibits a significant effect is about 15 mg%, while in lower doses, the blood level less than 10 mg% yields a significant effect. Thus, these appear to indicate that the administration of higher dose of NAPA with the intention of obtaining higher intensity of effect is not always advantageous apart from a possibility of appearance of toxic effect due to the high dose.

The increasing process of drug effect after the drug administration was sometimes better understood by relating with the drug level in a peripheral compartment of multicompartment open model.[15] The effect of NAPA obtained in this study, however, seems to be not reconciled with the level in a peripheral compartment of multicompartment open model. This is understood by plotting both E and the corresponding blood level of NAPA against time on a semilogarithmic scale. Figure 10 illustrates such a plot for intravenous experiment

15) *a*) J.G. Wagner, G.K. Aghajanian, and O.K.L. Bing, *Clin. Pharmacol. Therap.*, **9**, 635 (1968); *b*) G. Levy, M. Gibaldi, W.J. Jusko, *J. Pharm. Sci.*, **58**, 422 (1969).

of 360 mg/kg dose, similar patterns being obtained also in other doses. In multicompartment open model, NAPA in the blood should be assigned to the central compartment and the effect of NAPA should be assumed to be related to the level of NAPA in a peripheral compartment. So far as based on these prerequisites, the declining slope of E must be equal to or less than that of NAPA level in the blood. Unfortunately, Fig. 10 demonstrates clearly that E decreases more rapidly than the blood level of NAPA. In addition, it is shown in Fig. 1 that the time course of the blood level of NAPA after intravenous administration of NAPA is better interpreted by a single compartment open model. Thus, the attempt to relate E with the level of NAPA in a peripheral compartment of multicompartment open model has been suspended.

Relationship between Route of Administration of NAPA and the Effect

On comparing intravenous and subcutaneous data at the same dosage level, it is noted that the effects after subcutaneous administration are lower than those after intravenous administration even during the period from 30 to 60 min after the administration when the blood levels of NAPA after subcutaneous administration are higher. From this fact, it may be assumed that the analgesic effect of NAPA at any time after the peak effect is not depend on the NAPA level in the blood or in the site of action at the time when the effect is measured but rather on the peak drug level there and the elapse of time. Such circumstances are demonstrated in Fig. 11, which is for 360 mg/kg dose, similar graphs being also obtained for the other doses. It is also shown in Fig. 11 that the effects following intravenous and subcutaneous administration show both upward curvatures from 7.5 to 30 min after the administration while the corresponding blood levels at the same period are quitely different; downward curvature after intravenous administration and upward curvature after subcutaneous administration, suggesting that different kinetics may be concerned before and after the peak effect independently. Putting together all the experimental observation described above, even the possibility that analgesic effect elicited by NAPA may be inherently beyond the limits of the kinetic interpretation cannot be ruled out.

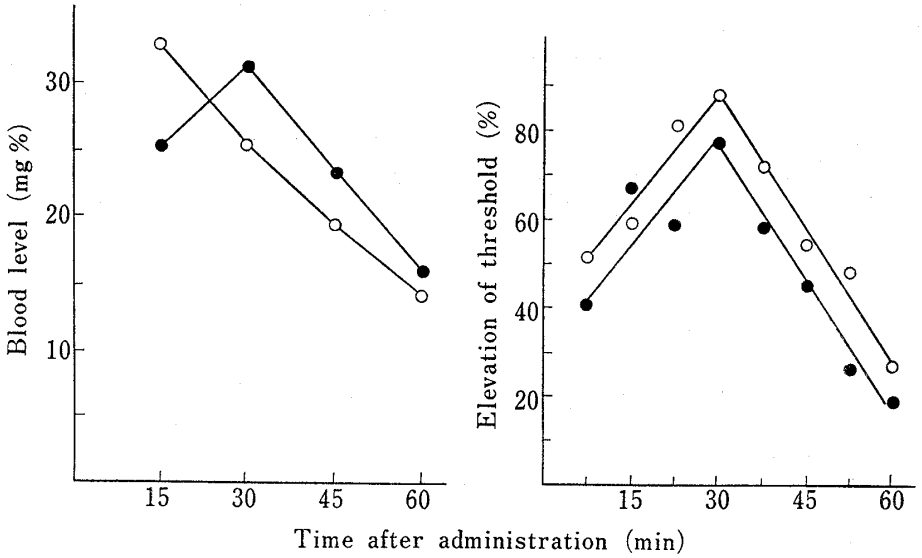

Fig. 11. Comparisons of Blood Level of NAPA and Analgesic Effect between Intravenous and Subcutaneous Experiments with Dose of 360 mg/kg NAPA

○, intravenous data; ●, subcutaneous data.

Classification of Types of Drugs from Viewpoint of Relating the Pharmacological Effects with the Levels in the Body

Recently, the ability of theophylline to increase the pain sensitivity in the rat combined with the determination of drug levels in the plasma after intravenous administration of the

drug was investigated by Paalzow.[16] The pharmacological activities were followed at 10—15 min interval during 3 hr after administration of the drug in doses of 6.25, 12.5, and 25.0 mg/kg. In the dose of 12.5 mg/kg, plasma levels of theophylline were also followed at appropriate time interval to be fitted by the levels in the central compartment of two compartment open model and high linear relationship was found between the pharmacological effect and logarithm of the amount of theophylline in the central compartment. Assuming now that the same kinetics at 12.5 mg/kg dose of theophylline may be applicable to the other doses, the theoretical plasma levels of 6.25 and 25.0 mg/kg doses at the time when the experimental data of pharmacological activities were available were calculated by the equation presented by Paalzow.[17] In Fig. 12, logarithms of standardized[18] theophylline levels in the plasma for three kinds of doses are plotted together against the corresponding effects, where highly linear relationship is observed being fairly different from the case of NAPA which is demonstrated in Figs. 6 and 7. It is, therefore, likely that in the case of theophylline the relationship between the plasma level and the pharmacological effect is consistent irrespective of dosage level. Judging from both the results for NAPA and theophylline, it may be deducible that drugs can be classified into two types from viewpoint of relating drug effect with its level in the body; one is a drug such as theophylline, the effect of which is directly reflected by the drug content in the body and the other is a drug such as NAPA, the effect of which is not predictable only from the drug content in the body.

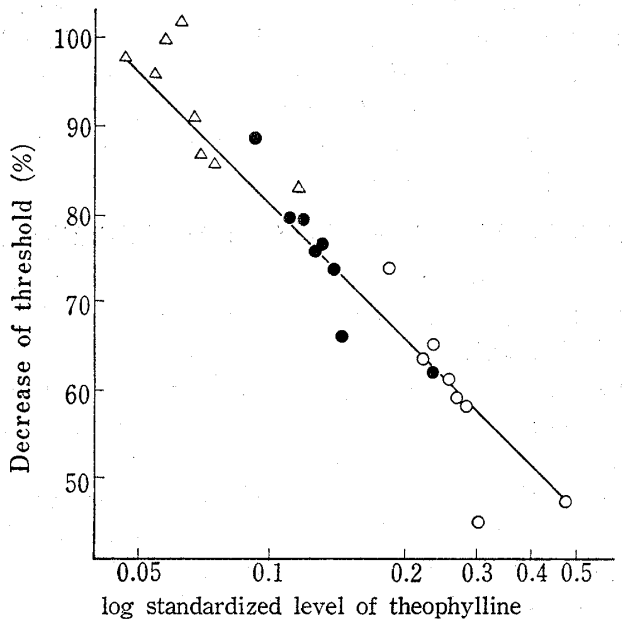

Fig. 12. Effect of Theophylline on the Threshold for Vocalization as a Function of Standardized Level of Theophylline in Plasma after Intravenous Administration of 6.25, 12.5, and 25.0 mg/kg to the Rat

The method for calculating the standardized level of theophylline is shown in the text. Data generated by Paalzow.[16]
△, 6.25 mg/kg; ●, 12.5 mg/kg; ○, 25.0 mg/kg.
$y=26.6-56.8x$ ($r=0.96$).

16) L.K. Paalzow, *J. Pharmacokin. Biopharm.*, 3, 25 (1975).
17) Equation presented by Paalzow for the plasma level of theophylline (C_p, μg/ml) at any time (hr) is: $C_p=36.6\ e^{-16.4t}+15.5\ e^{-0.15t}$, which is applied to 25.0 and 6.25 mg/kg doses by doubling and reducing to half two coefficients in the equation, respectively.
18) The standardized values are obtained by dividing each C_p by zero time C_p of 25.0 mg/kg dose (104.2 μg/ml).