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Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption. III.[1] Combination of Aminopyrine and Barbital. (1)

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The combination of aminopyrine and barbital has been recomended from pharmacological point of view and claimed as one of the representative examples of drug interactions. On the other hand, it will be necessary to investigate pharmacokinetically whether such a combination exhibits a tolerable potency or not. The significantly increased plasma level of aminopyrine in rabbit after simultaneous administration of barbital was observed. The most likely reason for positive effect of barbital was investigated in detail. But the potentiation of aminopyrine–barbital interaction in gastrointestinal absorption and the accelerated effect of barbital on metabolic and excretion process of aminopyrine could not be concluded from various *in vitro* and *in vivo* experiments. As one of the reasons, an increased rate of stomach emptying induced by barbital may be presented.

Numerous studies in pharmacological field have shown that the simultaneous administration of aminopyrine and barbital improved the antipyretic and analgetic efficacies, and conversely depressed the stimulative activity of aminopyrine and the anesthetic action of barbital. And this combination is claimed as one of the representative examples of drug interactions. At the same time, it is also necessary to investigate whether such a combination of drugs exhibits a tolerable potency from the pharmacokinetical aspects, that is, kinetical investigations of drug absorption, distribution, metabolism and excretion. Ohata[3] has studied on the mutual effect of aminopyrine and barbital (molar ratio of 2:1) from the changes of individual plasma concentration and physiological condition observed in rabbit. According to his report, it was concluded that the simultaneous intramuscular administration produced a small decrease in plasma concentration of aminopyrine through whole experimental time, a decrease of plasma concentration in an initial stage of barbital attaining a maximum in few hours and a prolongation of high plasma level after passing over the maximum as compared with single administration of barbital. Furthermore, it is supposed that such a mutual effect may be useful in the manner that weakens the acute toxicities of them and sustains its sedative activity for long time. And an assumption that the biological membrane permeability of drugs is changed by simultaneously administered components and then causes the physiological changes that differs from the case of single administration, may be developed. Ohata[4] has also studied on the mixture of aminopyine and barbital, in a molar ratio of 1:1. The result has almost coincided with that of experiment in molar ratio of 2:1.

A time course of plasma concentration of aminopyrine in rabbit following oral administration of mixture was investigated by Nito.[5] It was found that the plasma concentration reached the maximum value at a remarkable rate. Although aminopyrine was administered through different routes, the above result contradicted obviously with Ohata's result.

On the other hand, many attempts have been focused to potentiate the absorption of drug for a more rapid attainment of therapeutic blood levels. Therefore, the effects of co–

1) Part II: S. Goto, O. Tsuzuki and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **17**, 837 (1969).
2) Location: *Katakasu, Fukuoka.*
3) K. Ohata, *Nippon Yakurigaku Zasshi*, **53**, 542 (1957).
4) K. Ohata, *Yakugaku Zasshi*, **78**, 312 (1958).
5) S. Nito, *Yakugakukenkyu*, **35**, 105 (1963).

existences and adjuvants on drug absorption have recently evaluated with considerable attention.

The purpose of this paper is to investigate systematically the effect of barbital on gastrointestinal absorption of aminopyrine using rabbit and rat, and to reconsider pharmacokinetically the combination of aminopyrine–barbital which has already been recommended from pharmacological point of view.

Experimental

Materials——Aminopyrine, 4-aminoantipyrine and barbital were recrystallized from distilled water. N-Acetylaminoantipyrine was synthesized by method of Knorr[6] and recrystallized from toluene, mp 197—199°, colorless crystalline, UV λ_{max}^{EtOH} 245 and 275 mμ. Other reagents were of JIS special grade (Wako pure reagent).

Determination of Aminopyrine——(a) From Aqueous Solution: The colorimetric method developed by Ono[7] was used. To 1 ml of sample solution, add 2 ml of ammonium chloride buffer (pH 8), 1 ml of 0.2% phenol and 2 ml of 1% $K_3Fe(CN)_6$. Stand 30 min and shake vigorously with 10 ml of $CHCl_3$ for 30 sec. Extract a colored material of aminopyrine into $CHCl_3$ and aspirate aqueous layer. Dehydrate by adding of anhydrous Na_2SO_4 and then read at 460 mμ against a reagent blank.

(b) From Plasma or Urine: Add 1—3 ml of plasma or urine and 0.5 ml of 1 N NaOH to 8 ml of $CHCl_3$ in a glass stoppered bottle. Shake for 2 min and centrifuge. Remove the supernatant aqueous phase by aspiration. Filtrate $CHCl_3$ solution using dry filter paper. Then transfer 5 ml of filtrate into a glass stoppered test tube. Add 0.5 ml of Ac_2O to the filtrate and stand for 30 min at room temperature. Extract with 5 ml of 0.2 N HCl for 2 min and centrifuge. Transfer 2 ml of the supernatant aqueous phase into glass bottle containing 1.5 ml of 0.25 N NH_4OH and 5 ml of Michaelis buffer (pH 8.4). Add 0.5 ml of 0.5% phenol and 0.5 ml of 5% $K_3Fe(CN)_6$. Shake for 5 min gently. After 20 min standing, extract a colored material with 8 ml of $CHCl_3$ and aspirate aqueous layer. Dehydrate by adding of anhydrous Na_2SO_4 and then read at 460 mμ against a reagent blank.

(c) Establishment of Optimum Condition for Analysis from Plasma or Urine: (i) Color Development and pH: One ml sample solution containing aminopyrine (100 μg/ml) transfer into glass bottle. Add the Michaelis buffers (pH 7.2—8.8) and adjust to various pH values. The analytical procedure was described in the above section (b). In pH 7.6—8.4 range, the optimum condition for color development was obtained as shown in Fig. 1. Then pH 8.4 buffer was selected for analysis.

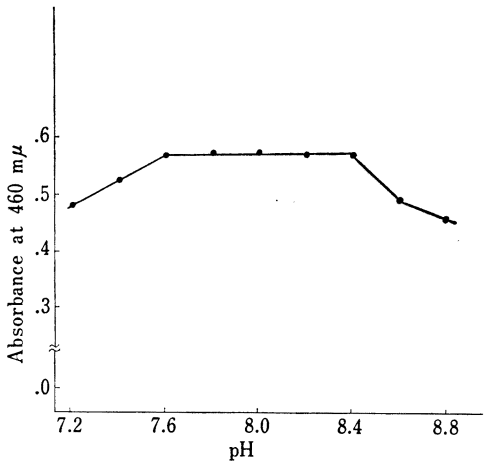


Fig. 1. Effect of pH on Absorbance of Colored Material of Aminopyrine

measured at 460 mμ, concentration of aminopyrine, 100 μg/ml

(ii) Acetylation of 4-Aminoantipyrine: For the removal of disturbance of co-existing 4-aminoantipyrine, one of the main metabolites, on the determination of aminopyrine in plasma or urine, it is advisable to acetylate 4-aminoantipyrine. And resulting N-acetylaminoantipyrine does not interfere with the aminopyrine analysis. First, 0.5 ml Ac_2O was added to the solution containing 4-aminoantipyrine (400 μg/ml). After standing in various minutes (0, 15 and 30 min), the absorbance at 460 mμ was measured. The result showes that the effect of 4-aminoantipyrine on the absorbance at 460 mμ can be perfectly removed by acetylation during 15 min period (see Table I).

The same results were also obtained in another amounts of 4-aminoantipyrine (100 and 200 μg/ml).

(iii) Calibration Curve for Aminopyrine: The absorbance of sample solution at 460 mμ was proportional to aminopyrine concentration within 50—400 μg/ml range.

(iv) Recovery for Aminopyrine: Aminopyrine in plasma or urine of rabbit in amounts from 150 to 400 μg/ml was recovered with adequate precision as shown in Table II.

6) L. Knorr and F. Stolz, *Ann.*, **293**, 58 (1896).
7) S. Ono, R. Onishi, M. Tange, K. Kawamura and T. Imai, *Yakugaku Zasshi*, **85**, 245 (1965).

Table I. Effect of Acetylation Time on Absorbance[a] of Sample Solution Containing 4-Aminoantipyrine[b]

Reaction time (min)	Sample No. 1	2	3	Mean
0	0.065	0.070	0.070	0.068
15	0.000	0.001	0.001	0.001
30	0.001	0.000	0.001	0.001

a) measured at 460 mμ *b*) concentration, 400 μg/ml

Table II. Recovery of Aminopyrine from Rabbit Plasma and Urine

	Aminopyrine (μg/ml)	4-Aminoantipyrine (μg/ml)	N-Acetyl-aminoantipyrine (μg/ml)	Absorbance	Recovery (%)
Plasma	150	—	—	0.227	100.0
	150	200	—	0.223	98.5
	300	—	—	0.440	100.0
	300	—	200	0.440	100.0
	300	1000	—	0.450	102.2
	300	500	500	0.445	101.2
Urine	150	—	—	0.226	100.0
	150	300	300	0.229	100.1
	150	300	700	0.224	99.8
	400	—	—	0.600	100.0
	400	—	400	0.600	100.0
	400	400	400	0.600	100.0

Determination of 4-Aminoantipyrine——Add 1 ml of 0.5 N HCl and 0.5 ml of 0.2% sodium nitrite solution to 1 ml urine and stand for 10 min. Further, add 0.5 ml of 1% ammonium sulfamate and leave for 3 min. Add 1 ml of 1% β-napthol dissolved in 1 N NaOH to the above solution. Stand for 10 min. Extract a colored material with 8 ml of $CHCl_3$. Filtrate $CHCl_3$ solution with filter paper. Measure at 450 mμ.

Determination of N-Acetylaminoantipyrine——Acidify with 1 ml of 0.5 N HCl 1 ml of aqueous solution, and extract by 8 ml of $CHCl_3$ for 5 min. Aspirate water layer and filtrate. Re-extract 5 ml of $CHCl_3$ solution with 8 ml of 0.5 N NH_4OH and centrifuge. Determine at 240 mμ spectrophotometrically.

Determination of *p*-Nitroaniline——To 10 ml of aqueous sample solution, add 1 ml of 1 N HCl and 0.5 ml of 0.2% $NaNO_2$. After 3 min shaking, add 0.5 ml of 1% ammonium sulfamate to the above solution. Further add 0.5 ml of 0.1% Tsuda reagent. Measure at 550 mμ against a reagent blank after 20 min standing.

Determination of 5-Nitrosalicylic Acid——Acidify 1 ml aqueous sample solution with 1 ml of 1 N HCl and extract with 8 ml of $CHCl_3$ for 5 min. Separate $CHCl_3$ layer and filtrate. Re-extract 5 ml of $CHCl_3$ extract with 8 ml of 0.5 N NaOH. Read at 410 mμ.

Analysis fof Interaction or Organic Molecules in Aqueous Solution——The method of Giles,[8,9] refractive index measurement, was applied. The refractive index of sample solution, n, was measured using Abbe refractometer (Zwiss) at 37° and pH 6.95. The standard solutions (1×10^{-2}M) of aminopyrine, barbital, *p*-nitroaniline and 5-nitrosalicylic acid were made using phosphate buffer, pH 6.95. These solutions thermostated at 37° and the sample solutions for measurement were prepared by adding together proper portions of two standard solutions. Total concentration of sample solution maintained at 1×10^{-2}M. Three combinations, aminopyrine–barbital, aminopyrine–5-nitrosalicylic acid and barbital–*p*-nitroaniline, were used for the measurement.

Determination of Stability Constant for Complex in Aqueous Solution (Solubility Method)——The modification of the method of Higuchi and co-worker[10] was used.

***In vitro* Circulation Method**——(a) Apparatus, A Wiseman type apparatus reformed by Matsumoto[11] was used.

8) C.H. Giles, *J. Chem. Soc.*, **1952**, 3799.
9) M. Samejima, *Yakugaku Zasshi*, **80**, 99 (1960).
10) T. Higuchi and D.A. Zuck, *J. Am. Pharm. Assoc.*, **42**, 138 (1953).
11) H. Matsumoto, *Yakugaku Zasshi*, **86**, 590 (1966).

(b) Procedure, Male Wister rats weighing 150—200 g were anesthetized with ether and remained under anesthesia throughout the operation. The abdominal region was incised along the midline, and small intestine was exposed. The intestine was removed carefully, rinsed with saline solution and sinked in Ringer solution containing 2% sodium citrate at 37°. And then the intestine was cut to give a length which required slight stretching to reach the lower projection from upper one of the apparatus. The end of intestinal segment was tied on the projections with ligature. Seventy ml of inner solution at 37° was poured into the upper chamber and circulated with flow rate of 13 ml/min. After 3 minutes' circulation, the lower chamber was filled with 70 ml of outer solution at 37°. Krebs-Ringer (pH 7) and phosphate buffer (pH 6—8) solutions were used for the outer and inner solutions, respectively. All apparatus was maintained at 37°. One ml of the outer solution was pipetted out at regular intervals and immediately 1 ml Krebs-Ringer solution was added into the lower chamber. The outer solution was analyzed. The volume change of inner solution and the adsorbed amount of drug on intestinal wall were almost negligible.

Intestinal Recirculating Perfusion Method[12,13]——Male Wister rats weighing 150—200 g and rabbit weighing 3 kg were fasted for 24 hr prior to the experiment. Water was allowed *ad libitum*. The animals were anesthetized with urethane (175 mg/kg), the duodenum and ileum were exposed through a midline incision and cannulated for recirculation using a Tokyo Kagaku Seiki perfusion pump of the CV-I type for rat or CV-II type for rabbit.[14] The intestine was first perfused with saline solution and followed 85 ml phosphate buffer solution (pH 6.95) with flow rate 9 ml/min for rat intestine and 32 ml/min for rabbit intestine. Phenol red was used for volume indicator of recirculating solution. One ml sample solution was pipetted out at regular intervals and analyzed.

Everted Intestine Method[15]——The apparatus is shown in Fig. 2. Male Wister rat was used and the small intestine was removed under ether anesthesia. It was rinsed with saline solution at 37° and sleeved onto a glass rod and everted carefully. The end of a 6 cm segment of intestine was closed tightly with a ligature. The opposite end of intestine was tied around the lower end of glass cannula. One ml of Krebs-Ringer solution (pH 7) was pipetted into the everted intestine tube. The intestine tube was immersed in 100 ml of aminopyrine or mixture of aminopyrine and barbital maintained at 37° and gassed continuously with a mixtue of 95% oxygen and 5% carbon dioxide. To obtain the samples of the serosal fluid a vent needle (A) was occluded. Under the force of the increased gas pressure, the serosal fluid rised into the upper portion of the glass cannula where it was available to a pipette. Every 10 minutes, the entire serosal fluid was withdrawn and the segment was rinsed twice with about 1 ml Krebs-Ringer buffer. After rinsing, new Krebs-Ringer buffer (1 ml) was introduced and was immersed into a new mucosal solution.

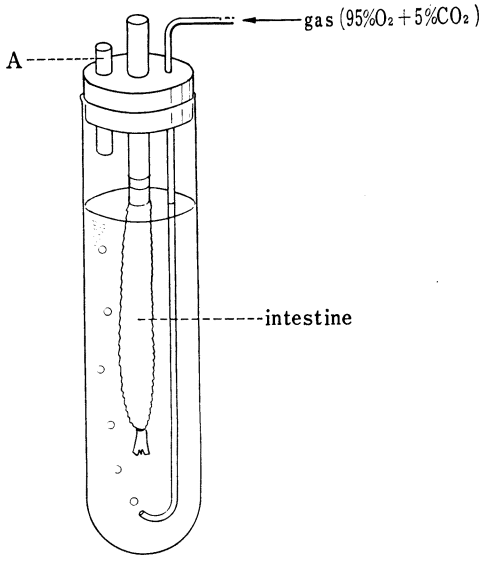


Fig. 2. Apparatus for Everted Intestine Method

Ligated Stomach Method——Male Wister rat and male rabbit were used. The procedure stated in the previous paper[16] was applied.

Plasma Concentration and Urinary Excretion Studies——Plasma and urine were collected at constant intervals and analyzed.

(a) Oral administration, Male rabbit weighing 3 kg was fasted for about 24 hours prior to the experiment. Aminopyrine (255 mg/kg) or mixture with barbital (85 mg/kg) dissolved in 40 ml distilled water was administered in stomach through rubber tube for stomach and 10 ml distilled water was also administered.

(b) Intravenous administration, Male rabbit weighing 3 kg was fasted for about 24 hours prior to the experiment. Aminopyrine (60 mg/kg) or mixture with barbital (20 mg/kg) was dissolved in 5 ml saline solution and then administered from ear vein within 1 min.

12) K. Kakemi, T. Arita and S. Ohashi, *Yakugaku Zasshi*, **82**, 348 (1962).
13) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
14) An intestine of 20 cm length measuring from pylorus was used for this experiment.
15) R. K. Crane and T.H. Wilson, *J. Appl. Physiol.*, **12**, 145 (1958).
16) S. Goto, R. Takamatsu, M. Shibao and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 332 (1968).

Result and Discussion

Plasma Level Following Oral Administration

The maximum plasma level of aminopyrine following the single administration (255mg/kg) was obtained in 3 hours using male rabbit. In the administration of combination with barbital (85mg/kg), the maximum developed with a remarkable rate within only 30 min as shown in Fig. 3. The same tendency was also observed in another three rabbits. As the most likely reason for the positive effect of simultaneous administration of barbital to the plasma level of aminopyrine, a decrease of both the metabolic and urinary excretion rates of aminopyrine or an increase of the gastrointestinal absorption rate of aminopyrine may be considered.

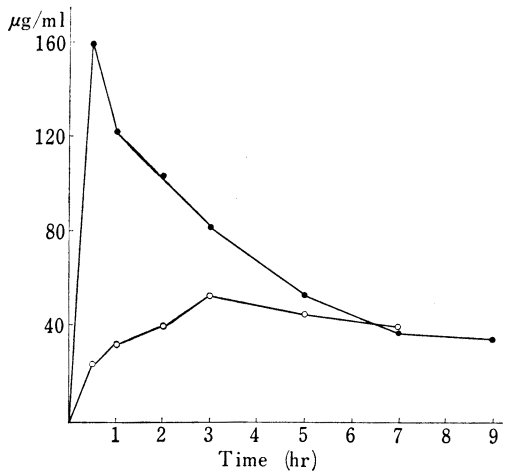
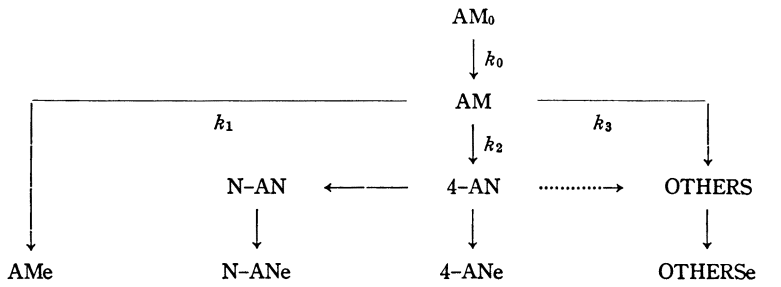


Fig. 3. Plasma Level of Aminopyrine after Oral Administration in Rabbit

—○—: aminopyrine 255 mg/kg
—●—: aminopyrine 255 mg/kg+barbital 85 mg/kg

Effect of Barbital on the Metabolic Process and the Urinary Excretion Process of Aminopyrine

Prior to the study of the effect of barbital on the pharmacokinetics of metabolic process or urinary excretion of aminopyrine, it is necessary to determine the rate constant for elimination of aminopyrine in rabbit. Under the assumption that the first-order kinetics is applied in each process in the model, the following consideration will be developed.



where AM_0 is the total amount of aminopyrine administered in the gastrointestinal tract; AM is the amount of aminopyrine in the compartment of distribution at any time; 4-AN is the amount of 4-aminoantipyrine in the compartment of distribution at any time; OTHERS is the total amount of other metabolites in the compartment of distribution at any time; AMe, N-ANe, 4-ANe and OTHERSe are the amounts of respective materials excreted in the urine at any time; and k_i ($i=0$ .... 3) represents the apparent first-order rate constants. An appropriate differential equation for above model is described as follows;

$$AM = f \cdot AM_0 \frac{k_0}{K-k_0}(e^{-k_0 t} - e^{-Kt}) \qquad (1)$$

where $K=k_1+k_2+k_3$ and f is the utility fraction of aminopyrine administered in gastrointestinal tract.

$$\frac{d\mathrm{AMe}}{dt} = k_1 \cdot \mathrm{AM} \tag{2}$$

Substituting eq. (1) to eq. (2), the following equation is obtained;

$$\frac{\Delta\mathrm{AMe}}{\Delta t} = f \cdot \mathrm{AM}_0 \frac{k_0 k_1}{K - k_0}(e^{-k_0 t} - e^{-Kt}) \tag{3}$$

If $k_0 \gg K$ and after the time that the absorption of aminopyrine from gastrointestinal tract is completed, eq. (3) is simplified to the following equation;

$$\frac{\Delta\mathrm{AMe}}{\Delta t} = f \cdot \mathrm{AM}_0 \frac{k_0 k_1}{k_0 - K} e^{-Kt} \tag{4}$$

or

$$\log \frac{\Delta\mathrm{AMe}}{\Delta t} = \log f \cdot \mathrm{AM}_0 \frac{k_0 k_1}{k_0 - K} - \frac{K}{2.3} t \tag{5}$$

where $\Delta\mathrm{AMe}/\Delta t$ is the amount of intact aminopyrine excreted in the urine in unit time. The log $\Delta\mathrm{AMe}/\Delta t$ is plotted against t and the rate constant for overall elimination of aminopyrine (K) which is proposed with the above assumptions, is calculated from the slope of the resulting straight line. The cumulative amounts appearing in the urine of intact aminopyrine were approached an asymptote at 24 hours following oral administration of aminopyrine in rabbit. The plot of log $\Delta\mathrm{AMe}/\Delta t$ *versus* t is shown in Fig. 4. The time t in figure represents the mid-point of the urinary collection intervals. The effect of barbital on the elimination rate of intact aminopyrine was observed in three rabbits but the data for one of the rabbits is presented in Fig. 4. It appears that the effect of barbital upon the elimination rate of intact aminopyrine is not significant. But the amount in urinary excretion in the presence of barbital was 2 to 3 times greater than that in the absence of barbital through all experimental time.

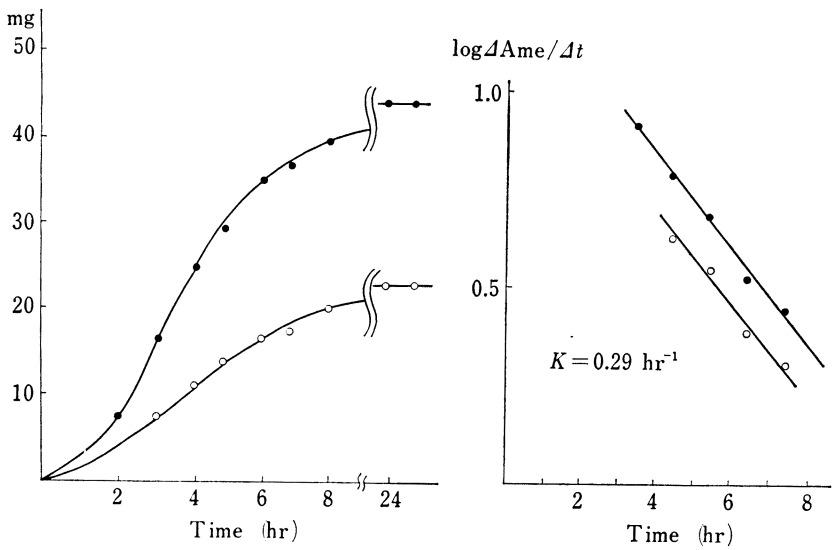


Fig. 4. Cumulative Excretion Curve and Excretion Rate Plot of Aminopyrine after Oral Administration in Rabbit

—○—: aminopyrine 255 mg/kg —●—: aminopyrine 255 mg/kg+barbital 85 mg/kg

Following the intravenous administration of aminopyrine (60 mg/kg), the concentration of aminopyrine in plasma was measured. The plasma level of aminopyrine decreased rapidly

and exponentially with time. And this rate was not affected by simultaneous administration of barbital (20 mg/kg) as shown in Table III. No difference between the administrations of aminopyrine alone and in the presence of barbital on the cumulative amounts of intact aminopyrine and 4-aminoantipyrine appearing in the urine were observed. The results are shown in Fig. 5.

TABLE III. Effect of Barbital on Disappearance Rate of Aminopyrine from Plasma after Intravenous Administration in Rabbit

	Disappearance rate constant(hr^{-1})		
Rabbit No.	1	2	3
Single administration[a)]	0.55	0.60	0.51
Simultaneous administration[b)]	0.57	0.69	0.57

a) aminopyrine 60 mg/kg
b) aminopyrine 60 mg/kg+barbital 20 mg/kg

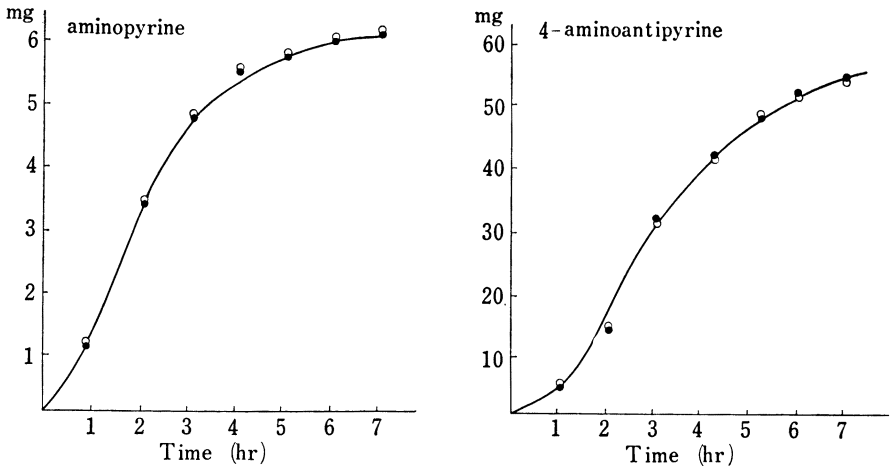


Fig. 5. Cumulative Excretion Curve of Aminopyrine and 4-Aminoantipyrine after Intravenous Administration in Rabbit

—○—: aminopyrine 60 mg/kg
—●—: aminopyrine 60 mg/kg+barbital 20 mg/kg

As shown in Table III and Fig. 4, the disagreement of the rate constants for intact aminopyrine obtained by oral administration and by intravenous injection is observed. These rate constants must be identified theoretically. But this will be the problem to be solved from a different angle.

Moreover, the ratio of metabolites, aminopyrine : 4-aminoantipyrine : N-acetylaminoantipyrine, in 24 hours-urine was almost constant regardless of whether barbital was administered. This fact may indicate that barbital does not affect with the metabolic process of aminopyrine.

Effect of Barbital on Gastrointestinal Absorption of Aminopyrine

In an attempt to obtain a better understanding, *in vitro* circulation method was undertaken to ascertain the increase of absorption of aminopyrine based on the existence of possible interaction between aminopyrine and barbital. The results obtained in pH 6.95 are summarized in Table IV. Each value is represented as the mean of transfer percentage in one hour period of three rats. Although these percentages are small, it is obvious that there is no significant difference between single and simultaneous administrations.

TABLE IV. Mutual Effect between Aminopyrine and Barbital across Intestine (pH 6.95)

Intestine segment	Aminopyrine		Barbital	
	Single[a] permeation %	Simultaneous[b] permeation %	Single[c] permeation %	Simultaneous[b] permeation %
Proximal	3.7	3.7	2.7	1.8
Middle	5.6	7.3	3.5	3.5
Distal	5.9	6.2	3.5	3.7

Each value is expressed as the mean of three rats.
initial concentration *a*) aminopyrine 1×10^{-3}M
b) aminopyrine 1×10^{-3}M, + barbital 1×10^{-3}M
c) barbital 1×10^{-3}M

TABLE V. Mutual Effect between Aminopyrine and Barbital on Absorption from Intestine (pH 6.95)

	Absorption rate constant (hr^{-1})					
	Single administration			Simultaneous administration		
Aminopyrine[a]	0.46			0.46		
	0.46			0.55		
	0.46	mean	0.46	0.57	mean	0.51
	0.46			0.46		
Barbital[a]	0.35			0.32		
	0.35			0.41		
	0.35	mean	0.33	0.41	mean	0.35
	0.28			0.25		

a) initial concentration: 1×10^{-3}M; When the initial concentrations of aminopyrine and barbital were changed into 5×10^{-3} or 1×10^{-2}M, no differences in between the absorption rate constants of single and simultaneous administration were observed.

Using intestinal recirculating perfusion method, the intestinal absorption of aminopyrine administered with barbital was compared with that of single administration at pH 6.95 in rat and rabbit. In this method, there is no significant difference between alone and together with drugs. The same tendency was also observed in rabbit. The result in rat was shown in Table V.

In previous papers[1,16] it has been shown that the gastric absorption rate of relatively absorbable drugs were remarkably depressed by administration of poorly absorbable drugs on view point of complex formation between them. On the other hand, it has been suggested that caffeine enhanced the gastric absorption of nonabsorbable drug by virture of their ability to form complex. In varing ratios of combination of aspirin and caffeine, an enhancing the gastric absorption of each drug was not observed. And the experimental fact that their gastric absorption rates were almost same degree is a reason which cannot be overlooked. Such an idea may also be applied in the combination of aminopyrine–barbital because their intestinal absorption rates are almost same degree.

TABLE VI. Effect of Aminopyrine[a] on Absorption of 5-Nitrosalicylic Acid[a] from Intestine (pH 6.95)

	Absorption rate constant (hr^{-1})		
Single administration	0.049	0.055	0.055
Simultaneous administration	0.15	0.17	0.17

a) initial concentration: 1×10^{-3}M

Two combinations, aminopyrine– 5-nitrosalicylic acid and barbital– *p*-nitroaniline, were selected. And the effect of aminopyrine and barbital to another drugs was observed by the intestinal recirculating perfusion method. *p*-Nitroaniline is one of the comparatively absorbable drugs from intestines of rat and rabbit, and 5-nitrosalicylic acid is opposite. Aminopyrine accelerated the absorption of 5-nitrosalicylic acid as shown in Table VI. The accelerated effect of *p*-nitroaniline on the absorption rate of barbital was observed evidently as shown in Fig. 6. These facts may be due to the complex formation between them in aqueous solution. By using both of the refractive index measurement and the solubility method, the complexation between them could be recognized at 37°. The results are summarized in Table VII.

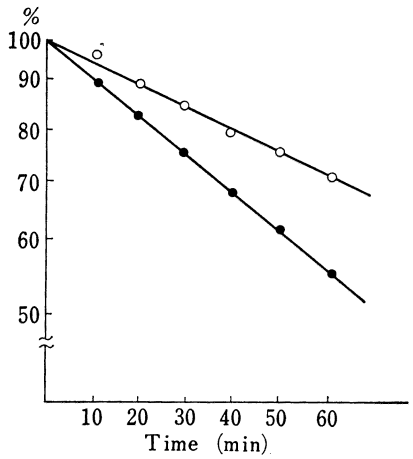


Fig. 6. Logarithmic Plots of Barbital Remaining in Recirculation Solution, pH 6.95

—○—: barbital 1×10^{-3}M
—●—: barbital 1×10^{-3}M+*p*-nitroaniline 1×10^{-3}M

No enhancement or deterioration in the transfer rate of aminopyrine across everted intestine of rat by barbital could be observed as despicted in Fig. 7.

Unfortunately, the remarkable plasma level of aminopyrine following oral administration with barbital in rabbit could not be explained by various gastric and intestinal absorption experiments using rat and rabbit. Then another attempts will be quite necessary to dissolve the above fact.

Table VII. Apparent Stability Constants and Mol. ratio of Complexes of Aminopyrine–Barbital, Aminopyine–5-Nitrosalicylic Acid and Barbital–*p*-Nitroaniline at 37°

Complexes	Mol. ratio[a)]	Apparent stability[b)] constant (M^{-1})
Aminopyrine–Barbital	1 : 1	12
Aminopyrine–5-Nitrosalicylic acid	1 : 1	57
Barbital–*p*-Nitroaniline	1 : 1	33

a) from refractive index measurement *b*) from solubility method

Another Attempt using Rabbit

The amount of aminopyrine remaining in rabbit stomach was measured in one hour after oral administration. The stomach level of aminopyrine was more than 1.3 times higher in the control group than in the simultaneous administration group. Recently, Riegelman and co-workers[17] have stated that it was almost impossible to obtain an empty stomach in rabbit by using the conventional method of fasting, and then the significantly increased plasma level of aminopyrine after simultaneous administration of barbital may be due to an increased rate of stomach emptying induced by barbital. This statement agrees very well with our observation.

The conclusion does not offer to support the potentiation of barbital-aminopyrine interaction in gastrointestinal absorption and that of barbital on metabolic and elimination rates

17) W.L. Chiou, S. Riegelman and J.R. Amberg, *Chem. Pharm. Bull.* (Tokyo), **17**, 2170 (1969).

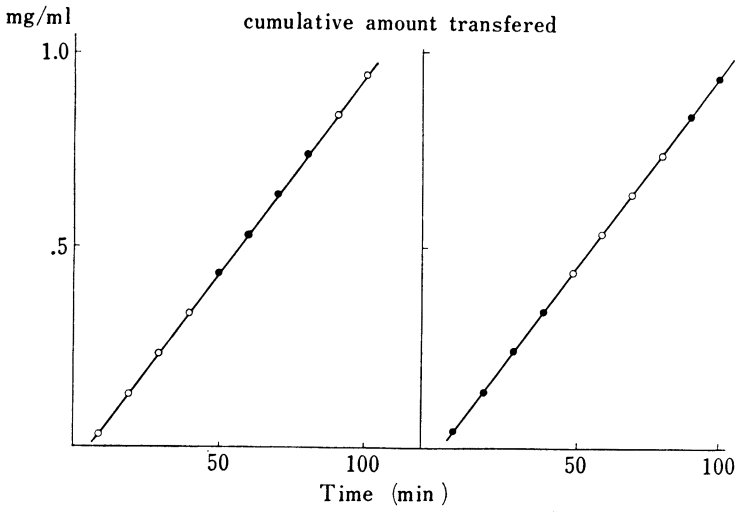


Fig. 7. Effect of Barbital on Transfer of Aminopyrine across Everted Intestine of Rat

—●—: mucosal solution, aminopyrine 3.5×10^{-3}M+barbital 3.5×10^{-3}M
—○—: mucosal solution, aminopyrine 3.5×10^{-3}M

of aminopyrine. After all, the possibility that the physiological conditions may be changed by barbital will be suggested as the most likely reason. But there are many problems to be solved, and the investigation will last for a long time.