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204. Keijiro Takagi,*1,*2 Tokuji Suzuki,*2 and Yukiya Saitoh*2 : Effect of Terephthalic Acid on the Blood Level of Sulfaethylthiadiazole.*3

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The effect of terephthalic acid on the blood level of sulfaethylthiadiazole in the rabbit was examined. About twice increase of the biologic half-life of sulfaethylthiadiazole in the range of 50 to 150 mg./kg. of terephthalic acid was shown at the simultaneous oral administration. No marked effect was shown by parenteral administration of terephthalic acid. The acute toxicity following simultaneous intraperitoneal administration was investigated in the mouse. The increase of the acute toxicity was not observed significantly judging from LD_{50} determined by the single administration of sulfaethylthiadiazole and of terephthalic acid.

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It has been reported that the blood levels of antibiotics of tetracycline family were increased by simultaneous administration of terephthalic acid (TPA),[1,2] and TPA has been used together with the antibiotics for treating and preventing diseases of poultry. It has been found, also, that when sulfonamide drugs are fed or given in drinking water in combination with sodium TPA, the rate of absorption of sulfonamide drugs is significantly increased and there is an increase in the amount of sulfonamide in the blood stream.[3] However, no quantitative explanation of this effect of TPA on the blood level of sulfonamide drugs has been reported.

In this report, the change of blood level of free sulfaethylthiadiazole (SETD, unacetylated) against time after the co-administration of TPA by various administration routes in the rabbit has been examined. The acute toxicity following simultaneous intraperitoneal administration of TPA and SETD also has been investigated in the mouse. It has been shown previously that oral doses of SETD are virtually completely absorbed, almost totally excreted in the urine, and a large portion of SETD absorbed is contained in the blood,[4,5] and that SETD has a relatively short biologic half-life (1.24 hr. in the rabbit[6]) with very low degree of acetylation.[7] Since the interpretation of the excretion data is not considered to be difficult from the facts mentioned above, SETD appeared to be a suitable drug for this study.

Experimental

Materials——Terephthalic acid (TPA) was a commercial product and used without further purification. Sulfaethylthiadiazole (SETD) was supplied from C.H. Boeringer Sohn, Ingelheim. SETD and TPA were neutralized by NaOH solution and sterilized for parenteral use before administration. Terephthalic acid-(carboxyl-^{14}C), ^{14}C-TPA, was supplied from Daiichi Pure Chemicals Co., Tokyo. The radiochemical purity of ^{14}C-TPA

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(10 μc./100 mg.) by paper chromatography developed in a mixture of ethanol–water–ammonia (80:60:4) was more than 99%. It was also neutralized by NaOH solution and sterilized.

Animals——Adult male white rabbits (2.8~3.9 kg.) were used. SETD in a dose of 100 mg./kg. or 400 mg./kg. and TPA in a dose of 50 mg./kg., 100 mg./kg. or 150 mg./kg. were administered orally by a stomach tube or intramuscularly. When TPA was administered by a different route from that for SETD, the respective doses were 100 mg./kg. The animals were fasted for 24 hours before oral administration. The same rabbits were used for both the control experiments (SETD alone) and the experiments of co-administrations of TPA and SETD. ^{14}C–TPA was administered orally or intravenously in a dose of 100 mg./kg. along with SETD in a dose of 100 mg./kg. The acute toxicity following intraperitoneal administration of SETD and/or TPA was investigated in the mouse. Five groups of 10 mice of d.d. strain were given graded doses of SETD and/or TPA intraperitoneally. SETD and TPA were neutralized by NaOH solution and injected as 5% solutions. The mice were observed for 24 hours after the administration, and LD_{50} was calculated by a graphic method of Litchfield-Wilcoxon.[8]

Analytical Method——Two ml. of blood was taken with a syringe containing 0.4 ml. of 3.8% sodium citrate at various intervals. The protein content of 1.2 ml. of the blood sample was precipitated with 4 ml. of 15% trichloroacetic acid, after 8 ml. of 0.1% saponin was added, and the mixture was filtered. SETD in 4 ml. of the filtrate was determined by the modified Tusda's method.[9,10] ^{14}C–TPA was determined by liquid scintillation counting, after the plasma sample was separated from 2 ml. of blood by centrifuging at 3000 r.p.m. The procedure is given in Chart 1. The supernatants A, B and C were mixed in a counting vial, and neutralized by 0.1*N* NH_4OH. This solution was counted for 10 min. in a Tri-Carbo 3203 liquid scintillation spectrometer (Packard). The counting efficiency was 50~60% which was determined with a channel ratio method. The recovery of ^{14}C–TPA added to rabbit plasma was 91~100% by this procedure.

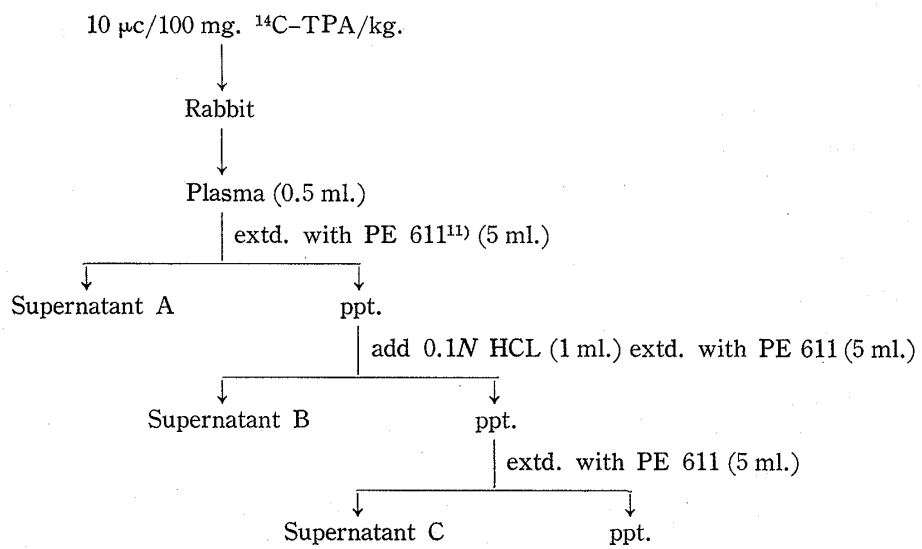

Chart 1. Procedure for Liquid Scintillation Counting of Terephthalic Acid-(carboxyl-^{14}C)($HOOC^{14}$-⟨⟩-COOH) in Rabbit Plasma

Results and Discussion

Typical plots of the logarithms of unacetylated sulfaethylthiadiazole (SETD) against time following oral administration of SETD with or without terephthalic acid (TPA) were shown in Fig. 1. The SETD blood level after the peak concentration followed a first-order disappearance, as reported previously on human.[5] The drug disappearance from the blood may be characterized by the following first-order rate expression[12] after termination of absorption from the digestive tract and attainment of diffusion equilibrium between the blood and tissues.

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$$\log C_t = -k_d t/2.303 + \log C_0$$

Where, t is a period of time after drug administration, C_t is SETD blood concentration at any time, and C_0 is a constant corresponding to a value of intercept on the concentration axis at zero time. The elimination of SETD from the blood stream may be described as a rate constant (k_d) or a biologic half-life $\left(t_{1/2}=\frac{0.693}{k_d}\right)$.

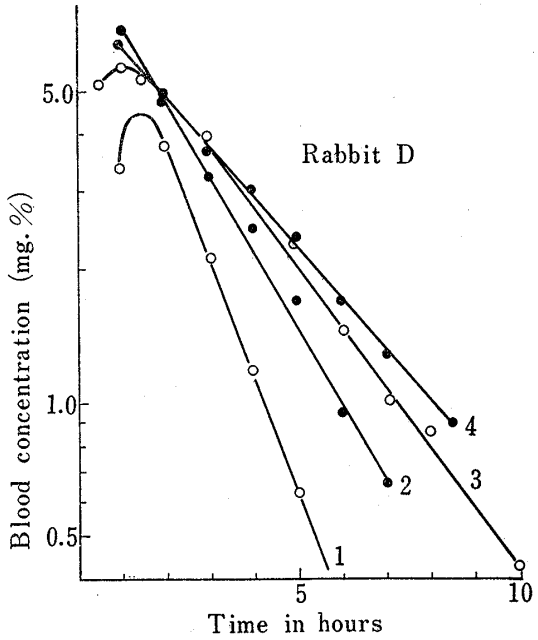

Fig. 1. Blood Concentration of Unacetylated Sulfaethylthiadiazole (SETD) following Oral Administration (100 mg./kg.) with and without Terephthalic Acid (TPA) in Rabbit D

Key: 1; SETD alone, 2; SETD+TPA (50 mg./kg.), 3; SETD+TPA (100 mg./kg.), 4; SETD+TPA (150 mg./kg.)

The effect of TPA on blood level of SETD was examined by comparing the biologic half-life following simultaneous oral administration of SETD and TPA with the control (SETD alone). The half-lives obtained were summarized in Table I. When TPA was administered together with SETD in a dose of 100 mg./kg., about twice increase of the biologic half-life in the range of 50 to 150 mg./kg. of TPA was demonstrated. The biologic half-life with an oral dose of 400 mg./kg. SETD also increased 2~3 times by TPA in a dose of 100 mg./kg.

The process of the elimination of SETD in logarithmic scale after intramuscular administration (SETD 100 mg./kg. and TPA 100 mg./kg.) are shown in Fig. 2-a. There was a lower elimination process for about 2 hours after the peak blood level, and thereafter the elimination rate constant of SETD was equal to that at single SETD administration. In order to confirm whether the less elimination in early stage after the peak was attributed to TPA administered together with SETD, TPA was additionally given once or twice in a dose of 100 mg./kg. by intramuscular administration after the initial co-administration (100 mg./kg. SETD and 100 mg./kg. TPA). The result is shown in Fig. 2-b. The portion of the lower elimination was extended by each administration of TPA. The biologic half-lives calculated from slower eliminated portion immediately after the peak were shown in Table II.

Table I. Biologic Half-Lives (in hr.) of Unacetylated Sulfaethylthiadiazole (SETD) following Oral Administration with and without Terephthalic Acid (TPA)

SETD / TPA / Rabbit	None	100 mg./kg. 50 mg./kg.	100 mg./kg. 100 mg./kg.	100 mg./kg. 150 mg./kg.	400 mg./kg. None	400 mg./kg. 100 mg./kg.
A	1.32	1.63 (1.23)[a]		2.89 (2.19)	2.45	5.37 (2.19)
B	1.21	2.19 (1.81)	2.21 (1.83)	2.45 (2.02)	1.97	5.37 (2.73)
C	1.32	2.11 (1.60)	1.91 (1.45)	3.01 (2.28)		
D	1.16	1.93 (1.66)	2.32 (2.00)	2.67 (2.30)		

a) The numbers in parentheses indicate the ratios of biologic half-lives to controls.

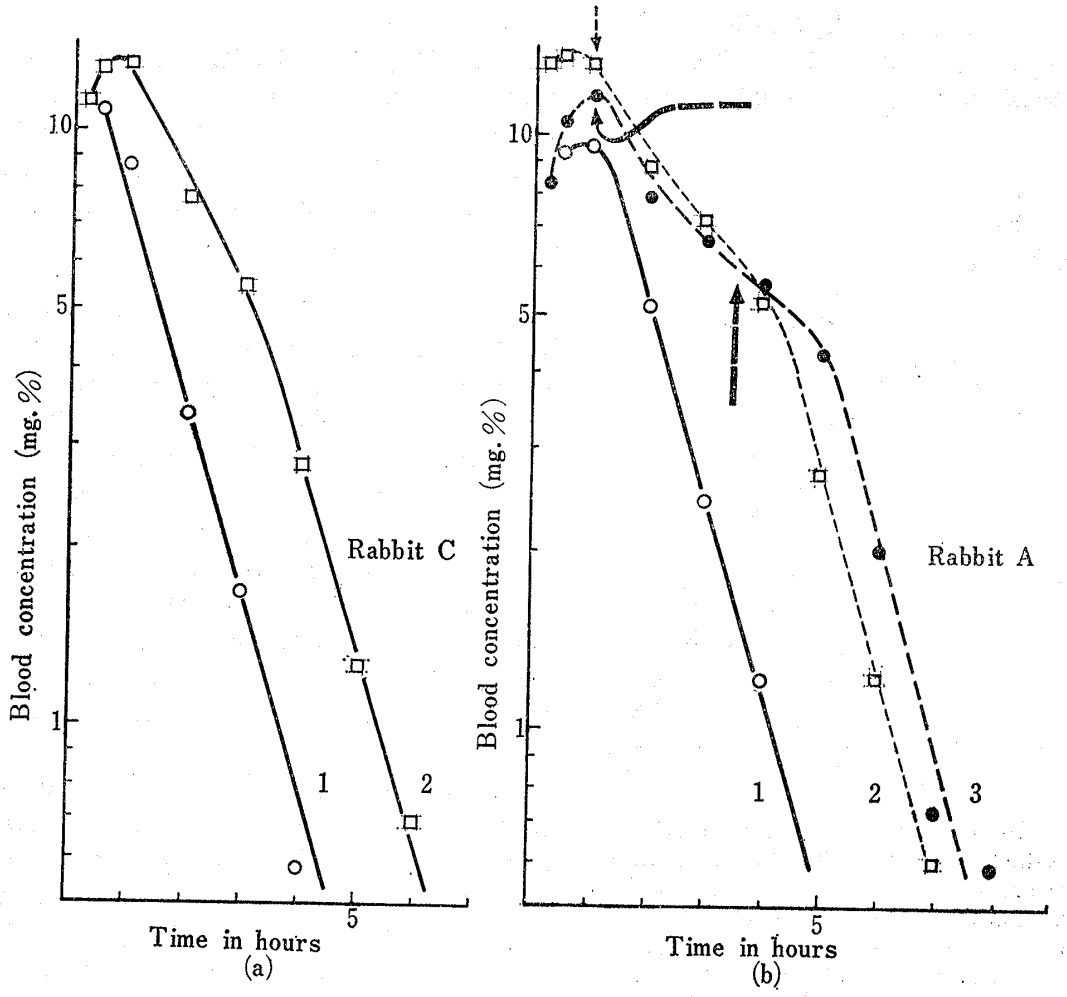

Fig. 2. Blood Concentration of Unacetylated Sulfaethylthiadiazole (SETD) following Intramuscular Administration (100 mg./kg.) with and without Terephthalic Acid (TPA)

(a): 1; SETD alone (100 mg./kg.). 2; SETD and TPA (100 mg./kg.) simultaneous administration.
(b): 1; SETD alone (100 mg./kg.). 2; TPA (100 mg./kg.) at 1 hr. after simultaneous administration (SETD 100 mg./kg. and TPA 100 mg./kg.). 3; TPA (100 mg./kg.) at 1 and 3.5 hr. after simultaneous administration (SETD 100 mg./kg. and TPA 100 mg./kg.).
The arrows mark the times of additive administration of TPA.

Table II. Increased Biologic Half-Lives (in hr.) calculated from Slower Eliminated Portion Immediately after the Peak Level of Unacetylated Sulfaethylthiadiazole (SETD) following Intramuscular Co-administration (100 mg./kg.) of Terephthalic Acid (TPA)

Rabbit	Control (SETD alone) (100 mg./kg.)	Simultaneous administration (SETD 100 mg./kg. and TPA 100 mg./kg.)	TPA (100 mg./kg.) at 1 hr. after simultaneous administration (SETD 100 mg./kg. and TPA 100 mg./kg.)	TPA (100 mg./kg.) at 1 and 3.5 hr. after simultaneous administration (SETD 100 mg./kg. and TPA 100 mg./kg.)
A	0.97		2.39 (2.46)[a]	2.97 (3.06)
B	0.89		1.83 (2.06)	1.86 (2.09)
C	0.84	1.62 (1.93)	2.29 (2.73)	
D	0.89	1.42 (1.60)	2.47 (2.78)	

a) The numbers in parentheses indicate the ratios of biologic half-lives to the controls.

It is reasonable to assume that the effect of TPA on the duration of the blood concentration of SETD was dependent upon its administration route (oral or parenteral). Half-lives of SETD following co-administration of SETD and TPA by different routes were examined, and the results were shown in Table Ⅲ. It was evident from Table Ⅲ

Table Ⅲ. Biologic Half-lives (in hr.) of Unacetylated Sulfaethylthiadiazole (SETD) (100 mg./kg.) following Administration of SETD and Terephthalic Acid (TPA) (100 mg./kg.) by Different Routes

SETD / TPA / Rabbit	Oral administration		Intramuscular administration	
	None[b]	Intramuscular administration at 1 hr. after oral SETD administration	None	Oral administration at 1 hr. before intramuscular SETD administration
A	1.32	1.39 (1.05)[a]	0.75	1.16 (1.55)
B	1.21	1.50 (1.24)	1.04	2.18 (2.10)
C	1.32	0.94 (0.71)	1.02	1.77 (1.74)
D	1.16	1.16 (1.00)	1.37	1.93 (1.41)

a) The numbers in parentheses indicate the ratios of biologic half-lives to the controls.
b) The values of controls in Table I for the corresponding rabbits.

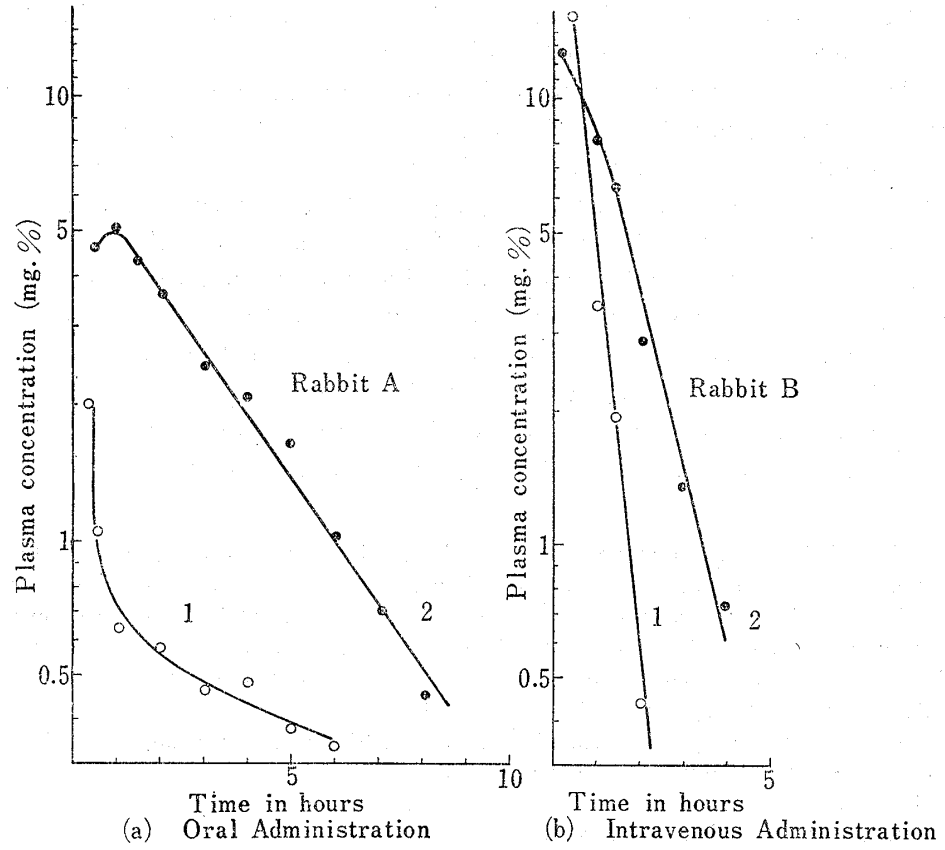

Fig. 3. Plasma Concentration of Terephthalic Acid (TPA) and Unacetylated Sulfaethylthiadiazole (SETD) following Simultaneous Oral and Intravenous Administration (each 100 mg./kg.) Key : 1; TPA, 2; SETD

that the rate constant of the elimination of SETD in the blood was influenced only by the orally administered TPA. Thus, it was postulated that the absorption process of TPA through the digestive tract was necessary for the duration of SETD blood concentration.

It was reported that phthalic acid were not metabolized in the rat,[13] the dog[14~16] and human.[14,17] An attempt was made to correlate the change of plasma level of TPA against time with that of SETD. ^{14}C-labelled TPA was used for the determination of plasma level of TPA following intravenous or oral administration together with SETD, and also the change of plasma level of SETD in logarithmic scale with time was shown in Figs. 3-a and 3-b. It was shown in Fig. 3-a that the maximum plasma level of TPA following the oral administration was attained within 1 hour, and TPA plasma levels were much lower than those following intravenous administration. However, the elimination of TPA was much slower after 1 hour and a small concentration of TPA was maintained for a longer period of time after the oral administration, while the decrease of TPA plasma level following the intravenous administration was very rapid with a biologic half-life of about 0.37 hours and no detectable amount of TPA was found at 3 hours from the administration. Thus, it is found that the minimum plasma level of TPA necessary to increase the biologic half-life of SETD is considerably low from the processes of the elimination of SETD following the intravenous and oral administration of SETD and TPA in Fig. 3-a and 3-b. Hoshi, *et al.*[18] reported that the elimination of TPA following intraperitoneal administration were quite rapid in the rabbit and the rat, and the half-life was determined to be 1.8 hours in the rabbit, while the half-life of elimination of TPA after peak plasma concentration following oral administration was 27 hours. These results correspond with those of our study. The much slower elimination following the oral administration of TPA than the intravenous administration might be attributed to slow absorption from the digestive tract.

The tubular reabsorption of an ionizable drug may be influenced by changing the pH of the tubular fluids, and the elimination processes of the drug from the blood are influenced by administration of agents which affect the acidity or alkalinity of the urine. It was reported that when an acidifying agent, such as ammonium chloride, was administered simultaneously with SETD, the decrease in pH of tubular fluid resulted in a low ionization of the weak acid (SETD, pKa 5.5) and a relatively good reabsorption of SETD.[19] This effect is a slower clearance rate from the blood than if the urine was less acidity, and the rate of elimination of SETD from the body is decreased by acidifying of urine. No significant pH change of the urine was shown in cases which TPA was administered with SETD in our study. The reason why a small amount of TPA caused the clearance rate of SETD to decrease could not well explained.*4

*4 After completion of this study, it was found[20] that Hoshi, *et al.* have carried out the study on the effect of TPA on sulfadimethoxine blood level, and it was suggested that the lower elimination of sulfadimethoxine from the blood of the rabbit after the co-administration of TPA by oral route may be caused from the inhibition of excretion from the kidney rather than the increase of absorption through the digestive tract. However, the substantial evidence on the inhibition was not shown in their report.

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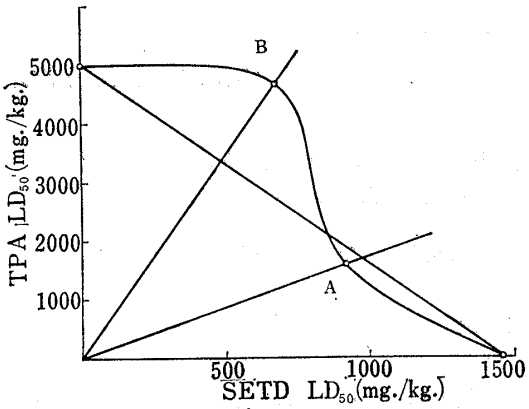

Fig. 4. Relation between LD_{50} of Single and Simultaneous administration for Sulfaethylthiadiazole (SETD) and Terephthalic Acid (TPA)-(See Text)

A: LD_{50} at a ratio of 2/3 SETD and 1/3 TPA for respective LD_{50}.

B: LD_{50} at a ratio of 1/3 SETD and 2/3 TPA for respective LD_{50}.

Although the rate of disappearance of SETD in the blood was delayed by simultaneous administration of TPA, this co-administration might cause an increase of the toxicity. Intraperitoneal LD_{50} of SETD and TPA were determined to be 1459 mg./kg. and 5040 mg./kg. in the mouse, respectively. When five graded doses of mixtures of SETD and TPA kept at a ratio of two-thirds of LD_{50} of SETD to one-third of LD_{50} of TPA were administered to mice, LD_{50} assigned to SETD was 918 mg./kg. and that of TPA was 1586 mg./kg. When five graded doses of mixtures of SETD and TPA kept at a ratio of one-third of LD_{50} of SETD to two-thirds of LD_{50} of TPA were administered to mice, LD_{50} assigned to SETD was 681 mg./kg. and that of TPA was 4707 mg./kg. (Fig. 4.). It is evident from Fig. 4 that the acute toxicity was decreased in the latter case, and a little increased in the former case, and that the increase of acute toxicity with the co-administration of the both drugs was not observed significantly.

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