Chapter 3

Results

1. Pharmacokinetic study

The standard curves of tetracycline in serum and in 0.1 M phosphate buffer, pH 4.5

The corrected standard values were plotted on two cycle semilogarithmic paper, using the tetracycline concentration as the ordinate (logarithmic scale) and the diameter (in mm) of the inhibition zone as the abscissa. And then the linear regression line equations were established.[46] The detail was shown in Figure 2.

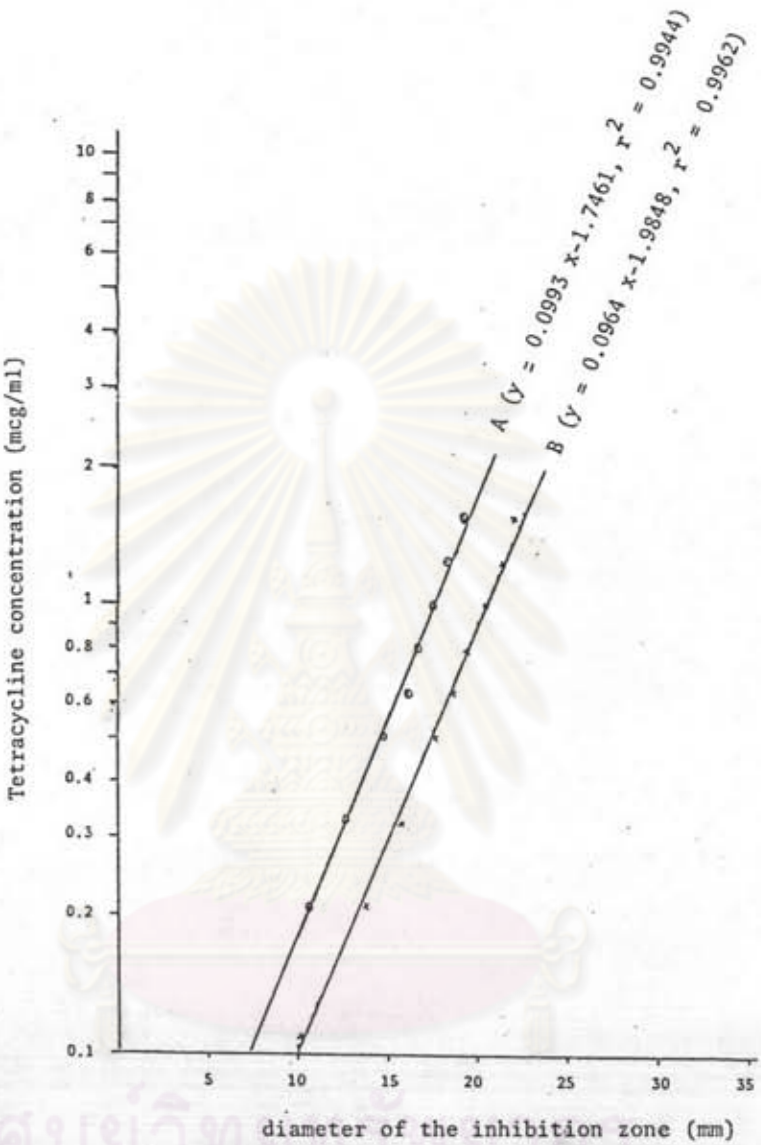

Figure 2 The standard curves of tetracycline in serum and in 0.1 M phosphate buffer, pH 4.5

A = the standard curve of tetracycline in serum

B = the standard curve of tetracycline in 0.1 M phosphate buffer, pH 4.5

y = log(tetracycline concentration)

x = diameter of the inhibition zone

r^2 = the coefficient of determination

Determination of the % recoveries of tetracycline in muscle and liver.

The recoveries of tetracycline after adding various amounts of tetracycline to muscle and liver were shown in Table 5. It was found that the average recoveries in muscle and liver were 72.77 ± 3.34 and 78.30 ± 3.48% respectively. These values were used for determining the theoretical drug levels in muscle and liver as the following equation.

A theoretical drug levels in muscle or liver

$$= \frac{\text{a determined drug level in muscle or liver}}{\text{\% recovery of the drug in muscle or liver}} \times 100$$

Table 5 % recoveries of tetracycline in muscle and liver

drug / muscle or liver	Tetracycline HCl added (mcg)								
10 gm (muscle)	3.0	5.1	6.4	8.0	10.0	12.5	15.6	30.0	50.0
% recoveries (muscle)	67.38 ± 2.83 (n = 3)	68.21 ± 1.59 (n = 3)	73.54 ± 2.49 (n = 3)	74.21 ± 0.84 (n = 3)	75.68 ± 4.11 (n = 3)	77.24 ± 3.14 (n = 3)	73.85 ± 4.74 (n = 3)	74.26 ± 5.11 (n = 3)	70.54 ± 1.30 (n = 3)
average % recovery (muscle)	72.77 ± 3.34								
1.0 0.1 gm (liver)	0.5	1.0	1.5	3.0	6.0	12.0			
% recoveries	81.26 ± 0.64 (n = 2)	75.06 ± 0.59 (n = 2)	80.70 ± 8.95 (n = 3)	81.22 ± 3.54 (n = 2)	78.42 ± 5.41 (n = 3)	73.12 ± 4.11 (n = 2)			
average % recovery	78.30 ± 3.48								

Intraperitoneal administration

At the initial time of observation, 0.5 h, tetracycline was detected in serum, muscle and liver being 1.04 ± 0.44 mcg/ml. 0.47 ± 0.19 mcg/gm and 8.72 ± 1.50 mcg/gm respectively. The highest levels of tetracycline in serum, muscle and liver were detected at 9 h, 9 h and 0.5 h being 4.21 ± 1.15 mcg/ml, 2.52 ± 0.31 mcg/gm and 8.72 ± 1.50 mcg/gm, respectively. And at the terminal time of observation, 144 h, tetracycline was also detected in serum, muscle and liver, being 0.16 ± 0.11 mcg/ml, 0.20 ± 0.08 mcg/gm and 0.32 ± 0.10 mcg/gm. The detail was shown in Table 6 and Figure 3-6.

The biological half-lives of tetracycline in serum and muscle were 37.87 h and 43.58 h, repectively. The AUC_0^{α} in serum and muscle were 146.11 mcg.h/ml and 130.25 mcg.h/gm, respectively. The detail was shown in Table 9.

Table 6 Drug levels of tetracycline in serum, muscle and liver in cat-fish after the intraperitoneal administration.

time (h)	organ	tetracycline (mcg/ml or gm)						
		sample 1	sample 2	sample 3	sample 4	sample 5	means	standard deviation
0.5	serum	1.52	-	8.51 R	0.66	0.94	1.04	± 0.44
	muscle	0.57	0.56	0.14	0.58	0.52	0.47	± 0.19
	liver	8.75	8.31	10.62	6.53	9.41	8.72	± 1.50
1	serum	2.04	6.07	0.92	5.84	4.79	3.93	± 2.32
	muscle	0.68	0.78	0.32	0.47	0.29	0.51	± 0.22
	liver	6.12	8.23	4.89	5.64	5.57	6.09	± 1.27
2	serum	3.20	1.07	-	2.46	1.20	1.98	± 1.02
	muscle	0.85	0.51	1.40	1.41	1.43	1.12	± 0.42
	liver	6.31	1.34	5.69	6.70	5.29	5.07	± 2.15
4	serum	2.04	2.90	4.11	3.18	3.42	3.13	± 0.76
	muscle	1.88	2.08	2.33	2.60	2.66	2.31	± 0.33
	liver	3.92	2.15	4.13	3.18	3.63	3.40	± 0.78
6	serum	6.25	3.93	2.56	3.32	3.32	3.88	± 1.41
	muscle	2.70	2.47	1.45	2.63	2.68	2.39	± 0.53
	liver	3.35	3.75	3.25	1.71	3.31	3.07	± 0.79
9	serum	3.50	3.17	5.84	3.55	4.97	4.21	± 1.15
	muscle	2.65	2.39	2.06	2.62	2.88	2.52	± 0.31
	liver	3.94	2.50	3.69	3.70	3.54	3.47	± 0.56
12	serum	2.92	3.50	4.11	2.50	2.22	3.05	± 0.76
	muscle	2.40	2.60	2.22	1.90	1.73	2.17	± 0.36
	liver	2.46	3.33	3.47	3.61	3.03	3.18	± 0.46
24	serum	1.74	1.75	1.38	1.07	1.15	1.42	± 0.32
	muscle	1.12	1.62	1.20	1.30	1.50	1.35	± 0.21
	liver	0.60	0.80	0.62	1.10	0.84	0.79	± 0.20
48	serum	1.10	0.80	0.94	0.94	0.69	0.89	± 0.16
	muscle	0.78	0.83	1.12	0.78	0.72	0.85	± 0.16
	liver	0.84	0.84	0.82	1.02	0.76	0.86	± 0.10
72	serum	0.33	0.75	0.94	0.84	0.72	0.72	± 0.23
	muscle	0.46	0.71	0.79	0.70	0.67	0.67	± 0.12
	liver	0.43	0.58	0.68	0.90	0.61	0.64	± 0.17
96	serum	0.42	0.32	0.33	0.34	0.56	0.39	± 0.10
	muscle	0.41	0.44	0.62	0.38	0.49	0.47	± 0.09
	liver	0.37	0.84	0.40	0.38	0.34	0.47	± 0.21
120	serum	0.32	0.22	0.14	0.23	0.33	0.25	± 0.08
	muscle	0.36	0.29	0.14	0.26	0.32	0.27	± 0.08
	liver	0.18	0.30	0.13	0.29	0.30	0.24	± 0.08
144	serum	0.18	0.31	0.16	0.16	-ve	0.16	± 0.11
	muscle	0.24	0.29	0.18	0.20	0.08	0.20	± 0.08
	liver	0.43	0.29	0.31	0.38	0.17	0.32	± 0.10

R = rejected value

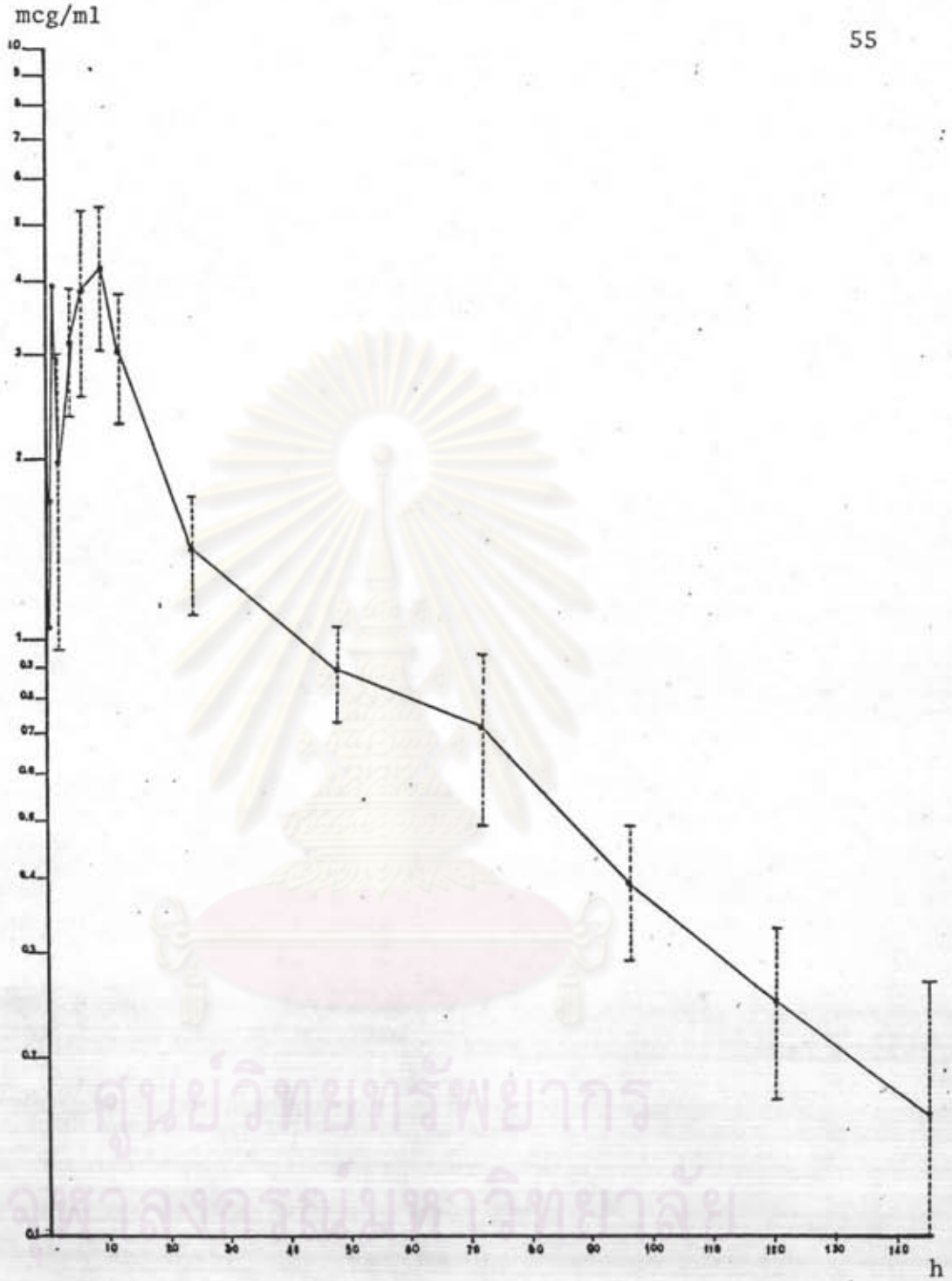

Figure 3 The tetracycline levels (mean ± S.D) in serum after the intraperitoneal administration (Each value was estimated from five fish except at h 0.5 (n = 3) and h 2 (n = 4))

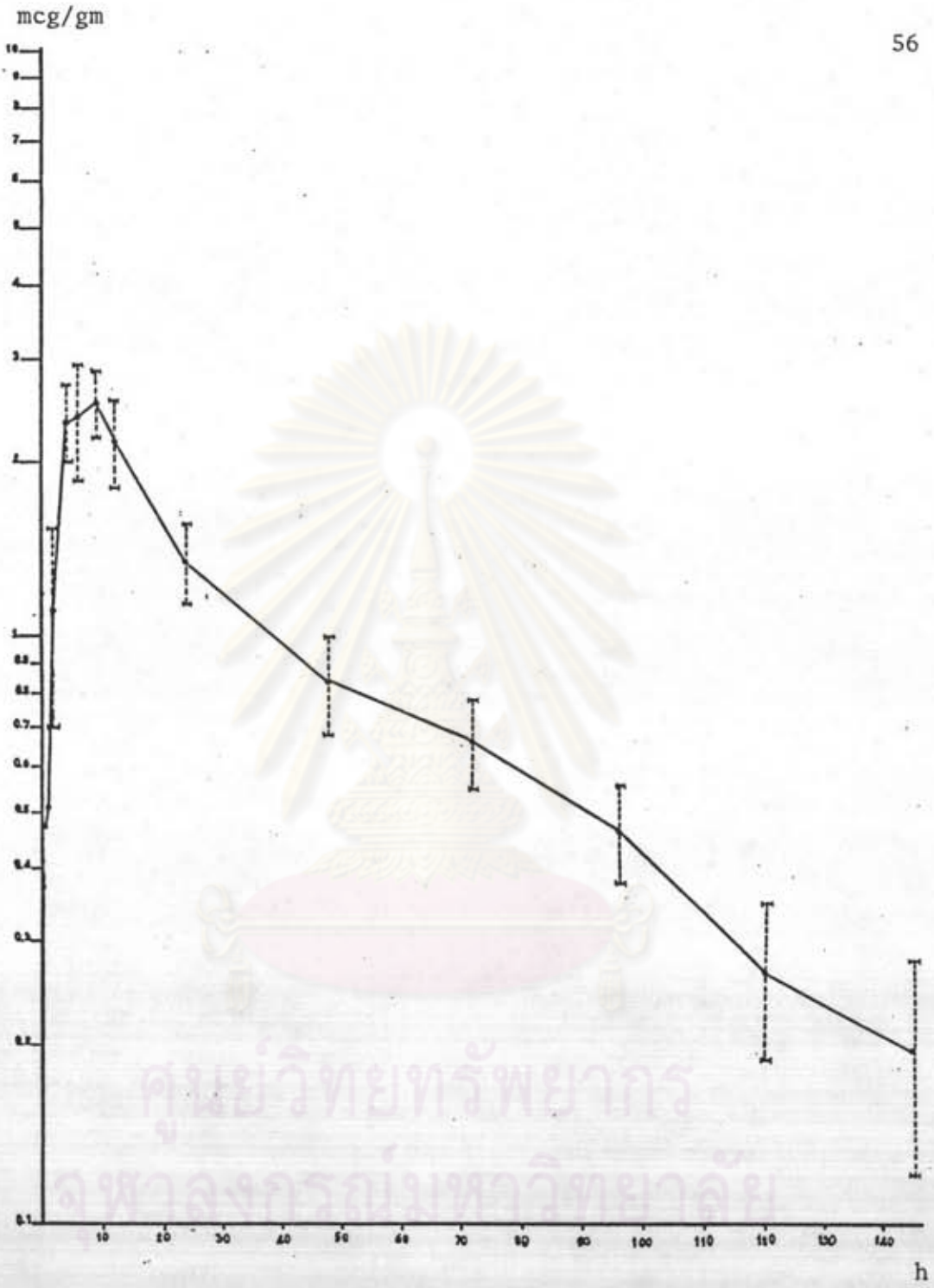

Figure 4 The tetracycline levels (mean ± S.D) in muscle after the intraperitoneal administration (Each value was estimated from five fish)

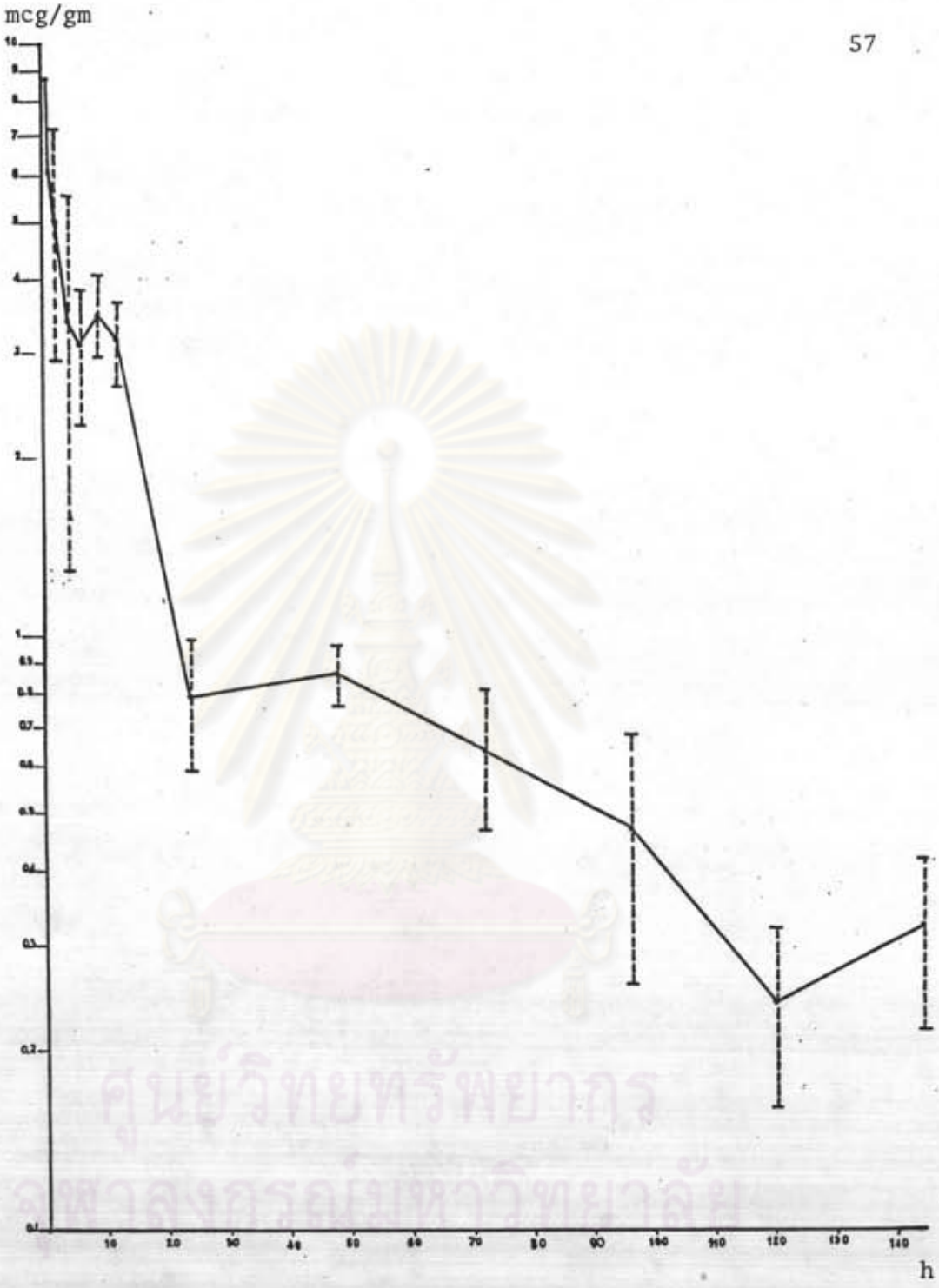

Figure 5 The tetracycline levels (mean ± S.D) in liver after the intraperitoneal administration (Each value was estimated from five fish)

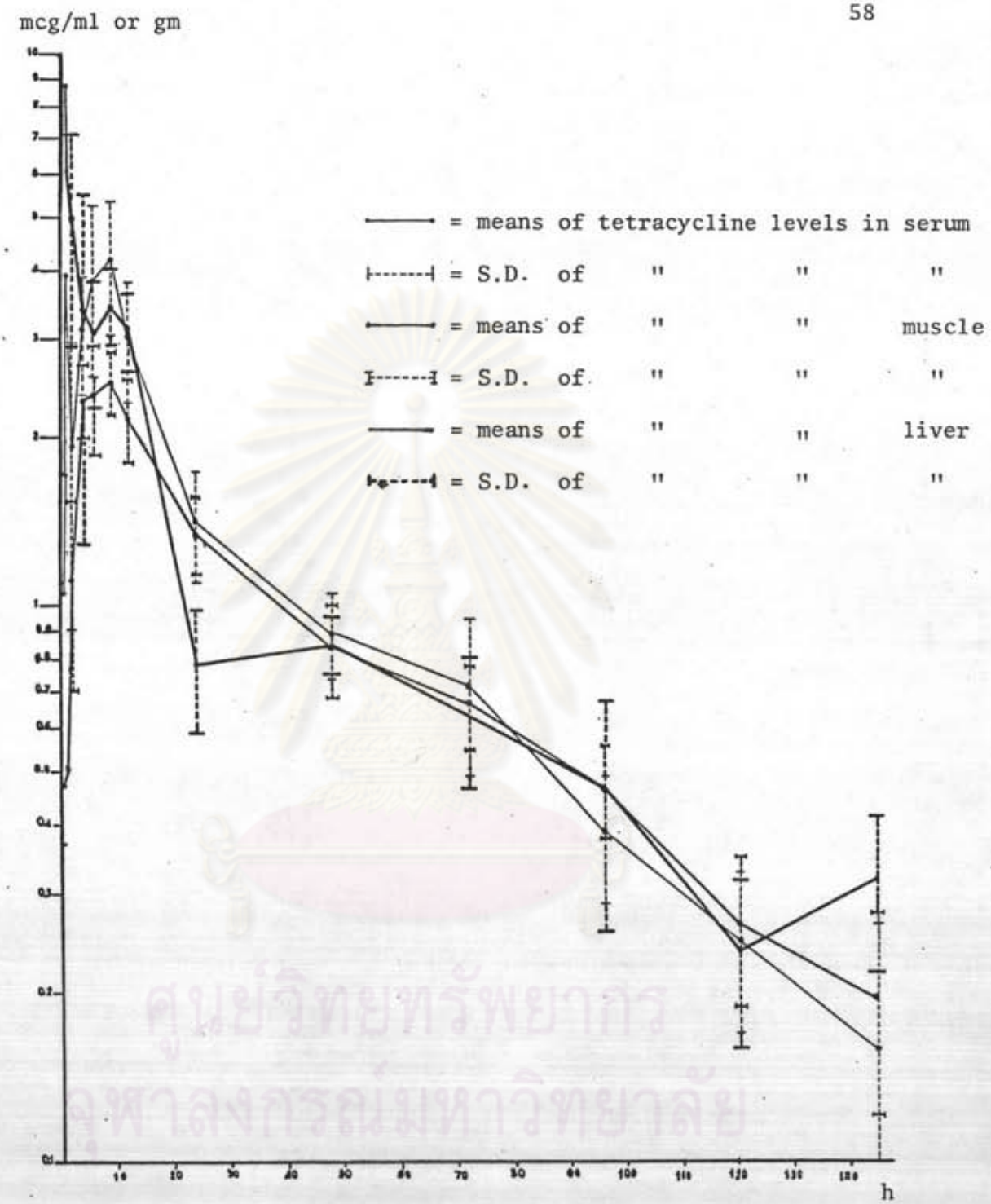

Figure 6 The tetracycline levels (mean ± S.D) in serum, muscle and liver after the intraperitoneal administration

Intramuscular administration

At the initial time of observation, 0.5 h, tetracycline was detected in serum, muscle and liver being 1.72 ± 0.30 mcg/ml, 0.51 ± 0.13 mcg/gm and 1.40 ± 0.26 mcg/gm, repectively. The highest levels of tetracycline in serum,muscle and liver were detected at 6 h, 6 h and 9 h being 2.34 ± 0.58 mcg/ml, 1.78 ± 0.23 mcg/gm and 2.46 ± 0.78 mcg/gm, respectively. And at the terminal time of observation, 120 h, tetracycline was also detected in serum, muscle and liver being 0.16 ±0.06 mcg/ml, 0.18 ± 0.07 mcg/gm and 0.18 ± 0.08 mcg/gm, respectively. The detail was shown in Table 7 and Figure 7-10.

The biological half-lives of tetracycline in serum and muscle were 33.16 and 32.69 h respectively. The AUC_0^{α}s in serum and muscle were 97.52 mcg.h/ml and 90.13 mcg.h/gm, respectively. The detail was shown in Table 9.

Table 7 Drug levels of tetracycline in serum, muscle and liver in cat-fish after the intramuscular administration.

time (h)	organ	Tetracycline (mcg/ml or gm)						
		sample 1	sample 2	sample 3	sample 4	sample 5	means	standard deviation
0.5	serum	1.23	1.74	1.75	2.02	1.86	1.72	± 0.30
	muscle	0.62	0.49	0.35	0.65	0.43	0.51	± 0.13
	liver	1.03	1.58	1.41	1.60	-	1.40	± 0.26
1	serum	1.62	3.12	2.66	2.18	1.32	2.18	± 0.74
	muscle	0.83	0.74	0.67	0.99	0.63	0.77	± 0.14
	liver	1.29	1.41	1.55	1.01	0.96	1.24	± 0.25
2	serum	1.56	2.13	1.94	2.52	3.25	2.28	± 0.64
	muscle	0.79	1.17	0.91	1.13	2.90	1.38	± 0.86
	liver	1.23	0.97	0.94	1.13	1.93	1.24	± 0.40
4	serum	2.34	2.19	1.62	1.81	2.58	2.11	± 0.39
	muscle	1.57	1.41	1.35	1.34	1.74	1.48	± 0.17
	liver	1.26	1.63	1.20	1.14	2.10	1.47	± 0.40
6	serum	2.57	2.04	1.49	2.69	2.92	2.34	± 0.58
	muscle	1.88	1.76	1.42	1.82	2.04	1.78	± 0.23
	liver	2.41	1.25	1.23	1.92	3.70	2.10	± 1.02
9	serum	1.69	1.91	1.58	2.30	3.13	2.12	± 0.63
	muscle	1.73	2.01	1.58	1.71	1.99	1.80	± 0.19
	liver	2.33	3.25	1.28	3.08	2.36	2.46	± 0.78
12	serum	2.44	1.80	2.87	2.55	1.97	2.33	± 0.44
	muscle	1.17	1.55	1.83	2.03	1.75	1.67	± 0.33
	liver	2.43	1.84	1.27	2.60	2.47	2.12	± 0.56
24	serum	1.26	0.94	0.94	1.00	0.95	1.02	± 0.14
	muscle	1.28	-	1.30	1.17	1.12	1.22	± 0.09
	liver	0.98	0.90	0.62	1.14	0.56	0.84	± 0.24
48	serum	0.71	0.80	0.54	0.76	0.89	0.74	± 0.13
	muscle	0.80	0.60	0.53	0.67	0.93	0.71	± 0.16
	liver	-	1.53	1.00	1.41	1.55	1.37	± 0.26
72	serum	0.16	0.40	0.42	0.20	-	0.30	± 0.13
	muscle	0.20	0.36	0.41	0.26	-	0.31	± 0.10
	liver	0.14	0.63	0.34	0.23	-	0.34	± 0.21
96	serum	0.46	0.11	0.14	0.10	-	0.20	± 0.17
	muscle	0.41	0.15	0.13	0.13	-	0.20	± 0.14
	liver	0.49	0.12	0.13	0.10	-	0.21	± 0.19
120	serum	0.11	0.20	-	-	-	0.16	± 0.06
	muscle	0.13	0.23	-	-	-	0.18	± 0.07
	liver	0.12	0.23	-	-	-	0.18	± 0.08

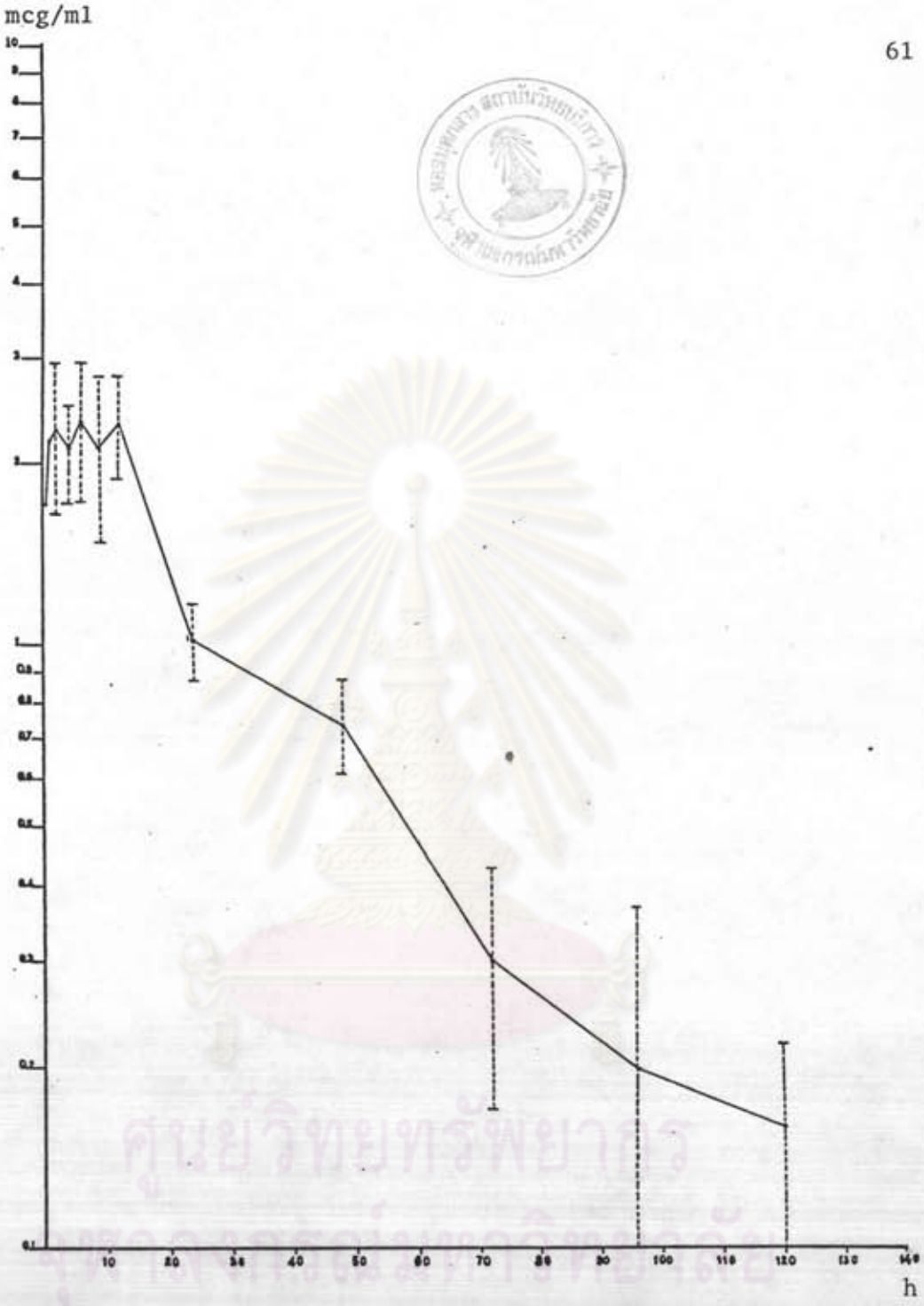

Figure 7 The tetracycline levels (mean ± S.D) in serum after the intramuscular administration (Each value was estimated from five fish except at h 72 (n = 4), h 96 (n = 4) and h 120 (n = 2))

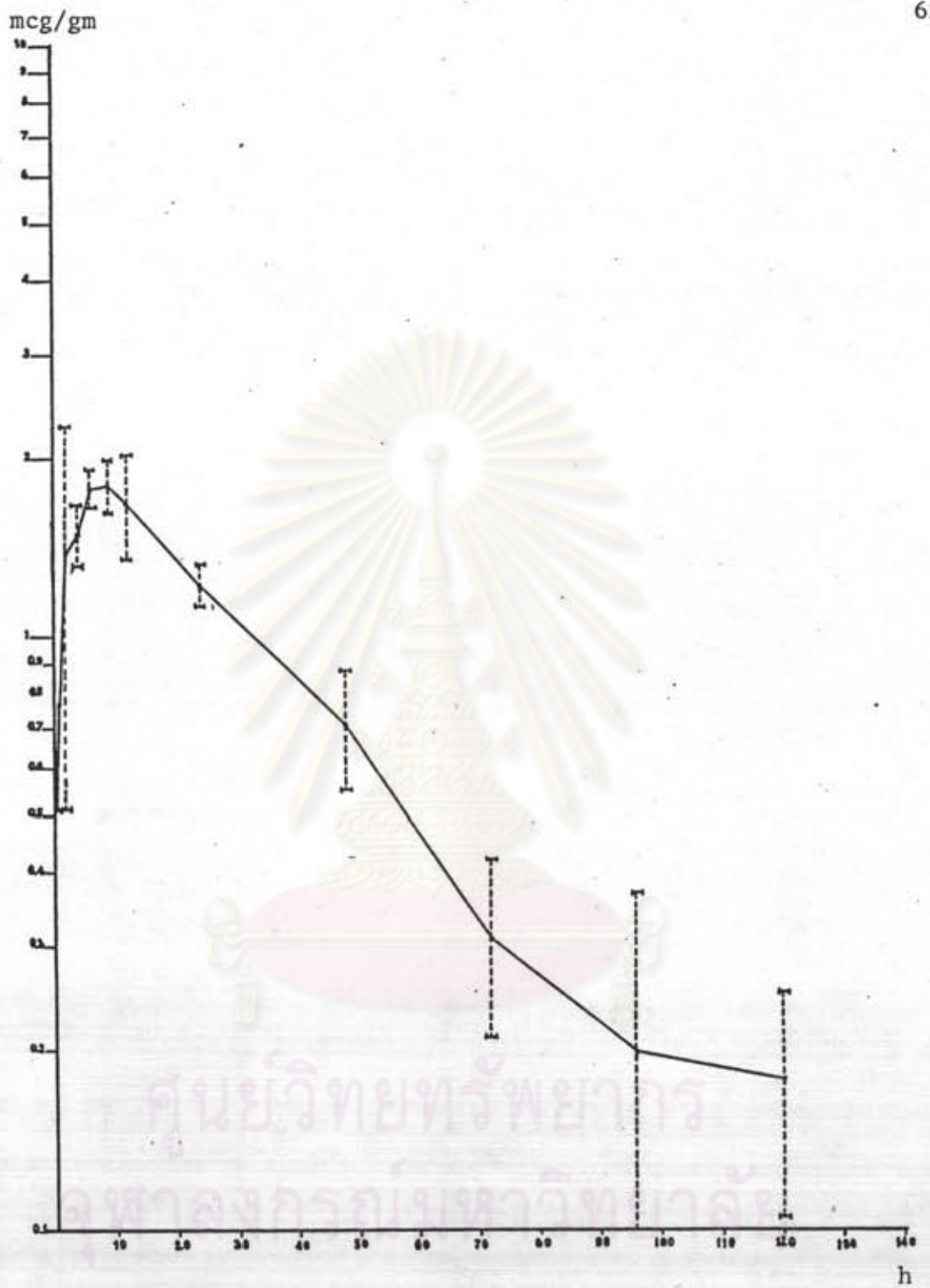

Figure 8 The tetracycline levels (mean ± S.D) in muscle after the intramuscular administration (Each value was estimated from five fish except at h 72 (n = 4), h 96 (n = 4) and h 120 (n = 2))

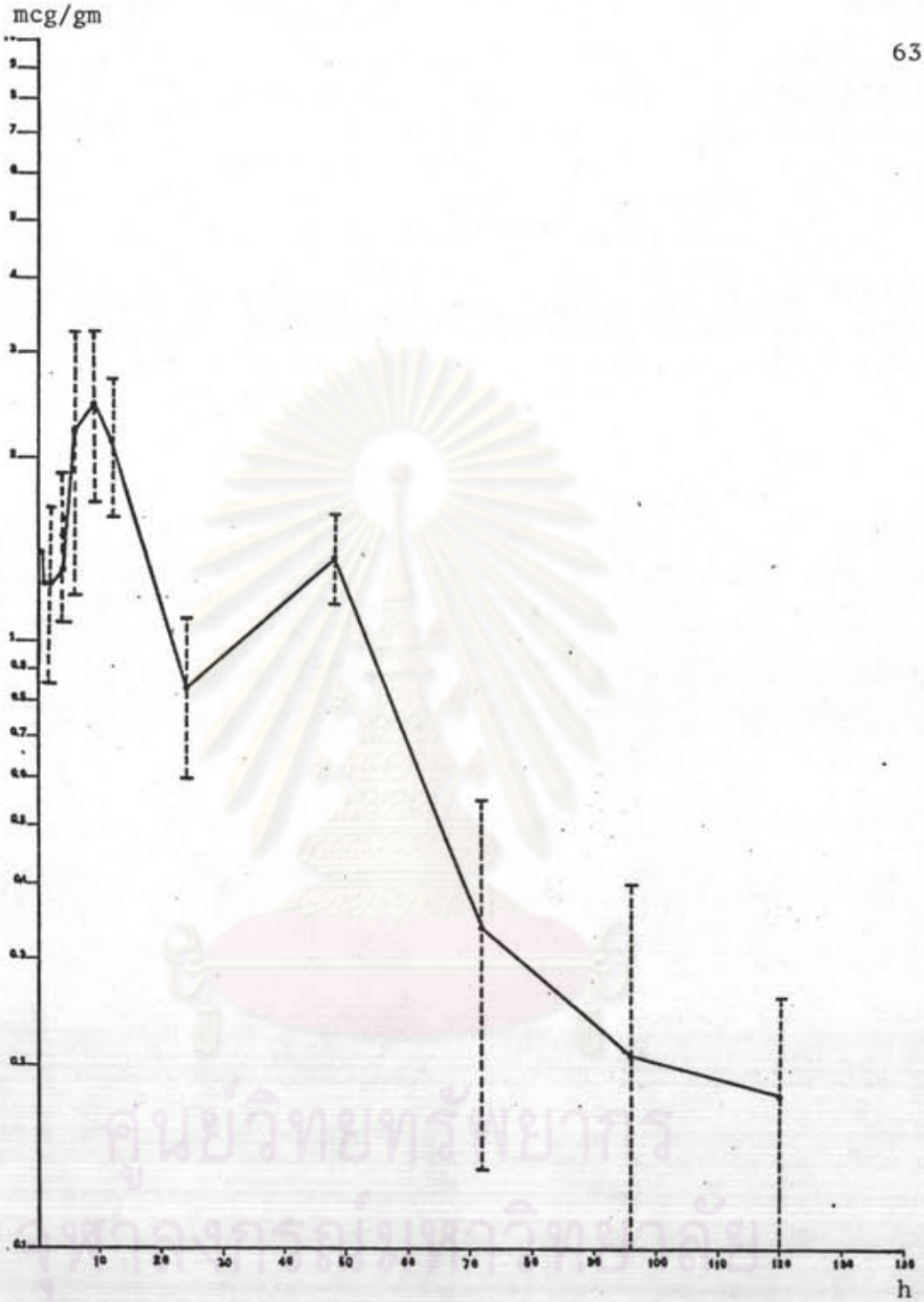

Figure 9 The tetracycline levels (mean ± S.D) in liver after the intramuscular administration (Each value was estimated from five fish except at h 0.5 (n = 4), h 72 (n = 4), h 96 (n = 4) and h 120 (n = 2))

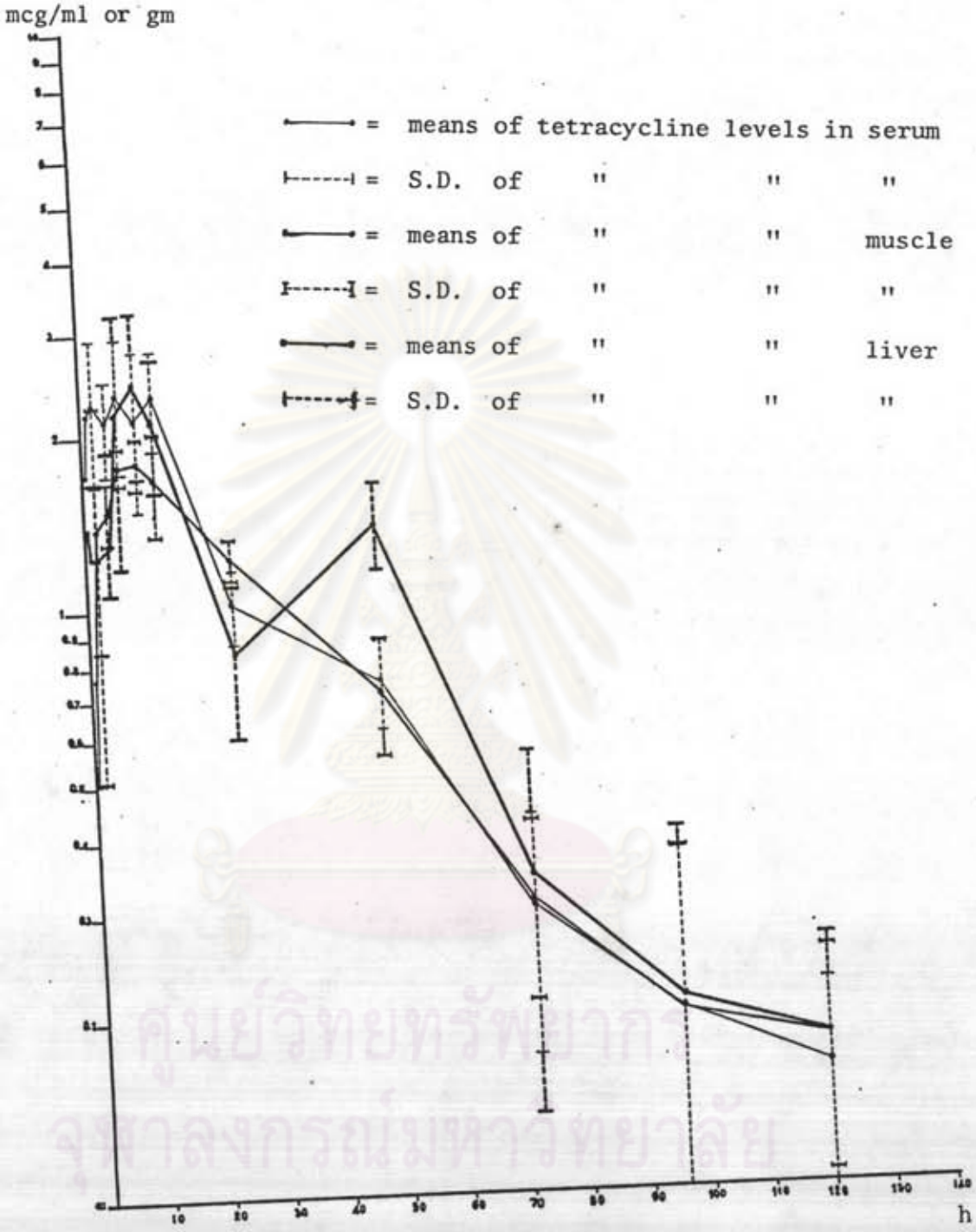

Figure 10 The tetracycline levels (mean ± S.D.) in serum, muscle and liver after the intramuscular administration.

Oral administration

At the initial time of observation, 0.5 h, tetracycline was detected in serum, muscle and liver being 0.26 ± 0.06 mcg/ml, 0.34 ± 0.16 mcg/gm and 2.08 ± 1.57 mcg/gm, respectively. The highest levels of tetracycline in serum, muscle and liver were detected at 2h, 6h and 2h being 1.28 ± 0.63 mcg/ml, 1.77 ± 0.90 mcg/gm and 2.93 ± 1.29 mcg/gm, respectively. And at the terminal time of observation, 96h, tetracycline were also detected in serum, muscle and liver being 0.19 ± 0.04 mcg/ml, 0.17 ± 0.03 mcg/gm and 0.15 ± 0.02 mcg/gm, respectively. The detail was shown in Table 8 and Figure 11-14.

The biological half-lives of tetracycline in serum and muscle were 28.28 and 24.57 h respectively. The AUC_o^{α}s in serum and muscle were 67.14 mcg.h/ml and 72.32 mcg.h/gm, respectively. The detail was shown in Table 9.

Table 8 Drug levels of tetracycline in serum, muscle and liver in cat-fish after the oral administration.

time (h)	organ	Tetracycline (mcg/ml or gm)						
		sample 1	sample 2	sample 3	sample 4	sample 5	means	standard deviation
...	serum	0.26	0.21	0.36	0.22	0.23	0.26	± 0.06
0.5	muscle	0.18	0.20	0.58	0.32	0.41	0.34	± 0.16
	liver	0.29	4.38	1.63	1.26	2.82	2.08	± 1.57
	serum	0.62	0.44	0.65	0.65	0.79	0.63	± 0.13
1	muscle	0.30	1.35	0.39	0.86	0.68	0.72	± 0.42
	liver	0,64	0.87	3.53	0.59	7.92	2.71	± 3.16
	serum	-	1.01	0.67	2.15	1.29	1.28	± 0.63
2	muscle	0.70	0.76	0.44	0.74	1.23	0.77	± 0.28
	liver	4.68	2.78	1.09	3.30	2.80	2.93	± 1.29
	serum	1.21	0.81	0.70	1.07	-	0.95	± 0.23
4	muscle	1.07	0.59	0.68	0.77	-	0.78	± 0.21
	liver	1.40	0.67	1.41	1.17	-	1.16	± 0.35
	serum	1.39	1.49	1.49	1.24	0.32	1.19	± 0.49
6	muscle	1.74	2.62	2.27	1.92	0.28	1.77	± 0.90
	liver	1.95	4.39	1.77	1.46	0.43	2.00	± 1.46
	serum	0.78	0.44	0.34	0.57	2.18	0.86	± 0.75
9	muscle	0.72	0.58	0.37	0.75	3.38	1.16	± 1.25
	liver	5.29	0.75	0.97	0.68	4.00	2.34	± 2.16
	serum	2.04	0.89	0.62	1.26	0.50	1.06	± 0.62
12	muslce	3.58	1.53	0.83	1.17	0.50	1.52	± 1.21
	liver	3.65	0.72	0.65	1.76	0.62	1.48	± 1.30
	serum	1.26	1.05	0.60	0.82	1.74	1.09	± 0.44
24	muscle	1.10	1.10	0.95	1.87	1.60	1.32	± 0.39
	liver	0.90	1.13	0.75	0.68	2.80	1.25	± 0.88
	serum	0.46	0.72	0.56	0.46	10.80[R]	0.55	± 0.12
48	muscle	0.49	0.49	0.51	0.41	9.24[R]	0.48	± 0.04
	liver	0.95	1.60	0.91	1.07	1.36[R]	1.13	± 0.32
	serum	0.23	0.35	0.34	0.24	-	0.29	± 0.06
72	muscle	0.19	0.41	0.28	0.15	-	0.26	± 0.12
	liver	0.20	1.20	0.66	0.20	-	0.56	± 0.48
	serum	0.17	0.25	0.15	0.20	-	0.19	± 0.04
96	muscle	0.16	0.21	0.16	0.15	-	0.17	± 0.03
	liver	0.17	0.14	0.13	0.17	-	0.15	± 0.02

R = rejected value

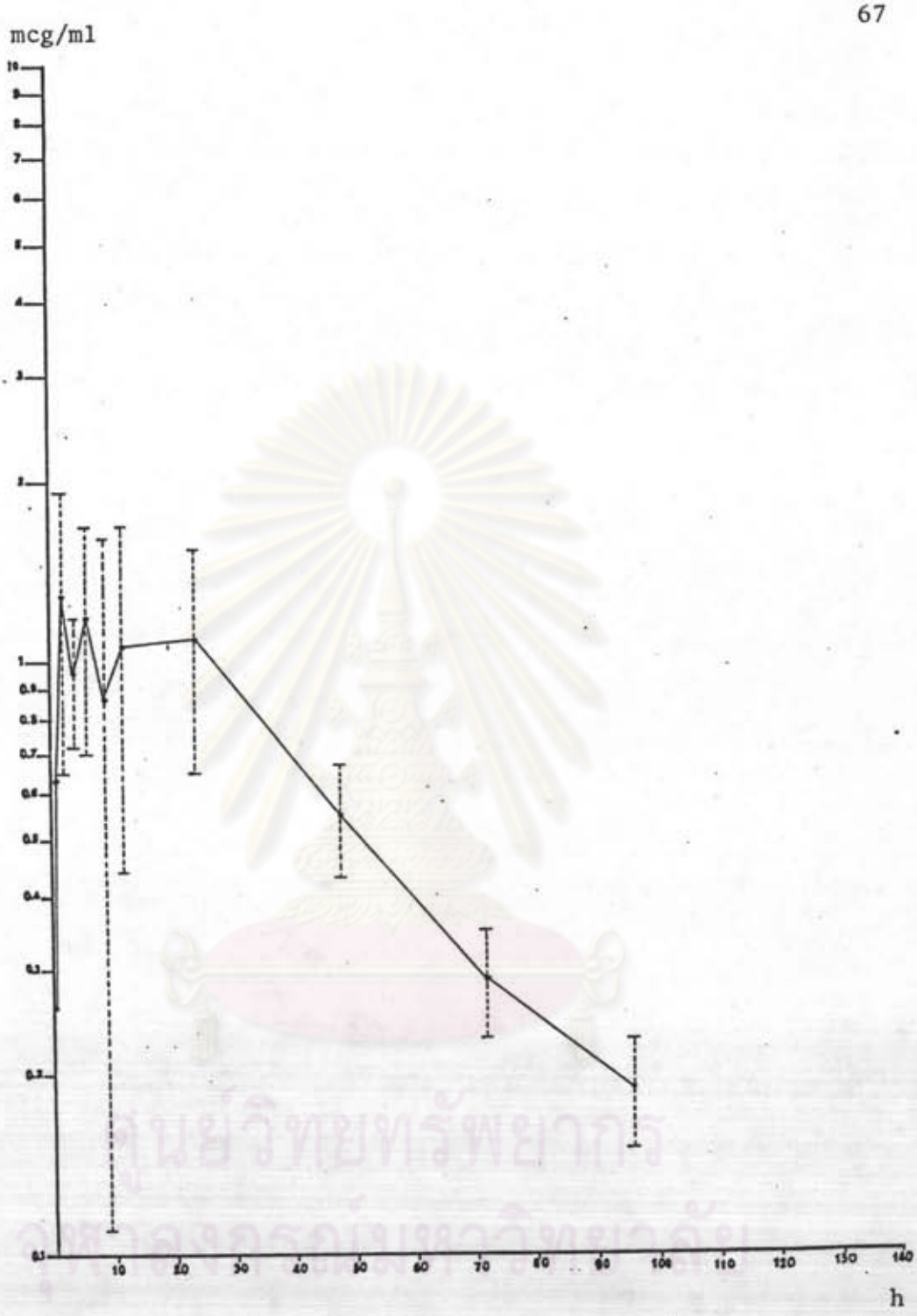

Figure 11 The tetracycline levels (mean ± S.D) in serum after the oral administration (Each value was estimated from five fish except at h 2 (n = 4), h 4 (n = 4), h 48 (n = 4), h 72 (n = 4) and h 96 (n = 4)).

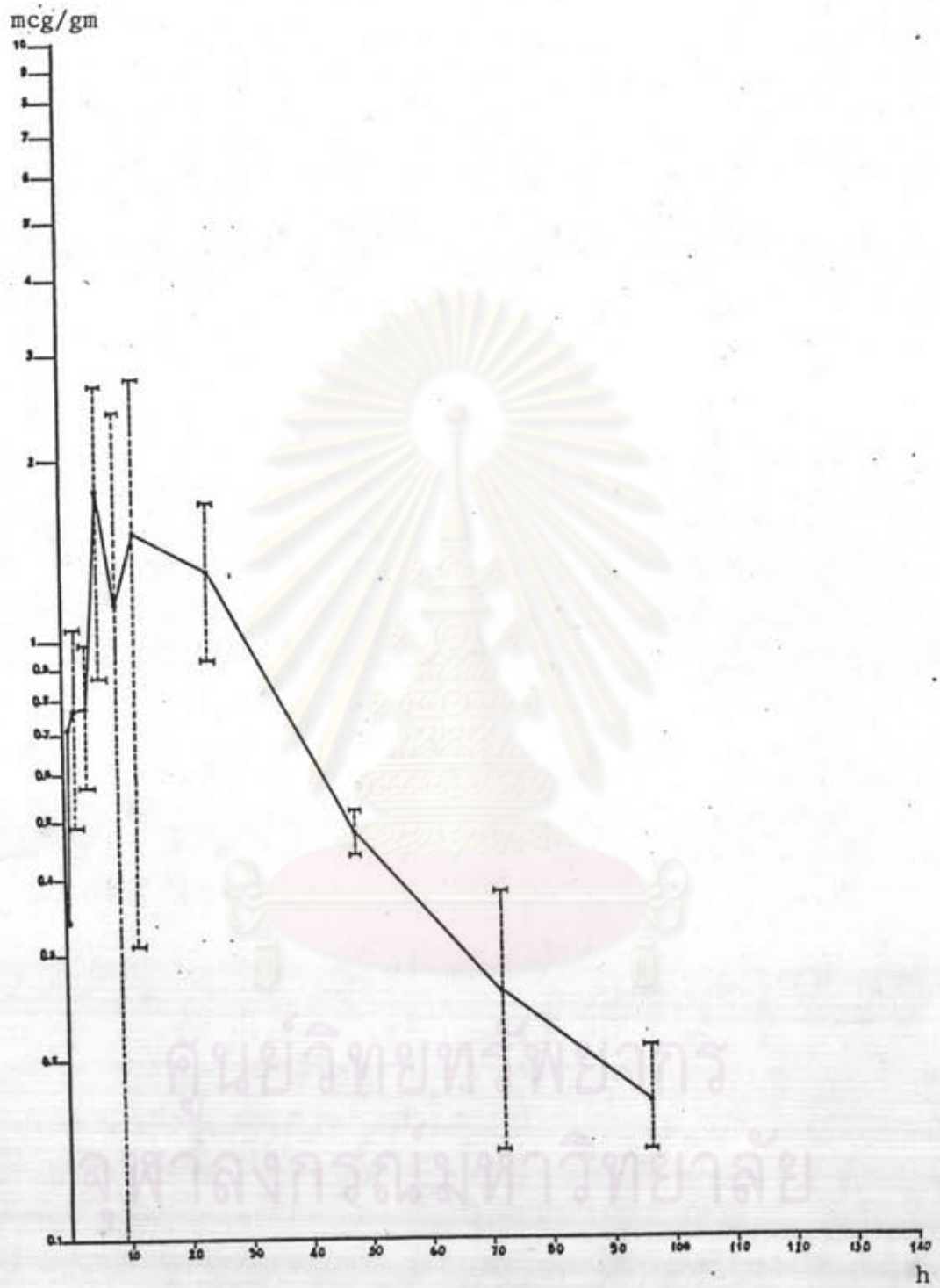

Figure 12 The tetracycline levels (mean ± S.D) in muscle after the oral administration (Each value was estimated from five fish except at h 4 (n = 4), h 48 (n = 4), h 72 (n = 4) and h 96 (n = 4))

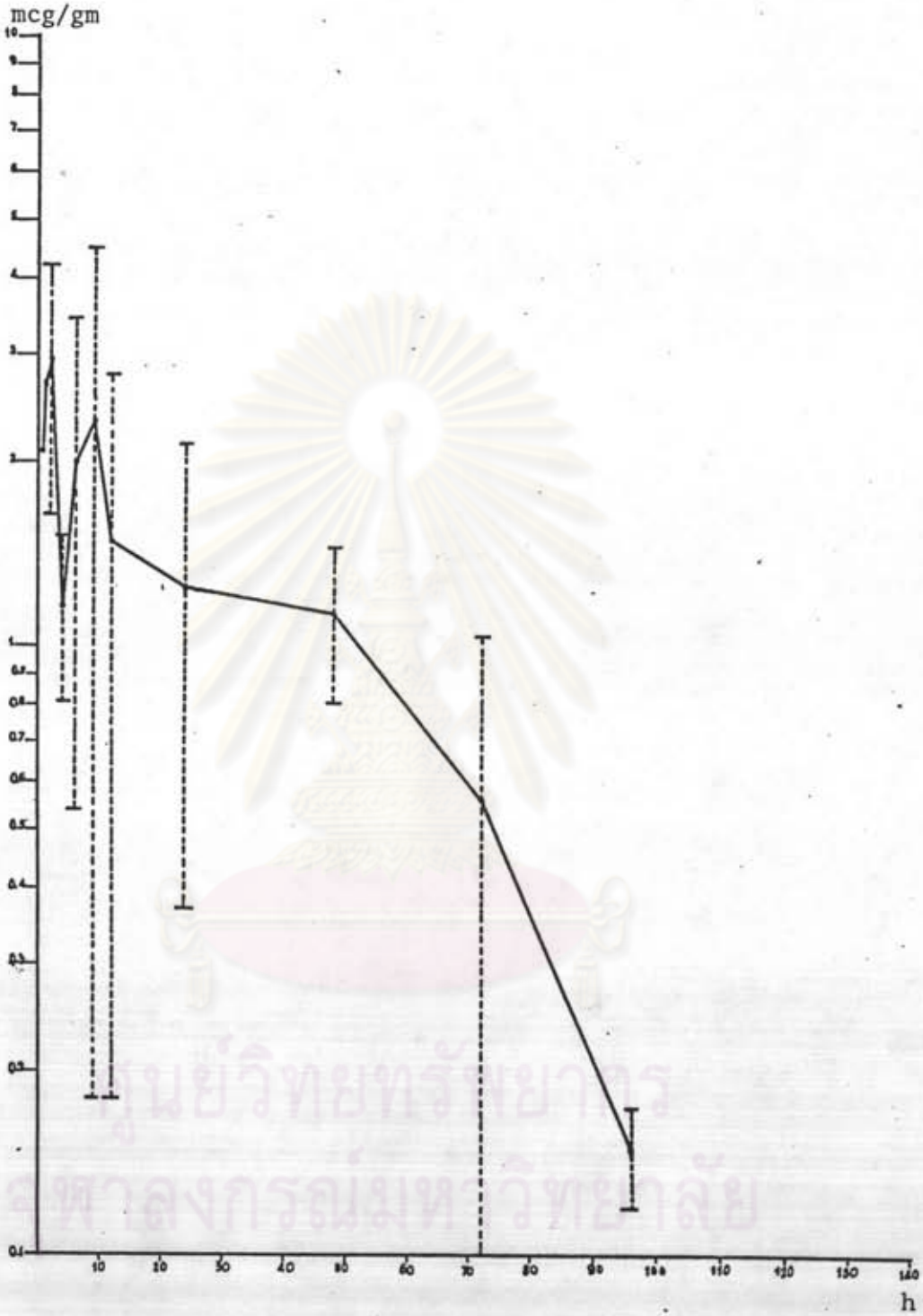

Figure 13 The tetracycline levels (mean ± S.D) in liver after the oral administration (Each value was estimated from five fish except at h 4 (n =4), h 48 (n = 4), h 72 (n = 4) and h 96 (n = 4))

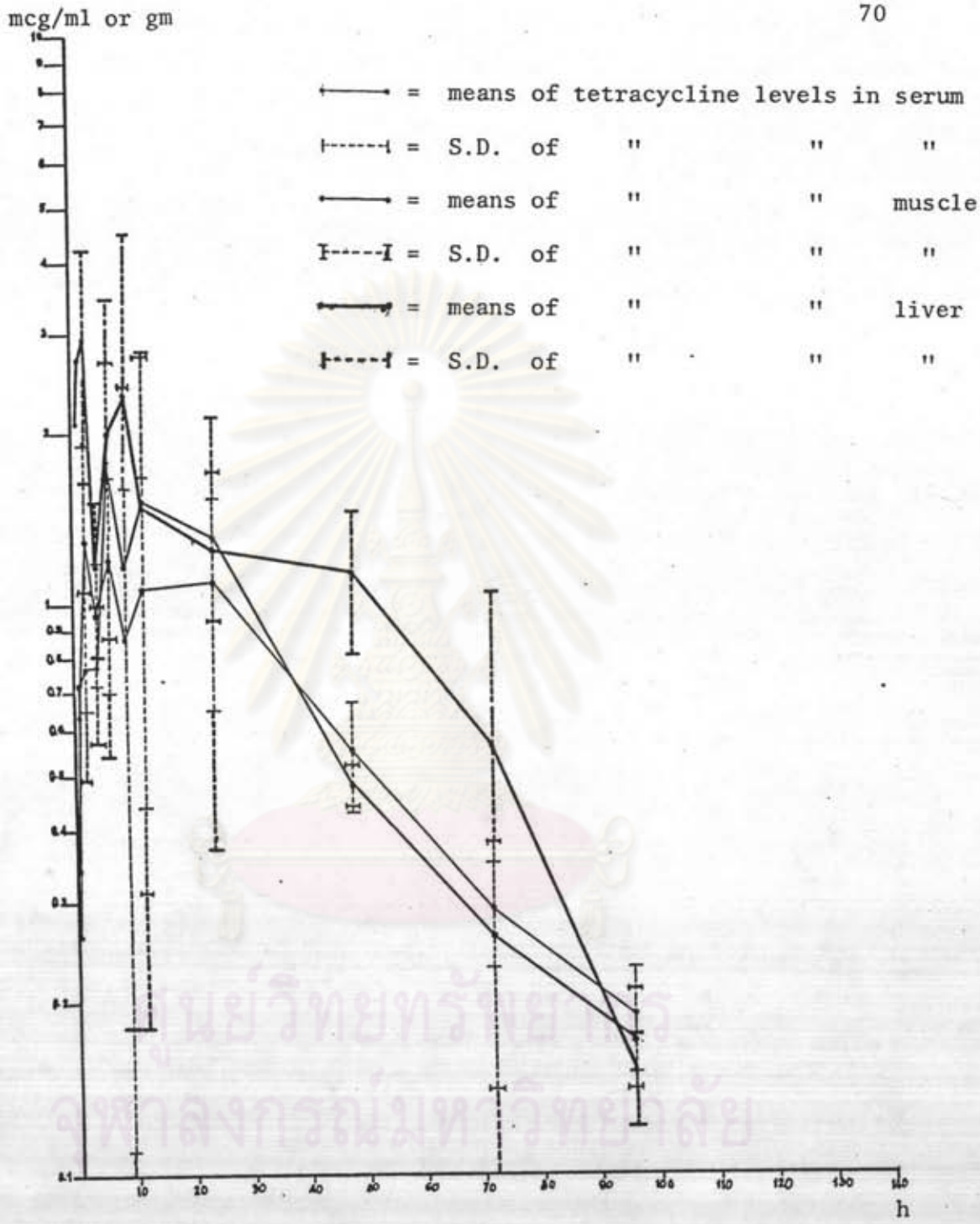

Figure 14 The tetracycline levels (mean ± S.D) in serum, muscle and liver after the oral administration .

Table 9 The biological half-lives ($t_{\frac{1}{2}}$) and AUC_0^{α} mcg.h/ml or gm after the IP,IM and oral administration. (AUC = area under concentration-time curve)

Route of Administration	Dose	$t_{\frac{1}{2}}$(h)		AUC_0^{α} mcg.h/ml or gm	
		in serum	in muscle	in serum	in muscle
Intraperitoneal	5 mg/kg catfish body weight	37.87	43.58	146.11	130.25
Intramuscular	5 mg/kg catfish body weight	33.16	32.69	97.52	90.13
Oral	50 mg/kg catfish body weight	28.28	24.57	67.14	72.32
	average	33.10 ± 4.80	33.61 ± 9.54		

Result Conclusion of the Pharmacokinetic Study

In general, it could be observed that after the 3 routes of administration, tetracycline was absorbed in to blood circulation and the tissues rapidly. The drug was detected within 0,5 h, sustained at high levels (more than about 1 mcg/ml or gm) until 24 h in serum, muscle and liver and then, after 24 h, the drug levels in serum and muscle were decreased. Within 96, 120 h and 144 h the drug levels in serum, muscle and liver of the oral, intramuscular and intraperitoneal administration were also detected, respectively. It was noticable that from 24 h to 48 h the drug levels in liver after the intraperitoneal and intramuscular administration were increased, by contraries, after the oral administration the drug levels were decreased, however the rate of decreasing was very slowly. In addition, from 120 h to 144 h, the drug levels in liver after the intraperitoneal administration were increased.

After the oral administration and intraperitoneal administration (about 0.5-2 h), the drug levels in liver were higher than in serum and muscle, by contraries, after the intramuscular administration, the drug levels in serum were the highest.

The biological half-lives of tetracycline averaged from the three routes of administration were 33.1 ± 4.80 h in serum and 33.61 ± 9.54 h in muscle (Table 9).

The AUC_0^{α}s (mcg.h/mlor gm) in serum and muscle after the 3 routes of administration were calculated (Table 9). The AUC_0^{α}s in serum and muscle of each administration were somewhat different, but the AUC_0^{α}s from the three routes of administration were distinctly different. The AUC_0^{α}s

from intramuscular administration were smaller than from intraperitoneal administration despite their equivalent doses. The AUC_o^αs from oral administration were the smallest in spite of the largest dose. The larger AUC_o^α implies the better absorption.

2. Determination of the minimal inhibitory concentrations (MICs) of tetracycline to 57 strains of A. hydrophila

It was found that the MICs of tetracycline to strains of A. hydrophila isolated from catfish were 0.5, 4.0, 32.0 and 128.0 IU/ml being 3.51%, 3.51%, 5.26% and 1.75% respectively. The MICs for the strains from shake-head fish were 0.5, 1.0, 4.0, 64.0 and 128.0 IU/ml being 17.54%, 5.26%, 3.51%, 5.26% and 17.54%, respectively. The MICs for the strains from environmental sources (water and soil in fish culturing ponds) were 0.5, 4.0, 128.0 IU/ml being 5.26, 3.51 and 5.26%, respectively. All strains from humans and JCM having the MIC, 0.5 IU/ml being 17.54 and 5.26%, respectively. In addition, the tetracycline resistant strains were found in catfish (50%), snake-head fish (46.42%), environmental sources (37.5 %), humans (0%) and JCM (0%). The detail was shown in Table 10-11.

Table 10 The Minimal Inhibitory Concentration (MIC) of tetracycline HCl to 57 strains of Aeromonas hydrophila.

A. hydrophila strains	Sources	Donors	MIC (IU/ml)
F 181	catfish	Div.of Microbiol.,Vet.Sc,Chula.Univers.	0.5
F 325	"	"	0.5
F 162	"	"	4.0
F 189	"	"	4.0
F 207	"	"	32.0
F 295	"	"	32.0
F 400	"	"	32.0
FK 297	"	"	128.0
F 510	snake-head fish	"	0.5
F 542	"	"	0.5
F 551	"	"	0.5
F 3004	"	"	0.5
F 3012	"	"	0.5
F 3246	"	"	0.5
F 3296	"	"	0.5
F 3298	"	"	0.5
F 3313	"	"	0.5
FK 432	"	"	0.5
F 532	"	"	1.0
FK 287	"	"	1.0
FK 514	"	"	1.0
F 3038	"	"	4.0
F 3076	"	"	4.0
FK 278	"	"	64.0

Table 10 The Minimal Inhibitory Concentration (MIC) of tetracycline HCl to 57 strains of Aeromonas hydrophila. (continued)

A. hydrophila strains	Sources	Donors	MIC (IU/ml)
FK 293	snake head fish	Div. of Microbiol.,Vet.Sc,Chula Univers	4.0
FK 559	"	"	4.0
FK 588	"	"	128.0
F 3051	"	"	128.0
FK 12	"	"	128.0
FK 14	"	"	128.0
FK 18	"	"	128.0
FK 31	"	"	128.0
FK 276	"	"	128.0
FK 304	"	"	128.0
FK 337	"	"	128.0
FK 351	"	"	128.0
FK 89	water	"	0.5
FK 105	"	"	0.5
FK 368	"	"	0.5
FK 51	"	"	4.0
FK 361	"	"	4.0
FK 65	"	"	128.0
FK 152	"	"	128.0
FK 363.1	soil	"	128.0
1	humans	Dr. Somanee, Mahidol University	0.5
2	"	"	0.5
25	"	"	0.5

Table 10 The Minimal Inhibitory Concentration (MIC) of tetracycline HCl to 57 strains of Aeromonas hydrophila. (continued)

A. hydrophila strains	Sources	Donors	MIC (IU/ml)
29	humans	Dr.Sommanee,Mahidol University	0.5
32	"	"	0.5
33	"	"	0.5
36	"	"	0.5
44	"	"	0.5
52	"	"	0.5
55	"	"	0.5
ATCC 7966	-	JCM	0.5
JCM 1027	-	"	0.5
JCM 2359	-	"	0.5

Table 11 The resistant A. hydrophila strains derived from the interpretation of disc susceptibility test (A resistant strain has ≥ 12 mcg/ml tetracycline MIC)[47]

Isolated sources	% tetracycline resistant strains (as calculated from the total strains of each group)
Catfish	50
Snake-head fish	46.42
Environment	37.5
Humans	0
JCM	0

3. Prophylaxis testing of tetracycline against A. hydrophila infection in catfish

After a single dose (5 mg tetracycline HCl/kg catfish body weight) of intraperitoneal administration, each catfish was challenged with 10^9 viable cells of A. hydrophila F181. It was found that tetracycline could prevent the infection 100% within 3 days. At the d4, d6 and d7, the percentage of protection was decreased to 80 , 0 and 0 respectively. The detail of the experiment was shown in Table 12.

The correlation of drug levels in serum and muscle with the percentage of protection was shown in Figure 15.

Table 12 The prophylaxis testing of tetracycline against Aeromonas hydrophila infection in catfish (clarias batrachus).

Time after IP administration	A. hydrophila F 181 viable cells/ml	catfish in each group	Catfish weight (mean ± S.D.)		Ulcerative development				Percentage of protection
					Control group		Treated group		
			Control group	Treated group	Ulcer (+ ve)	No ulcer (- ve)	Ulcer (+ ve)	No ulcer (- ve)	
6 h	0.98×10^9	10	200.2 ± 28.8	181.5 ± 38.7	9	1[a]	-	10	100
1 d	1.32×10^9	10	174.2 ± 46.2	156.6 ± 39.6	10	-	-	10	100
2 d	3.32×10^9	5	120.0 ± 26.2	125.2 ± 12.8	5	-	-	5	100
3 d	2.97×10^9	5	149.2 ± 25.4	145.2 ± 19.8	5	-	-	5	100
4 d	0.57×10^9	5	137.0 ± 27.0	123.0 ± 30.6	5	-	1	4	80
5 d	-	-	-	-	-	-	-	-	-
6 d	4.10×10^9	5	115.4 ± 11.4	150.4 ± 18.4	5	-	5	-	0
7 d	1.18×10^9	5	161.5 ± 62.8	155.6 ± 14.8	5	-	5	-	0

[a] Even though the catfish was repeatedly challenged with about 10^9 viable cells of the bacterial suspension after 24 h of the first challenge, there was no ulcerative development.

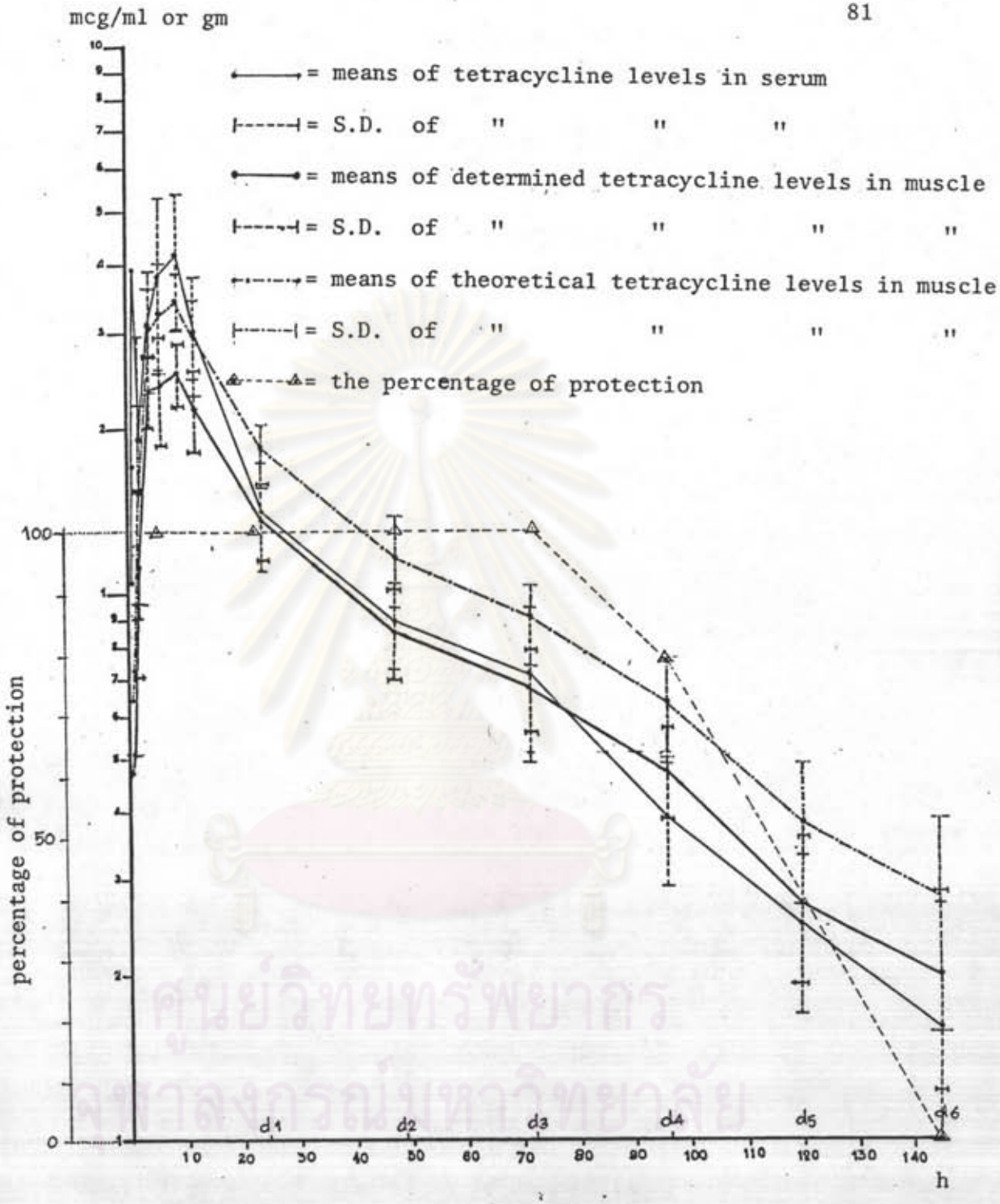

Figure 15 The tetracycline levels (mean ± S.D) in serum and muscle after the intraperitoneal administration, and the percentage of protection of the catfish to A. hydrophila F181 infection.