CHAPTER III

Results

3.1 The patient population

One hundred and thirty one patients were initially included in this study according to the inclusion criteria listed in materials and methods. All were biten by suspected rabid animals such as dogs, cats, etc and needed to receive rabids vaccine and rabids immunoglobulin according to the latest recommendation of Thai Red Cross Soeicty and World Health Organization(26,35). 77 were males and 54 were females. Their ages ranged from 15 to 60 with the mean age of 31.15 $\pm$ 11.6

However, only 102 patients (77.86 %) come for schedule follow-up which sequential serum specimens were obtained. Therefore, only these patients were included in subsequent serologic and immunologic analysis.

Additional 16 patients with clinical diagnosis of serum sickness were also included in this study as " positive controls ". Their clinical history and immunological tests were summarized in table IV,V and VI. Skin test of the Arthus type reaction was positive in all of the 10 patients tested. The reaction was best performed

with 0.1 ml ERIG diluted 1:10 and the reaction read at 6 hours. For a control group of 16 individual prior to injection of ERIG, none was positive. In addition, 10 individuals without serum sickness, none had a positive Arthus reaction on Day 7.

3.2 Skin test results

Because the patients had to receive ERIG (40 IU/Kg body weight) due to their finanicial restraints, immediate (Type I) skin test was performed using 0.02 ml or 3 mm bleb of ERIG diluted 1 : 10 with normal saline. According to the criteria of positive skin test reaction as deseribed in materials and methods, 108 or 82.44 % were negative, 19 or 14.5 % were positive and 4 or 3.05 % were borderlined. It was shown in table VII.

3.3 Clinical significance of immediate skin test results

Patients with positive and borderlined skin test were offered the use of HRIG instead of ERIG, but none could afford for price of HRIG. Therefore, ERIG was given

to these patients under close observation with the IV line in place. Approximately half of the ERIG dose was infiltrated around the bite site and the other half was injected intramuscularly at gluteal areas. PVRV was concomittantly given by the intradermal route according to the Thai Red Cross Society (TRCS) regimen, namely 2 of the 0.1 ml of reconstitued PVRV were given intradermally at both deltoid areas on days 0, 3, 7 and 1 injection(0.1 ml) intradermally on days 28 and 90 (35). The patients were observed for at least 4 hours before discharge and asked to report back to the investigator as soon as possible if any side-effects occured as a result of treatment.

Of the 131 patients who received ERIG, none developed immediate or delayed type I allergic reactions such as urticaria or asthma up to 48 hours after administration of ERIG irrespective of the skin test reactivity.

3.4 Patients with Serum Sickness.

Each recipient of ERIG and PVRV was questioned about any side-effects at each clinic visit, ie. days 3,7, 28 and 40. The patient was also instructed to return as soon as possible if any adverse reaction accured as stated above. Two of the 131 patients or 1.5 % developed symptoms

compatible with serum sickness.

The first patient was 24 year old female who was biten by a run-away dog at left leg one day prior to coming to TRCS Rabies clinic. Her skin test to 1:10 dilution of ERIG was negative. 8.8 ml of ERIG was given according to the split sites as described. Six days after initiation to the treatment,she developed urticaria at site of injection (left buttock) and later progressed to generalized urticaria. She also had fever, generalized urticaria and weakness. On day 7 which was the next scheduled vacci nation. She reported to the QSMI clinic.

On examination, the patient had urticarial rash on her entire body as well as over the left buttcok. No sign of arthritis were noted inspite of the complaints of arthralgia. Only the lymph nodes at the inguinal regions were enlarged which was more tender on the left side.
No fever was noted however the patient was treated with antipyretics and antihistamine with prompt response.

The second patient was a 32 year old male who was biten by a proven rabid dog at left foot 3 days prior to vaccination. The immediate skin test for ERIG was border-line. He was given PVRV and 12 ml of ERIG in the usual manner. 9 days after ERIG injection, the patient developed

intense pruritic inflammatory reaction at the right buttock (site of injection) as well as a few scattered urticarial rashes on the body. He also had fever and headache but no arthralgia. He treated himself with calamine lotion during the first 3 days. The symtoms persisted which brought him to see the doctor at QSMI.

On examination,generalized urticaria and bilateral inguinal lymphadenopathy were noted. No arthritis or fever was noted. The patient was treated with antihistamine with resolution of symtoms within 4 days.

Diagnosis of equine serum-induced serum sickness-like reaction was established by seeing a typical Arthus reaction when ERIG was re - injected intradermally or subcutaneously but no reaction when PVRV was similarly applied. (Data not shown).

3.5 Heterophile antibody test.

Heterophile antibody responses were determined by Paul-Bunnell sheep cell test (Presumptive test) and by Davidsohn differential test utilizing 20% guinea pig kidney suspension and 20 % ox red blood cell suspension to absorb heterophile antibody in the serum. The results were shown in Figure 1 and 2. Fig 1, showed geometric mean titer of

heterophile antibody of 100 patients(from presumptive test) on day 0 = 1:30 (range 1:10 - 1:80), day 7 = 1:35 (range 1:14 - 1:88), day 14 = 1:90 (range 1:30 - 1:260).

To do differential test,if the titer of heterophile antibody on day 0 and day 7 or day 7 and day 14 whose heterophile antibody titer was at least 2 fold dilution higher than the initial (day 0) or preceding (i.e.,day 7) serum specimen would be tested by Davidsohn's differential test. In this study, Davidsohn's differential test was done in 50 patients. 45 out of 50 patients were positive for heterophile antibody of serum sickness according to the criterial listed in Table III in the Materials and Methods. Figure 2, demonstrate the geometric mean titer (GMT) before and after absorption. GMT before absorption test of 50 patients = 1:167 (range 1:77-1:354) GMT after absorption by 20 % guinea pig kidney and 20 % ox red blood cell suspension were 1:9 (range 1:5-1:15) and 1:11 (range 1:6-1:21) respectively. When only the GMT of the positive sera for serum sickness were calculated (i.e., 45 patients).

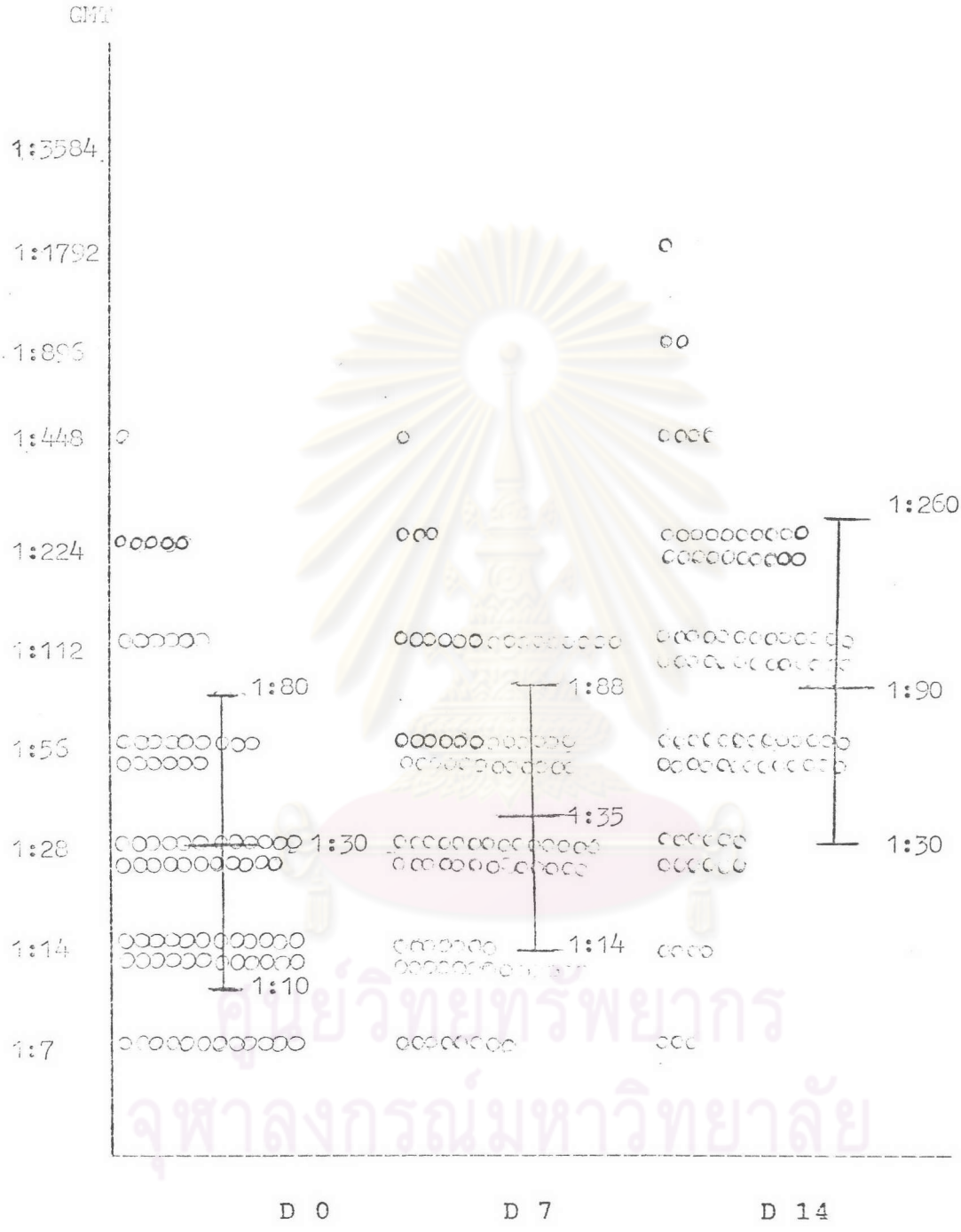


Figure 1 Heterophile antibody responses in 100 patients determined by Paul Bunnel sheep cells test (Presumptive test)

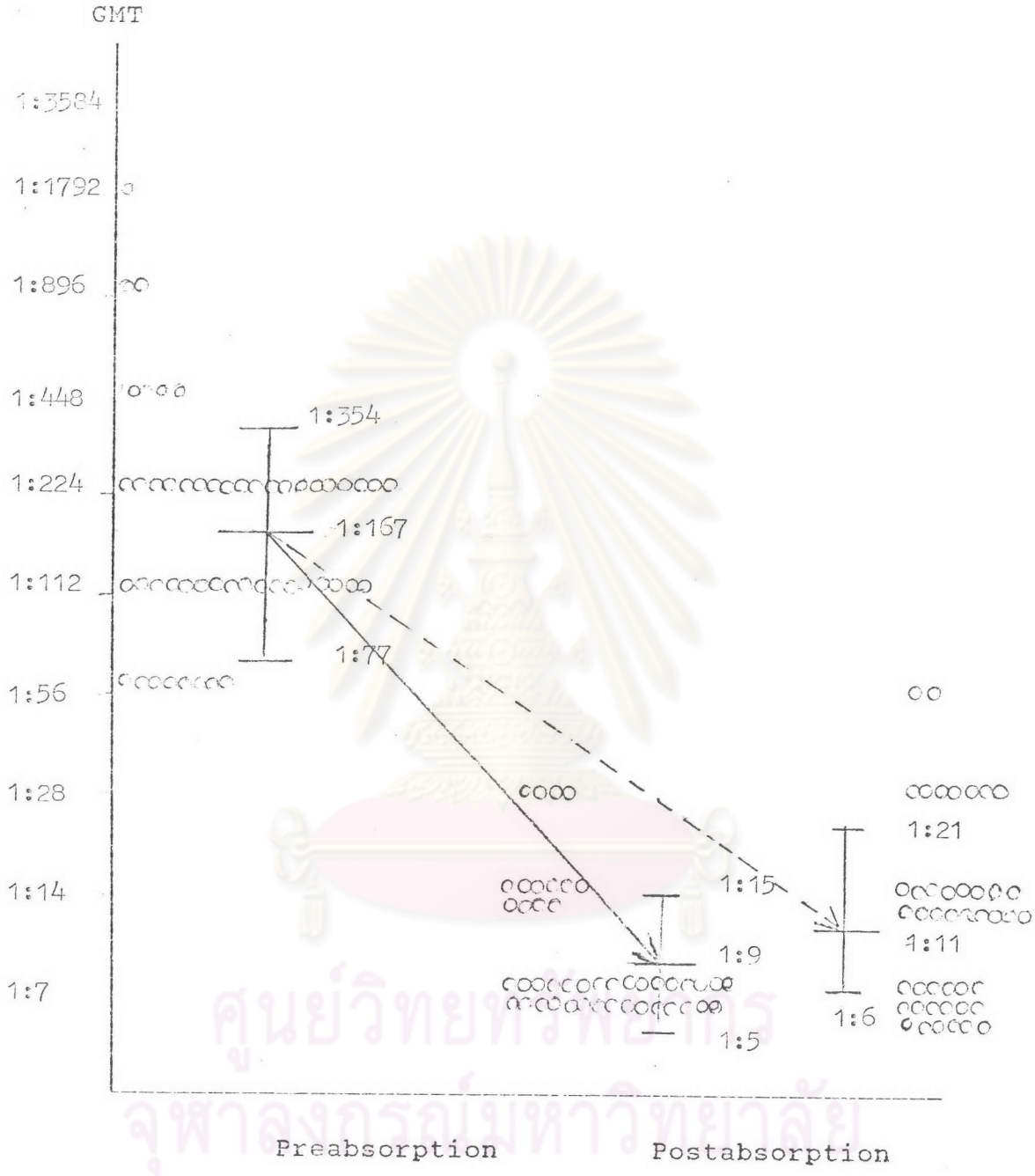


Figure 2 Heterophile antibody after Davidsohn's differentid1 test, They were shown as geometric mean titer (GMT).

------ Absorbed by guinea pig kidney.

----- Absorbed by ox red blood cell.

3.6 C_3 levels

Serial measuremens of serum C_3 levels in 102 patients revealed that mean serum C_3 levels on day 0,7 and 14 were 172±21(SD)mg%,176±20 mg% and177±19 mg% respectively. Numbers of patients whose C_3 levels less than X-SD of baseline values (i.e., day 0) or 151 mg% were 12 (11.8%), 10 (9.8%) and 13 (12.7%) on day 0.7 and 14 respectively (Figure 3). These results indicated that there was no significant change in C_3 levels with time after ERIG administration. C_3 levels of our 2 serum sickness patients were also in normal limits except one patient who had C_3 levels less than 151 mg% on day 14. When 16 additional patients day±7 (range 5-12) sera of patients with documented serum sickness were added to the 2 patients with serum sickness in the prospective study,the mean C_3 level on day 7 between symtomatic (N=18)and asymtomatic patients (N=100) were 166.4±29.7 and 175±20 mg% respectively (Figure 4). The results indicated that although mean C_3 level in serum sickness appeared lower than the non serum sickness group but the difference did not reach statistical significance. However, when the number of patients with low C_3 (less than mean -1 SD or 151 mg%) was compared, it was found that low C_3 not correlated significantly with serum sickness by chi-square test ($P > 0.05$) (Table VIII)

When C_3 levels were analysed against the heterophile antibody status, the mean C_3 levels on day 7 of the heterophile antibody positive (N=57) and the heterophile antibody negative (N=61) individuds were 173.5±20.6 and 174.7±21.5 mg% respectively (Figure 5). That is, there is no difference in C_3 levels between the heterophile antibody positive and heterophile antibody negative individuals by Student t test although heterophile antibody is more sensitive for serum sickness than C_3 level by McNemar chi-square (Table IX)

In addition, based on heterophile antibody status, 4 of 45 (8.9%) heterophile antibody positive group had C_3 level less 151 mg% where 10.9% (6/55) of the heterophile antibody negative group did (Figure 6). The difference was statistically significant by McNemar chi-square test (table X).

3.7 CIC levels

Mean serum CIC levels in 102 patients on day 0, 7, and 14 were 6.8±3.7, 6.95±4.58 and 7.25±4.3 respectively. Number of patients whose CIC level higher than X+SD of day 0 or 10.5% were 11 (10.8%), 9 (8.8%) and 12 (11.8%)on days 0,7 and 14 respectively (Figure 7). The CIC levels in our 2 serum sickness patients were within the normal ranges both on day 7 and day 14. Even when the large group of serum sickness patients was combined, the CIC levels

between the serum sickness group (N=18) and the non serum sickness group (N=100) were not significantly different, i.e., 6.3±4.9 and 6.8±4.7% respectively (Figure 8). Similarly, chi-square analysis of individuals with CIC higher than 10.5% (i.e., X+ 1 SD of normal or day 0 value) also revealed no correlation with serum sickness(table XI) CIC level of heterophile antibody-positive and heterophile antibody negative groups were 7.3±5.7% and 6.8±4.2% respectively (Figure 9). They were not significant different by Student T test even though heterophile antibody was more sensitive for serum sickness than CIC level by McNemar chi-square (Table XII)

3.8 Combined Immunologic Abnormalitus in Serum Sickness

Of the 18 patients with serum sickness, 5 (27.7%) had positive heterophile antibody and low C_3, 3 (16.7%) had positive heterophile antibody and high CIC, 2 (11.0%) had combined low C_3 and high CIC and 2 (11.0%)had positive heterophile antibody low C_3 and high CIC (Table 6). Similaryly, of the 100 asymtomatic ERIG recipients, 7 (7%) had positive heterophile antibody and low C_3, 8 (8%) had positive heterophile antibody and high CIC, 2 (2 %) had combined low C_3 and high CIC and 0 (0 %) had positive heterophile antibody, low C_3 and high CIC. There is no subclinical serum sickness (Table XIII).

Table IV Clinical data of 16 patients who developed serum sickness following ERIG administration

No	Sex	Age	Clinical Diagnosis	Dose	Day of onset of symtoms and signs
1	M	50	dog bite	12 ml	D8
2	F	35	cat bite	11 ml	D13
3	F	40	dog bite	10 ml	D7
4	F	42	dog bite	9.6 ml	D6
5	M	46	cat bite	10.6 ml	D7
6	F	24	dog bite	11 ml	D7
7	F	33	dog bite	11.2 ml	D8
8	F	15	dog bite	16 ml	D7
9	F	20	dog bite	19 ml	D7
10	M	37	dog bite	10.4 ml	D6
11	F	40	cat bite	11.4 ml	D7
12	F	40	dog bite	10 ml	D5
13	M	17	dog bite	10 ml	D7
14	F	22	dog bite	11 ml	D8
15	M	39	dog bite	11.6 ml	D9
16	F	26	dog bite	9 ml	D7

Table V Symptoms and signs of 16 serum sickness patients.

No	Faver	Skin Rash	Pruritus	Arthralgia/ arthritis	L.N.
1	-	*	-	-	-
2	-	*	-	+/-	+
3	-	**	-	-	-
4	-	*	+	-	-
5	+	***	-	+/-	-
6	+	*	+	-	-
7	-	*	-	-	-
8	+	*	-	-/+	-
9	-	*	-	-	-
10	-	*	-	+/-	-
11	+	*	+	-	-
12	+	-	+	-	+
13	-	-	+	-	+
14	+	*	+	-/+	-
15	+	*	+	-	-
16	-	***	+	+/-	-

- Negative , + Positive

* Generalized urticaria,

** Morbilliform rash, *** Urticaria at injection site

Table VI Data of heterophile antibody, C3, CIC levels and Arthus reaction in 16 serum sickness patients

No	Het-Ab	C3	CIC	Arthus reaction
1	-	161	3.9	ND
2	-	152	2.7	+
3	+	160	7.0	+
4	+	118	8.8	ND
5	+	155	2.7	+
6	+	125	15.3	+
7	+	170	3.52	ND
8	+	170	11.6	+
9	+	144	21.2	+
10	+	162	3.94	+
11	-	200	3.05	+
12	+	185	4.15	ND
13	+	135	3.49	ND
14	+	118	3.52	+
15	-	165	2.98	+
16	+	155	2.68	ND

+ = Positive

- = Negative

ND = Not done

Table VII Distribution of patients with skin test positive, borderline and negative.

Skin test	No. of patient	Percent
Negative	108/131	82.44
Positive	19/131	14.5
Borderline	4/131	3.05

Table VIII Chi-Square test between C3 and serum sickness

C3	Serum sickness		Total
	+	-	
Low	6	16	22
Normal	12	84	96
Total	18	100	118

Table IX McNemar chi-square test between C3 and Heterophile antibody,

C3	Heterophile Antibody		Total
	+	-	
Low	5	6	11
Normal	52	55	107
Total	57	61	118

Table X McNemar Chi-square test between C_3 and heterophile antibody among asymtomatic group,

C3	Heterophile Antibody		Total
	+	-	
Low	4	6	10
Normal	41	49	90
Total	45	55	100

Table XI Chi-Square test between CIC and serum sickness

CIC	Serum Sickness		Total
	+	-	
High	3	7	10
Normal	15	93	108
Total	18	100	118

Table XII Mc Nemar Chi-Square test between CIC and heterophile antibody.

CIC	Heterophile antibody		Total
	+	-	
High	7	5	12
Normal	50	56	106
Total	57	61	118

Table XIII Summary of individual and Combined Immunologic Abnormalities in Serum Sickness and in asymtomatic patients.

Immunologic Abnormalitis	Serum Sickness (N=18)	Asymptomtic (N=100)
Positive Heterophile Ab + low C3	5 (27.7%)	7 (7%)
Positive Heterophile Ab + high CIC	3 (16.6%)	8 (8%)
Low C3 + high CIC	2 (11.1%)	2 (2%)
Positive Heterophile Ab + low C3 + high CIC	2 (11.1%)	0 (0%)
Postive Heterophile Ab	12 (66.6%)	45 (45%)
Low C3	6 (33.3%)	9 (9%)
High CIC	3 (16.6%)	10 (10%)

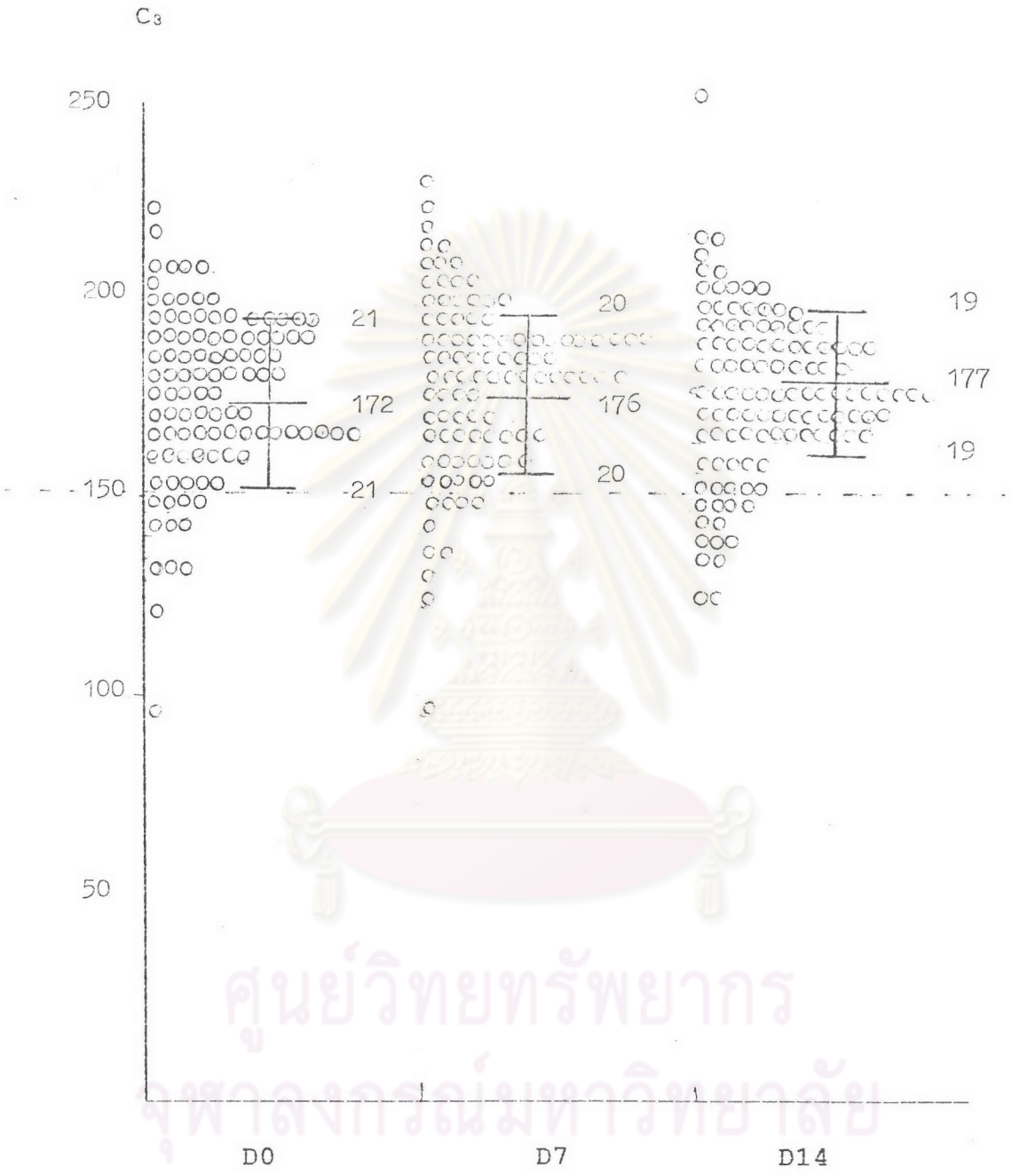


Figure 3 Distribution of C_3 levels in all ERIG recipients on day 0,7, and 14 to illustrate the individuals with levels less than mean-1SD or 151 mg.%

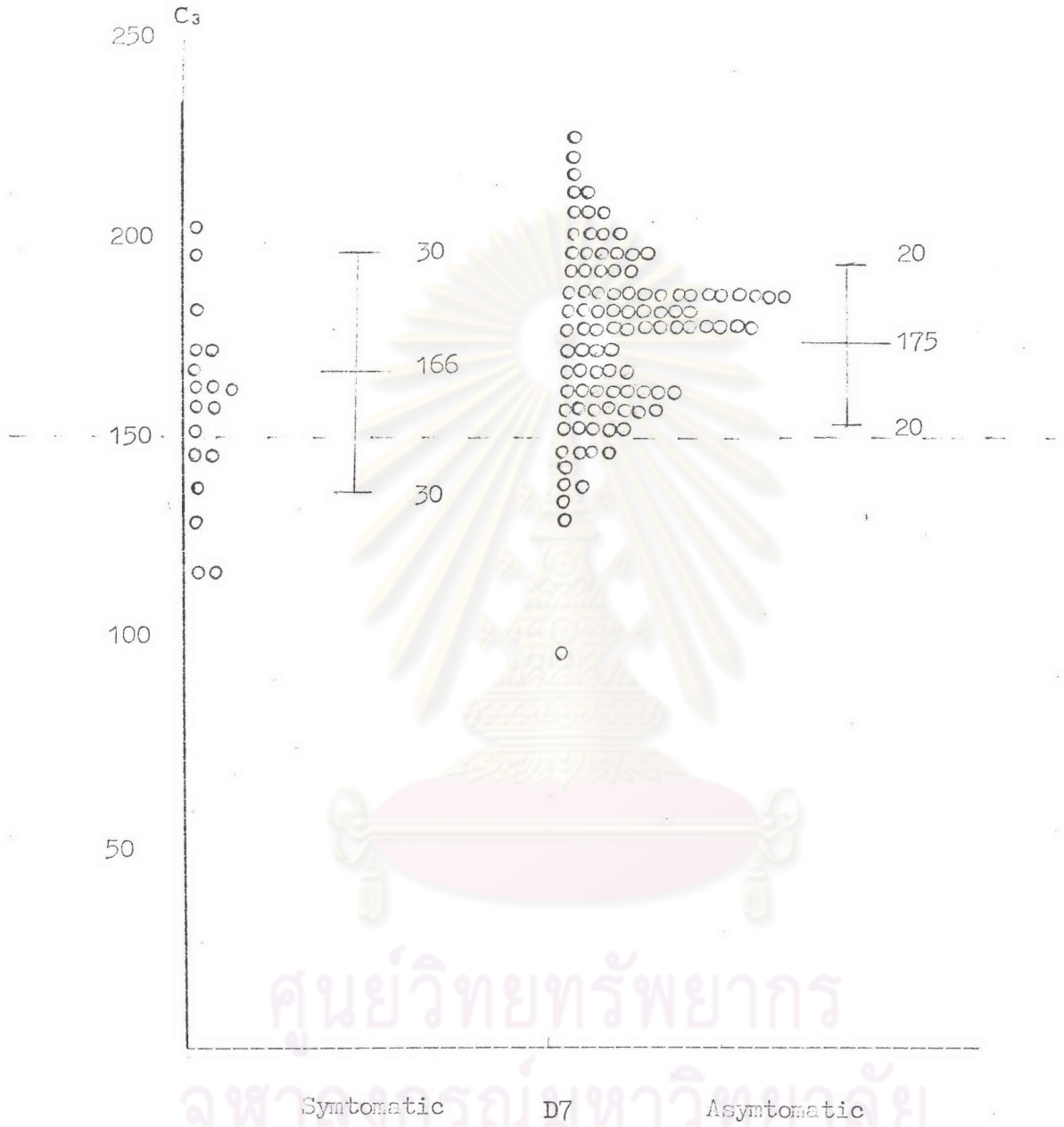


Figure 4 Distribution of C_3 levels in all ERIG r cipients on day 7 between symtomatic (N = 18) and asymtomatic (N = 100) patients of serum sickness to illustrate the individuals with levels less than Mean-SD or 151 mg%

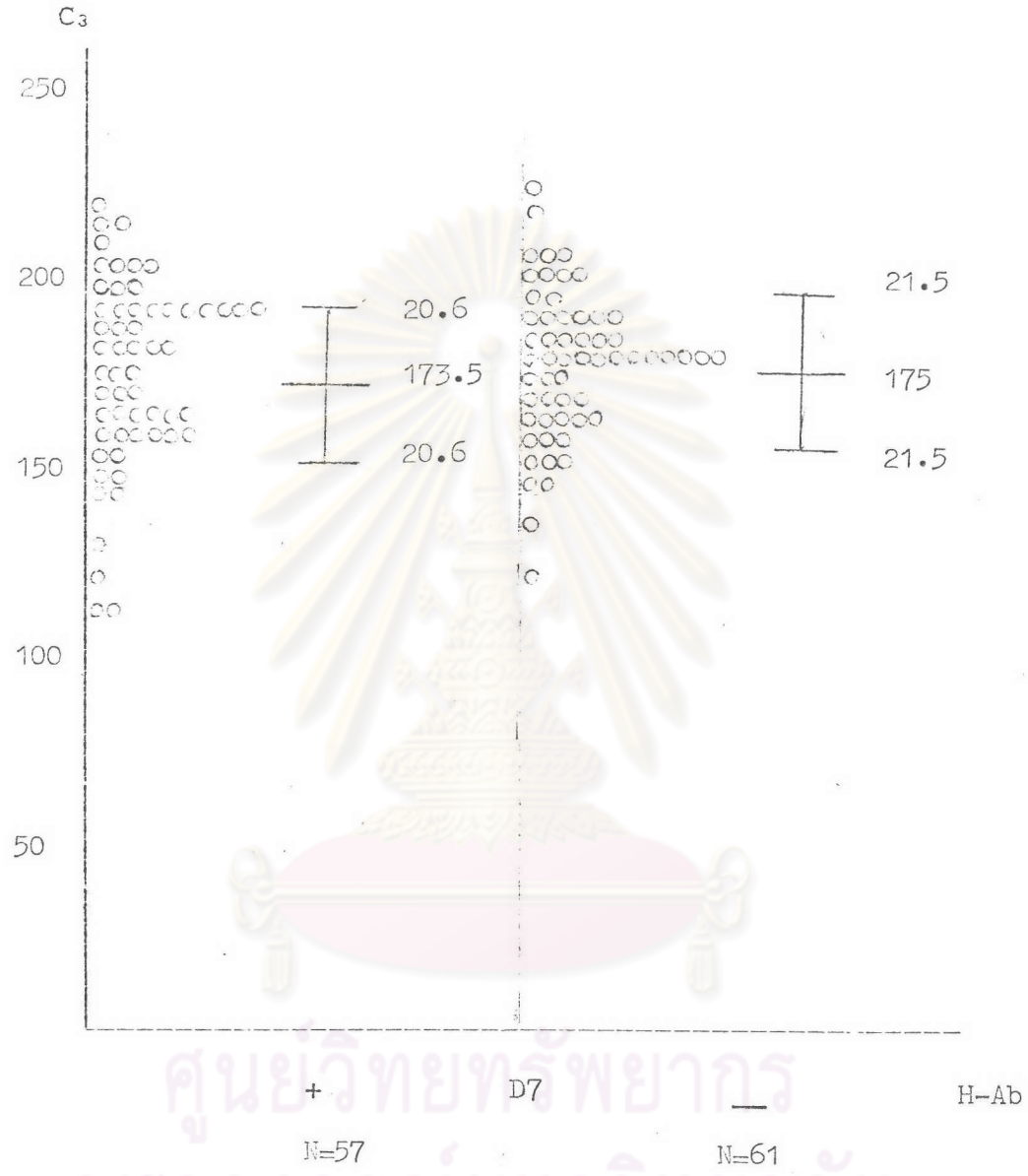


Figure 5 Compare C_3 levels in the patients with heterophile antibody positive (N = 57) and those with heterophile antibody negative (N = 61).

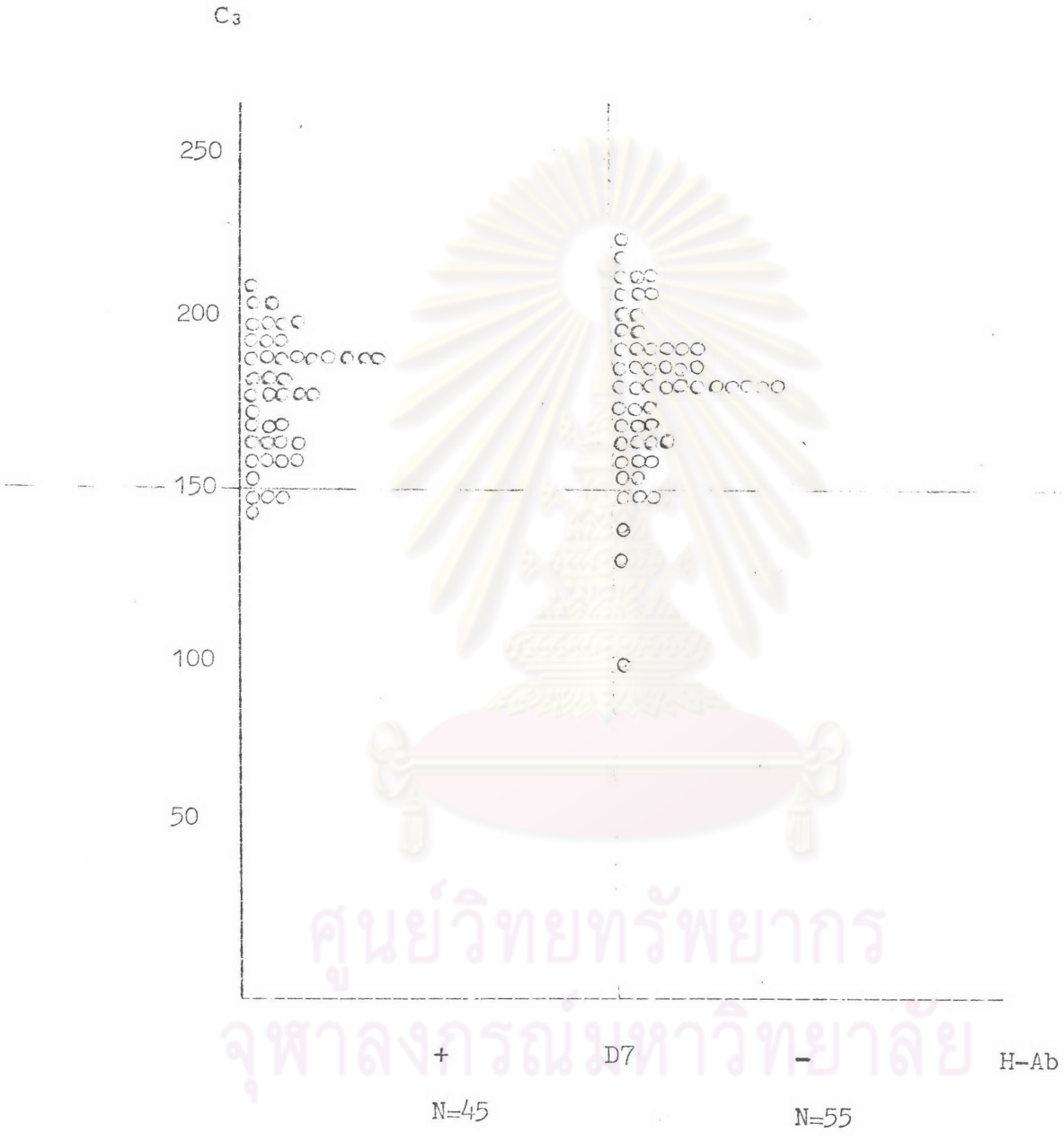


Figure 6 Distribution of C_3 levels in asymtomatic patients (N=100) on day 7 to illustrate the individuals with levels less than 151 mg% against heterophile antibody

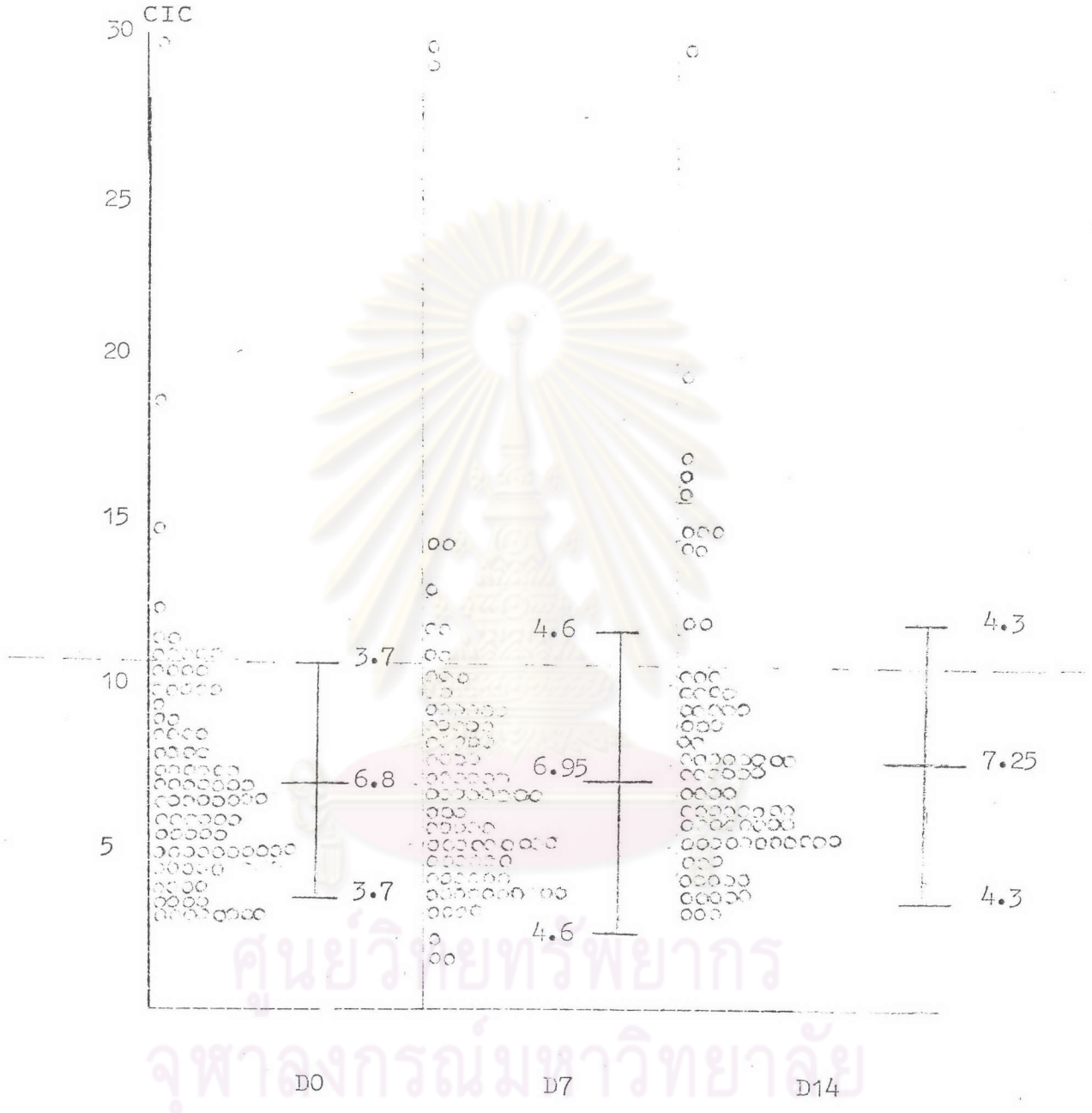


Figure 7 Distribution of CIC levels in all ERIG recipients on days 0,7 and 14 to illustrate the individuals with levels higher than Mean + 1 SD or 10.5%

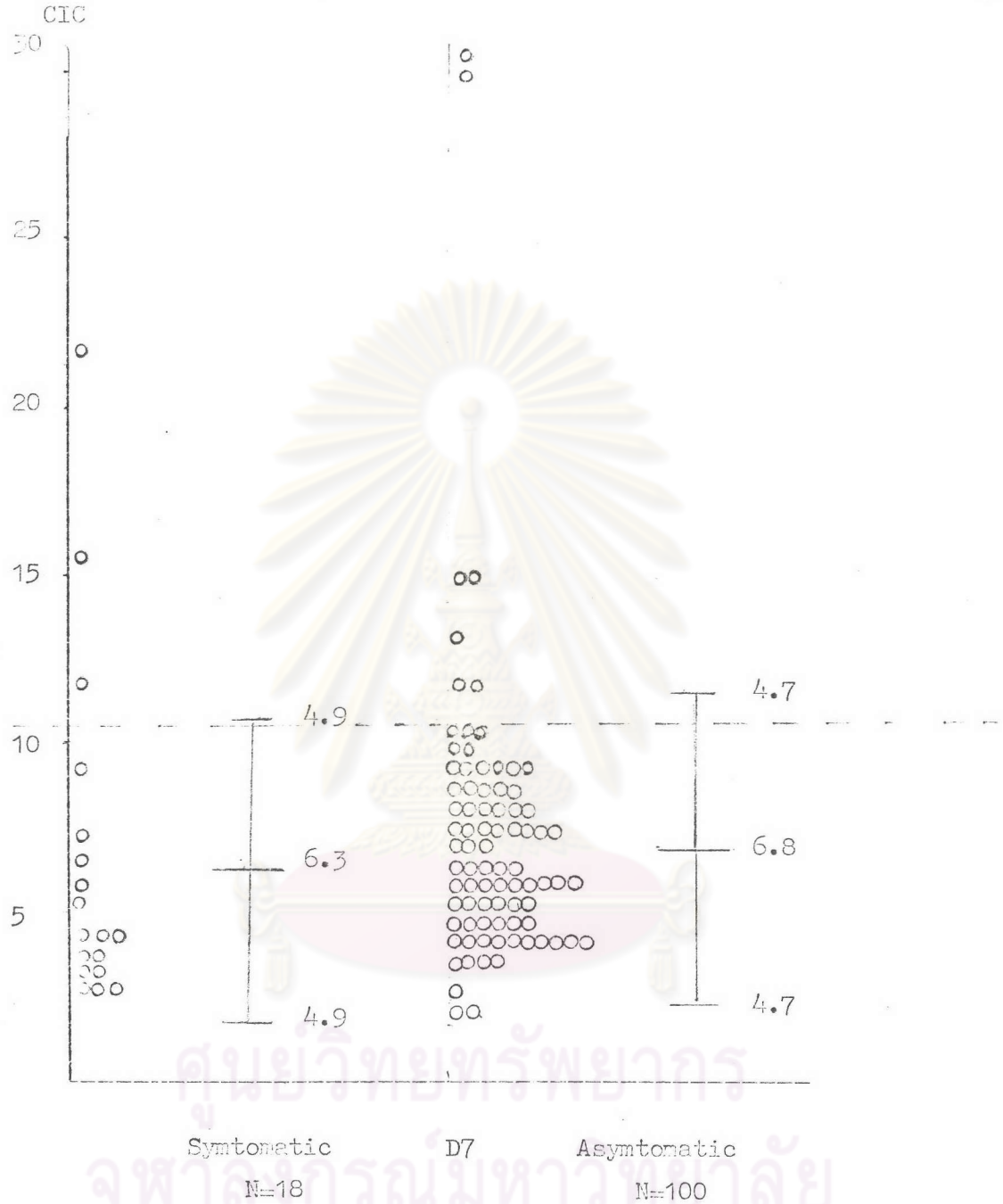
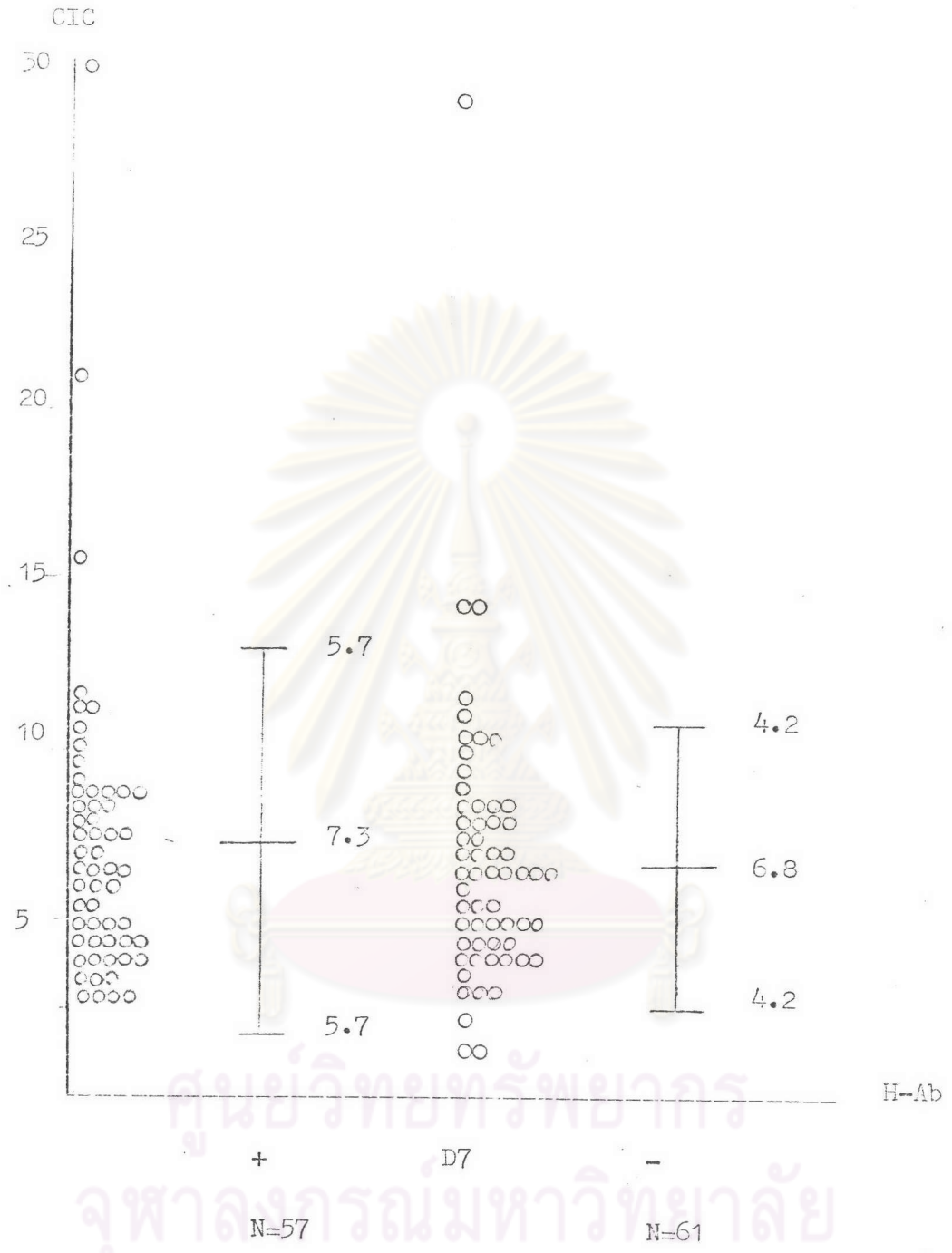


Figure 8 Distribution of CIC level in all ERIG recipients on day 7 between symtomtic (N=18) and asymtomatic (N=100) patients of serum sickness to illustrate the individuals with level higher than Mean + ISD or 10.5%.



Figrue 9 Compare CIC levels in the patients with heterophile antibody positive (N=57) and those with heterophile antibody negative (N=61).