Study for Etiology of Nephritis

I. Clinical Study

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THOUGH it is generally said that acute glomerulonephritis follows the infection of pneumococci or of *Str. viridans*, on many occasions it is supposed that the nephritis sets on 1 to 3 weeks following the infection of upper respiratory tract (tonsillitis, pharyngitis), or occasionally the cutaneous infection caused by group A streptococci. The first instance on this subject has been reported by SCHICK¹⁾ in respect to *Str. scarlatinae*.

Usually acute nephritis rises sporadically, but it also rises epidemically in one area or in a family as well. In 1908, WAHRER²⁾ reported that he had found 35 patients of acute hemorrhagic nephritis developing in a group of 195 patients of scarlet fever. During World War I beginning about February, 1915, a large epidemic of nephritis (termed trench nephritis) occurred in Allied and German troops³⁾. In a five-month-period there were more than 1000 cases reported among British troops alone. In the latter case, although etiological studies were meager, it is reported that β -hemolytic streptococci are isolated from oropharynx of some patients⁴⁾, and that there is similarity to nephritis which follows scarlet fever both clinically and pathologically⁵⁾. In 1926, PEACE⁶⁾ observed 8 cases each of whom developed nephritis 12 to 14 days after an attack of acute tonsillitis. These patients lived in the same neighborhood within 50 yards of distance among each other. Apart from such relatively large scaled occurrence as above, ADDIS,⁷⁾ KLEIN⁸⁾ and PRATAS⁹⁾ et al. reported the cases of small epidemics of nephritis, or a number of the outbreaks within family units. Thus it has come to be considered by many authors

that the outbreak of acute nephritis occurs 1 to 3 weeks after the streptococcal infection and its development mechanism is a kind of allergic reaction caused by the infection of group A streptococci.

In 1953, RAMMELKAMP¹⁰⁾ and his co-workers concluded from past data of outbreak ratio of nephritis which followed scarlet fever that there were some types of group A streptococci leading to the development of nephritis. They named them "nephritogenic types or strains" and reported¹¹⁻²³⁾ that the rises of epidemic nephritis in the United States were caused by types 12, 6 and 4 and Red Lake strain (later named type)⁴⁹⁾. Also, the outbreak of epidemic nephritis in the period of 1955-1956 in Japan, are reported²⁴⁻³⁰⁾ to have been caused by types 12, 6 and 4. On the other hand, SASLOW³¹⁾ reported cases of school pupils who had been infected by the so-called nephritogenic types and strains in a long run but had not developed nephritis.

Now, regarding to the relationship between group A streptococci and the development of nephritis, there is a case in which those who have been infected by type 12 which, according to RAMMELKAMP is classified as nephritogenic have not developed nephritis; there is also a case (reported by GOLDSMITH)³²⁾ in which type 1 which is usually considered as irrelevant to nephritis caused the development of nephritis; from these it is possible to conclude, as it seems, that throughout group A streptococci the possibility to have "nephritogenic substance" is fairly extensive, and that type 12 has particularly high possibility in this matter.

The reason why the development of nephritis

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as a result of the infection of β -hemolytic streptococci happens after a certain period of incubation is as it is argued, not because nephritis develops as a direct result of cellular and extracellular substances of organisms, but from inference of the diminishment of serum complement³³⁾³⁴⁾⁷³⁾ and development mechanism of Masugi's nephritis,³⁵⁾³⁶⁾ because of antigen-antibody reaction, and especially because of tissue allergy of Prof. M. MAEKAWA.³⁷⁾³⁸⁾

According to the report of RAMMELKAMP, STETSON and others,¹⁸⁾ two days after the development of pharyngitis caused by the infection of type 12, microscopic hematuria shows high grade, then diminishes for a while, and again rises, leading to the development of nephritis. However, in cases of the infection of types 3, 6 and 19, microscopic hematuria, after 2 days of the development of pharyngitis, is not as high as in cases of type 12 in grade and diminishes, never causing the development of nephritis.

It is supposed from this fact that there might exist substance in type 12 which damages renal tissue at the time of epidemic outbreak of nephritis. Also there is an interesting report made by RAMMELKAMP¹⁹⁾ saying that nephritogenic strain can be bacteriolysed by bacteriophage.

Among patients of nephritis who visited us, few came at the acute stage of its development, but mostly either at chronic stage or at the stage of postnephritic albuminuria. From the view point of pursuit in pathogenesis of

nephritis, we classified chronic nephritic patients in accordance with whether the development of chronic nephritis was apparently initiated by acute nephritis or not, and whether the chronic nephritis is exacerbated or not employed the method of throat culture and titration of ASLO (antistreptolysin O); and studied the relationship between nephritis and streptococcal infection. Along with the course of our study, not only the relationship between the β -hemolytic streptococcal infection and the development of acute nephritis as well as the exacerbation of chronic nephritis, but also the relationship between viral infection and the development of nephritis in part were covered. Also at tonsillectomy of nephritic patients, streptococcal culture and histological examination of extracted tonsils were done, and clinical symptom at tonsillectomy and its impact afterwards were also carefully studied. The following is the report on the above research.

METHODS

1) Clinical Materials; (Table I)

58 nephritic patients who were admitted to Maekawa's clinic of Kyoto University Hospital from 1957 to 1961. As control, 191 patients except those of rheumatic fever and rheumatic diseases. Among the above 58 nephritic patients, there are;

- 5 healed cases of acute glomerulonephritis
- 2 active cases of acute glomerulonephritis
- 14 cases of postnephritic albuminuria
- 4 cases of subacute stage of nephritis
- 10 cases of non exacerbation of chronic nephritis initiated by acute nephritis

TABLE I MATERIALS

Renal Diseases			Number of Case	Control Groups		Numbes
Acute Nephritis	Healed Group		5	Congenital Heart Diseases		18
	Active Group		2	Arachnoiditis		62
Postnephritic Albuminuria			14	Other Cardiac Diseases		67
Subacute Stage of Nephritis			4	Septicaemia		18
Chronic Nephritis initiated by Acute Nephritis	Exacerbation	(-)	10	Other Diseases		26
		(+)	6	Total		191
Chronic Nephritis not initiated by Acute Nephritis	Exacerbation	(-)	5			
		(+)	3			
Interstitial Nephritis			1			
Nephrotic Syndrome			8			
Total			58			

- 6 cases of exacerbation of the same as above
- 5 cases of non-exacerbation of chronic nephritis not apparently initiated by acute nephritis
- 6 cases of exacerbation of the same as above
- 1 cases of interstitial nephritis
- 8 cases of nephrotic syndrome.
- 2) Bacteriological and serological examination on β -hemolytic streptococcus
 - i) Throat Culture

From the time of admission, patients were asked, at an appropriate interval, to swabb the surface of tonsil and visible part of throat with disinfected cotton before morning toileting and to cough several times. Secretion extracted from the cotton swabb was washed off in 5 c.c. of Brain-Heart Infusion Broth (Difco), and one streak of the broth was cultured, in accordance with Brown's method,⁴²⁾ in Heart Infusion Agar (Difco) which was added 5% defibrinated horse blood as soon as possible; and then the character of colony was examined after 24 hours, 48 hours and occasionally 72 hours. The basis of β -hemolysis⁴²⁾⁴³⁾ was made at two points where rest red blood cell is not observable near colony and where green-tone is unobservable around hemolytic ring. When dubious, after isolation, mix culture was repeated until character of colony could be affirmed, and at the same time, colony character of hemolysis on surface was taken into account. For subculturing, Brain Heart Infusion (Difco) was employed. Serological classification of β -hemolytic streptococci into Lancefield's groups and types⁴⁶⁾ was made in accordance with the capillary method of H. F. SWIFT⁴⁴⁾⁴⁵⁾. Antigens were produced as follows. By surface culture of isolated strain, colony type was determined, organisms extracted from the matt colony⁴⁷⁾ were inoculated in Todd-Hewitt's broth (40 c.c.). The broth was incubated for 18 hours at 37°C, centrifugated for 20 minutes for 4000 r.p.m., collected organisms were added 1/5 N. HCl physiological NaCl solution (0.5 c.c.), extracted the antigen by adding heat for 10 minutes in boiling water, after stirring with glass bar, kept for 2 hours at the room temperature, centrifugated again for 20 minutes for 4000 r.p.m., added phenol red (a droplet of 0.02% solution) to the supernatant as the indicator, added 1/5 N. NaOH solution till the color approximated light pink, centrifugated again, and extracted the supernatants. The supernatants thus extracted were kept in ice box over night after adding a droplet of 0.1% marzonin solution, made the supernatant "precipitin". For precipitation, using capillary tube of inside diameter 0.9 mm, after incubation of 2 hours at 37°C., the result was observed. And after keeping it in ice box for a night, again observation was made. Antisera against groups and types used were those obtained (a) from assistant

Prof. TADOKORO of Infectious Disease Institute of Tokyo Univ., (b) from Kitazato Research Institute and (c) from Dr. KUSAMA of National Preventive Hygiene Institute, and there were 7 kinds of anti-sera against groups (i.e.) (A, B, C, G, K, N, and (O) and 19 kinds of anti-sera against types (i.e.) (5, 6, 9, 10, 14, 15, 18, 23, 26, 28, 29, 32, 33, 36, 37, 40, 44). Todd-Hewitt's broth⁴⁴⁾ used was both made from Todd-Hewitt's broth dry powder of Difco, and produced by us from purchased horse flesh.

ii) Culture of extracted tonsil

After tonsillectomy, a part of the extracted tonsil was grinded as soon as possible, thus making emulsion. Examination on β -hemolytic streptococcus was done in the same way as applied to the throat culture.

iii) Titration of ASLO

Following the method of RANZ-RANDALL⁴⁹⁾⁴⁹⁾, ASLO was titrated from the time of admission onward. Streptolysin O trial used here was that obtained from assistant Prof. TADOKORO of Infectious Diseases Research Institute (Tokyo Univ.), and was based on ASLO standard serum (Difco).

iv) CRP (C-Reactive Protein)

Method employed was the capillary tube method of MC CARTY AND ANDERSON⁵⁰⁾, and the trial used was that produced by Difco. Co.

v) Measurement of serum complement

Ogata's method⁵¹⁾ was applied. Hemolysin obtained from Assistant Prof. TOKUDA of Virus Institute (Kyoto University) was used. Sheep red cell was also used. Complement titer was based on serum consumptive quantity.

vi) Virus serum complement fixation reaction

Measurement there of was asked to Virus Institute of Kyoto Univ. mainly with regard to Influenza A and B virus⁵²⁾ and Adeno-virus.

vii) Histological findings of the extracted tonsil

After tonsillectomy, a part of the extracted tonsil was fixed in 10% formaline solution. Then the object stained with hematoxylin-eosin was examined by microscope.

viii) Rheumatoid Arthritis Test (RAT)⁵³⁾

The trial used was that made by Difco. Co. This test was made in Central Laboratory of Kyoto University Hospital.

RESULTS

1) Materials (Table I)

When classified 58 cases of nephritis according to their onset patterns, running patterns after the onset and renal biopsy findings, there were

5 healed cases of acute glomerulonephritis

TABLE II PERIOD BETWEEN PRECEDING INFECTION AND ATTACK OF NEPHRITIS

		Number of Case	Infection (+)	within 1 week	w. 2 w.s	w. 3 w.s	w. 4 w.s	Unclear
Acute Nephritis	Healed Group	5	4	1	2	1		1
Postnephritic Albuminuria	Active Group	2	0					2
Subacute Stage of Nephritis		14	9	8		1		5
Chronic Nephritis initiated by Acute Nephritis	Exacerbation (-)	4	4	3	1			
	at onset (+)	10	4	1	1	2		6
	(at exacerbation)	6	5 (5)	2 (5)	2		1	1 (1)
Chronic Nephritis not initiated by Acute Nephritis	Exacerbation (-)	5						
	at onset (+)	3						
	(at exacerbation)		(2)		(1)		(1)	(1)
Interstitial Nephritis		1	1		1			
Nephrotic Syndrome		8	2	2				6
Total		58	29 (7)	17 (5)	7 (1)	4	1 (1)	21 (2)

2 active cases of acute glomerulonephritis
 14 cases of postnephritic albuminuria
 4 cases of subacute stage of nephritis
 10 cases of non-exacerbation of chronic nephritis initiated by acute nephritis
 6 cases of exacerbation of the same as above
 5 cases of non-exacerbation of chronic nephritis not initiated by acute nephritis
 6 cases of exacerbation of the same as above
 1 case of interstitial nephritis
 and 8 cases of nephrotic syndrome.

As control, in 191 cases except those of rheumatic fever and other rheumatic diseases throat culture and titration of ASLO were carried out.

2) Preceding Infection and Period from Preceding Infection to the Attack

As is shown in table II, findings of the onset stage of acute nephritis indicated the preceding infection which supposed to have caused the development of nephritis in 26 cases (63.4%) out of 41 cases. In the rest of cases, it was not clear whether there was any preceding infection.

Among the 26 cases above mentioned, the cases which developed nephritis within a week after preceding infection were 15; within 2 weeks, 6; within 3 weeks, 4; and within 4 weeks, 1. A case of interstitial nephritis developed 2 weeks after the infection.

As to exacerbation of chronic nephritis, out of 6 cases initiated by acute nephritis 5 cases

(83.3%) indicated preceding infection all of which developed within a week.

As to exacerbation of chronic nephritis out of 3 cases not initiated by acute nephritis, 2 cases (66.7%) indicated preceding infection in which the one developed within 2 weeks and the other, within 4 weeks.

As to 8 cases of nephrotic syndrome, in only 2 cases (25%) preceding infection was indicated, both of which developed within 1 week.

Therefore it may well be concluded that in cases of development of acute nephritis and of exacerbation of chronic nephritis preceding infection was frequently indicated, but in cases of nephrotic syndrome it was not so.

3) Period between Onset of Nephritis and Admission

Since this period is almost paralleled by that between preceding infection and onset of nephritis, it relates to the isolation ratio of β -hemolytic streptococci at throat culture at the time of admission. Also rise of ASLO⁵⁴⁾ is impossible to catch when patients are admitted long after onset (after 3 months).

As the table III shows, in the healed cases of acute nephritis, all were admitted at the early stage, while among 14 cases of post-nephritic albuminuria, only 3 were admitted within 1 month after onset and the rest were admitted long after onset for the purpose of a close investigation of persisting albuminuria.

TABLE III PERIOD BETWEEN ONSET OF NEPHRITIS AND HOSPITALIZATION

		Number of Case	within 1m.	w. 2m.	w. 3m.	w. 6m.	w. 1y.	w. 2y.s	w. 5y.s	w. 10y.s	more than 10y.s
Acute Nephritis	Healed Group	5	2	2	1						
Postnephritic Albuminuria	Active Group	2		1					1		
Subacute Stage of Nephritis		14	3			1	1	5	3		1
Chronic Nephritis initiated by		4			1	1	1		1		
Acute Nephritis (Exacerbation~Hospitalization)	Exacerbation (-)	10	2				2		3		3
Chronic Nephritis not initiated by	Exacerbation (+)	6	1 (4)		(1)	(1)	2		1		2
Acute Nephritis (Exacerbation~Hospitalization)	Exacerbation (-)	5			1	1	2		1		
Interstitial Nephritis	Exacerbation (+)	3				1		1		1	
Nephrotic Syndrome		1		1							
Total		58	9 (6)	4	3	5	8	9	10	2	6

TABLE IV TONSILLAR FINDINGS OF NEPHRITIC PATIENTS

		Number of Case	Tonsillar Findings (+)			Total Positive Cases	%	normal
			Hypertrophy + Congestion	Hypertrophy	Congestion			
Acute Nephritis	Healed Group	5	2	1		3	60	2
Postnephritic Albuminuria	Active Group	2		2		2	100	
Subacute Stage of Nephritis		14	2	6	3	11	79	3
Chronic Nephritis initiated by		4	2	1	1 *(1)	4 *(1)	100	
Acute Nephritis (Exacerbation~Hospitalization)	Exacerbation (-)	10	2	2		4	40	6 *(1)
Chronic Nephritis not initiated by	Exacerbation (+)	6	2		1	3	50	3
Acute Nephritis (Exacerbation~Hospitalization)	Exacerbation (-)	5	2	1		3	60	2
Interstitial Nephritis	Exacerbation (+)	3	2			2	66	1
Nephrotic Syndrome		1			1	1	100	
Total		8			1	1	13	7
Total		58	14	13	7	34		24

*() Number of case after tonsillectomy

TABLE V TONSILLAR FINDINGS OF CONTROL GROUPS

	Number of Case	Tonsillar Findings (+)			Total Positive Cases	%	normal
		Hypertrophy + Congestion	Hypertrophy	Congestion			
Congenital Cardiac Diseases	18	8	4	0	12	70.6	5
Other Cardiac Diseases	67	6	9	5	20	29.9	47
Arachnoiditis	62	16	10	8	34	54.8	28
Septicaemia	18	5	1	3	9	47.0	9
Other Diseases	26	4	5	4	13	50.0	13
Total	191	39	29	20	88		102

As to cases of subacute stage of nephritis and of chronic nephritis very few were admitted at the early stage. The rest of cases came long after developing chronic nephritis.

However, in case of exacerbation of chronic nephritis, many came at the early stage. In case of nephrotic syndrome, only a few came at the early stage.

As to 8 cases of acute nephritis admitted at the early stage, only 2 cured, while albuminuria persisted in 3 cases and chronic nephritis developed in 3 cases. Therefore it is possible to say that acute nephritis treated at the early stage some times leads to the chronic nephritis.

4) Pharyngeal findings at the time of admission (especially of tonsil)

As a result of pharyngeal findings, those which indicated hypertrophy and injection were; as shown in table IV and V.

60% for the healed cases of acute nephritis

100% for the active cases of acute nephritis

79% for the cases of postnephritic albuminuria

100% for the cases of subacute stage of nephritis.

Low ratio was shown for the cases of nephrotic syndrome, when compared with control. The ratio shown in the cases of chronic nephritis initiated by acute nephritis was almost the same as that of the control. In groups of control, only cases of congenital heart disease show-

ed high ratio of 70.6% which seems to be the result of inclusion of infants.

5) Isolation ratio of β -hemolytic streptococci

It is possible to examine the existence or non-existence of β -hemolytic streptococci, when nephritic patients come at the early stage, but when patients come long after the onset of nephritis, it is hard to argue on preceding infection from the results of throat culture conducted at the time of admission.

Table VI shows the results of comparative study upon cases admitted within 3 months after onset, cases admitted after 3 months, and cases of exacerbation. As a result of the comparative study, in a group of patients admitted within 3 months after onset, 7 instances of throat culture conducted upon 11 acute glomerulonephritic patients showed β -hemolytic streptococci and none of throat culture conducted upon interstitial nephritic patients and nephrotic syndrome showed.

In the group of exacerbated nephritic patients, despite chronic nephritic patients initiated by acute glomerulonephritis indicated preceding infection, it was impossible to isolate β -hemolytic streptococci. However, in cases of exacerbation of chronic nephritis not initiated by acute glomerulonephritis β -hemolytic streptococci were isolated in 2 cases out of 3. In the group of nephritic patients admitted

TABLE VI STREPTOCOCCAL ISOLATION RATIO AT THE HOSPITALISATION

		Number of Case	Hospitalisation within 3 months after onset of Nephritis		Hospitalisation after more than 3 months after onset of Nephri- tis.	
			Number of Case	Strept. (+)	Number of Case	Strept. (+)
Acute	Healed Group	5	5	3		
Nephritis	Active Group	2	1	1	1	0
Postnephritic Albuminuria		14	3	1	11	2
Subacute Stage of Nephritis		4	1	1	3	0
Chronic Nephritis initiated by	Exacerbation (-)	10	2	1	8	0
Acute Nephritis (after Exacerbation)	(+)	6	1	1	5	0
Chronic Nephritis not initiated by	Exacerbation (-)	5	0	0	5	2
Acute Nephritis (after Exacerbation)	(+)	3	2	2	1	0
Interstitial Nephritis		1	1	1	0	0
Nephrotic Syndrome		8	2	0	6	0
Total		58	18	11	40	4

more than three months after onset, a case of acute nephritis did not reveal β -hemolytic streptococci at all. Out of 11 cases of post-nephritic albuminuria, only one case isolated atypical β -hemolytic streptococci. Out of 3 cases of subacute stage of glomerulonephritis, only one case isolated atypical β -hemolytic streptococci. Out of 13 cases of chronic glomerulonephritis initiated by acute nephritis, none. Out of 4 cases of non-exacerbation of chronic glomerulonephritis not initiated by acute ne-

phritis, 2 cases isolated atypical β -hemolytic streptococci. Out of 5 cases of nephrotic syndrome, none.

6) Relationship between Pharyngeal Findings and Isolation Ratio of β -hemolytic Streptococci

Examination was performed to study whether there is any relationship between the results of throat culture on β -hemolytic streptococci and findings of tonsils.

Either in cases of admission within 2 months

TABLE VII RELATIONSHIP BETWEEN TONSILLAR FINDINGS AND ISOLATION RATIO OF β -HEMOLYTIC STREPTOCOCCI

		Case of Number	Cases of Hospitalisation within 3 months after the Onset of Nephritis				Cases of Hospitalisation over 3 months after the Onset of Nephritis				not Clear
Streptococci in Throat			(+) (−)		(−) (+)		(+) (−)		(−) (+)		
Finding of Tonsil			(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	
Acute	Healed Group	5	2	1		1					1
Nephritis	Active Group	2		1						1	
Postnephritic Albuminuria		14	1			1	1	1		9	1
Subacute stage of Nephritis		4				1				3	
Chronic Nephritis initiated by	Exacerbation (−)	10		1		1				6	2
Acute Nephritis	(+)	6		1						2	2
Chronic Nephritis not initiated by Acute Nephritis	Exacerbation (−) (+)	5 3				1	1	1	1	1	
Interstitial Nephritis		1		1							
Nephrotic Syndrome		8				1	5	1			1
Total		58	3	5	1	5	7	5	10	18	4

TABLE VIII CLASSIFICATION OF ISOLATED STREPTOCOCCI FROM NEPHRITIC PATIENTS

		Number of Case	Case of Throat Culture	Strept. Positive	Group		B	C	G	O	Ungroup- able
					A	(A-12)					
Acute Nephritis	Healed Group	5	4	3	2	(2)					1
	Active Group	2	2	1	1	(1)			1		
Postnephritic Albuminuria		14	13	3	1	(1)			1		1
Subacute Stage of Nephritis		4	4	1							1
Chronic Nephritis initiated by	Exacerbation (−)	10	10	1	1	(1)					
Acute Nephritis	(+)	6	6	1							1
Chronic Nephritis not initiated by	Exacerbation (−)	5	5	2							2
Acute Nephritis	(+)	3	3	2	1				1		
Interstitial Nephritic		1	1	1							1
Nephrotic Syndrome		8	8	0							
Total		58	56	15	6	(5)			3		7

TABLE IX CLASSIFICATION OF ISOLATED STREPTOCOCCI FROM CONTROL GROUPS

	Number of Case	Strept. Positive	Group A (A-12)	B	C	G	O	Ungroup- able
<i>Congenital Heart Diseases</i>	18							
<i>Other Heart Diseases</i>	67	10	3			1		6
<i>Arachnoiditis</i>	62	17	1	1	2	2		11
<i>Septicaemia</i>	18	1				1		
<i>Other Diseases</i>	26	7	1	3		1	2	
<i>Total</i>	191	35	5 (0)	4	2	5	2	17

TABLE X ASLO TITER OF NEPHRITIC PATIENTS

		Cases of Hospitalisation within 3 months after Attack			Cases of Hospitalisation over 3 months after Attack			Exacerbated Cases		
		Number of Case	ASLO ≥166	250 ≥ not clear	ASLO ≥166	250 ≥ not clear	ASLO ≥166	250 ≥ not clear	ASLO ≥166	250 ≥ not clear
<i>Acute</i>	<i>Healed Group</i>	5	1	4						
<i>Nephritis</i>	<i>Active Group</i>	2		1		1				
<i>Postnephritic Albuminuria</i>		14	1	1	1	10		1		
<i>Subacute Stage of Nephritis</i>		4	1			3				
<i>Chronic Nephritis initiated by</i>	<i>Exacerbation (-)</i>	10	1	1		7		1		
<i>Acute Nephritis</i>	<i>(+)</i>	6	1		1	1		4		2
<i>Chronic Nephritis not initiated by</i>	<i>Exacerbation (-)</i>	5	1			4				
<i>Acute Nephritis</i>	<i>(+)</i>	3						1	2	
<i>Interstitial Nephritis</i>		1	1							
<i>Nephrotic Syndrome</i>		8	2		1	4		2		
<i>Total</i>		58	9	7	3	29	1	3	6	2

or in those admission, more than 3 months after onset (see table VII), no relationship could be found.

7) Classification of isolated β -hemolytic streptococci (Table VIII and IX)

Among those admitted at the developmental stage of acute glomerulonephritis, out of 11 instances of throat culture, β -hemolytic streptococci were isolated in 7 cases.

In these 7 cases; group A was found in 5 cases (4 cases of type 12 and one case of unknown type); unknown group, in one case; and atypical β -hemolytic streptococci, in one case.

In cases of the exacerbation of chronic nephritis not initiated by acute glomerulonephritis, 2 cases out of 3 revealed β -hemolytic streptococci one of which was group A (unknown type) and the other was group G. Among those admitted more than 3 months after onset, in one case of postnephritic albuminuria out of two in

which β -hemolytic streptococci were isolated proved group G, the other being of unknown group.

In one case out of a group of subacute nephritis and in two cases of chronic nephritis not initiated by acute glomerulonephritis, β -hemolytic streptococci were isolated, in all of which they were atypical and of unknown group as well.

As to patients taken as control, in 40 cases out 191, β -hemolytic streptococci were isolated 7 of which, was group A (not belonging to type 12); 4 of which, group B; 2 of which, group C; 5 of which, group G; 2 of which, group O, and 20 of which, of unknown group respectively. Regarding to β -hemolytic streptococci of unknown group, a part of strain showed no difference from typical β -hemolytic streptococci, speaking from the results of culture. But hemolytic rings these of streptococci of

unknown group were small, and the sizes thereof were remarkably uneven. Sometimes, red blood cells could be microscopically observed. Hemolytic ring appeared sometimes unclear. And while culture being conducted in Todd Hewitt's broth, these hemolytic streptococci of unknown group grew with unusually slow speed or incompletely or did not grow at all. But even after being kept for a night in ice box, enlargement of hemolytic ring was not recognizable.

8) The relationship of ASLO response to incidence of acute nephritis and exacerbation of chronic nephritis (Table X)

Cases are grouped according to whether patients were admitted within 3 months or after 3 months since onset or at the time of exacerbation.

Taken 166 Todd units and less of ASLO as normal⁵⁵⁻⁵⁸⁾, out of 14 cases admitted within 3 months after onset of acute glomerulonephritis, ASLO was determined in 12 cases. And out of the above 12 cases, 7 cases (58.3%) recorded the ASLO of 250 Todd units or above. In one case of chronic nephritis not initiated by acute glomerulonephritis, in one case of interstitial nephritis, and in 2 cases of nephrotic

syndrome all of which were admitted within 3 months after onset, none recorded that high ASLO titer. Among those admitted more than 3 months after attack in one case out of active group of acute glomerulonephritis, ASLO showed 250 Todd units or above, but in 29 cases of the groups other than the active group, ASLO was all 166 Todd units or less. As for cases of exacerbation, while all of 4 exacerbation cases of chronic nephritis initiated by acute glomerulonephritis recorded 166 Todd units or less, 2 cases out of 3 exacerbated chronic nephritis not initiated by acute glomerulonephritis shown 250 Todd units or above, the remaining one showing 166 Todd units or less.

As for other patients taken as control, only 15.9% showed ASLO titer of 250 Todd units or above.

9) Culture and Histological Findings of Extracted Tonsils

While it is still controversial as to whether or not tonsillectomy brings about a good result for the prognosis of nephritis, for the purpose of learning about the role taken by hypertrophied tonsil as focus of β -hemolytic streptococci, we did the following researches. We operated tonsillectomy upon patients who had

TABLE XI CLINICAL FINDINGS, STREPTOCOCCAL CULTURE AND HISTOLOGY AT TONSILLECTOMY

		Streptococci		Change of ASLO	Change of CRP	Change of Blood Pressure	Change of Erythrocyte in Urine	Change of Protein in Urine	Histology of Extracted Tonsil
		Throat Culture before Tonsillectomy	Culture of Extracted Tonsil						
Healed Group of Acute Nephritis	A.O. ♂						(+)		II
Postnephritic Albuminuria	K.O. ♂	(-)	(-)	(-)	(-)	(-)	(-)	(-)	III
	H. ♂	(-)	Ungroupable (+)	(-)	(+)			(+)	II
	H. ♂	(-)	(-)				(-)	(+)	I
	S. ♂	(-)					(+)	(-)	IV
	H. ♀	(-)	(-) Staphylo (+)	(-)		(-)	(-)	(+)	I
Non-Exacerbation Group of Chronic Nephritis initiated by Acute Nephritis	H. ♀						(-)	(-)	I
	Y. ♂	(-)	(-)	(-)	(+)	(+)	(-)	(-)	I
	K. ♂	(-)	(-)	(-)		(+)	(-)	(-)	I
Exacerbation Group of Chronic Nephritis initiated by Acute Nephritis	K. ♂	(-)	Gr. B (+)			(+)	(+)	(-)	I
	T. ♂	(-)				(+)		(+)	IV
	T. ♂	(-)	(-)	(-)	(-)	(-)	(+)	(+)	IV
Exacerbation Group of Chronic Nephritis not initiated by Acute Nephritis		Gr. A (+) → Ungroupable (+)	Ungroupable	(-)	(+)	(+)	(-)	(-)	I
Total		1	3	0	3	5	4	5	I=7, II=2 III=0, IV=3

TABLE XII SENSITIVITY OF STREPTOCOCCI TO ANTIBIOTICS

Group	Penicillin		Streptomycin		Oxytetracycline (Tetracycline)		Chloramphenicol		Chlortetracycline		Sulfaisoxazol		Erythromycin
(Type)	Strains	Concentration of Sensitivity Disc 10u. 1.0u. 0.5u.	Concentration of Sensitivity Disc 100mg 10mg 1.0mg	Concentration of Sensitivity Disc 60mg 30mg 10mg	Concentration of Sensitivity Disc 60mg 30mg 10mg	Concentration of Sensitivity Disc 60mg 30mg 10mg	Concentration of Sensitivity Disc 250mg 50mg 10mg	Concentration of Sensitivity Disc 15mg 5mg 2mg					
(12)	Y. S.	+	+	+	+	+	+	+	+	+	+	+	
	T. O. I	+	+	+	+	+	+	+	+	+	+		
	T. O. II	+	+	+	+	+	+	+	+	+	+		
	T. O. III	+	+	+	+	+	+	+	+	+	+		
	Y. I.	+	+	+	+	+	+	+	+	+	+		
(12)	Y. D.	+	+	+	+	+	+	+	+	+	+		
	T. H.	+	+	+	+	+	+	+	+	+	+		
B	M. Y.	+	+	+	+	+	+	+	+	+	+		
	I. M.	+	-	(T. +)	+	+	+	+	+	+	+		
	M. u.	+	+	-	(T. +)	+	+	+	+	+	+		
C	I. S.	+	+	+	+	+	+	+	+	+	+		
G	S. M.	+	+	+	+	+	+	+	+	+	+		
	I. K.	+	+	+	+	+	+	+	+	+	+		
Unknown group	I. F.	+	+	-	+	+	+	+	+	+	+		
	A. u.	+	+	+	+	+	+	+	+	+	+		
	M. R.	+	+	+	+	+	+	+	+	+	+		
	H. U.	+	+	+	+	+	+	+	+	+	+		
	R. R.	+	+	+	+	+	+	+	+	+	+		
	K. T.	+	+	+	+	+	+	+	+	+	+		
	H. F. I.	+	+	+	+	+	+	+	+	+	+		
	H. F. II.	+	+	+	+	+	+	+	+	+	+		
	H. F. III.	+	+	+	+	+	+	+	+	+	+		

passed through acute stage of nephritis and who had been sufficiently administered with antibiotics. Culture of β -hemolytic streptococci was conducted on extracted tonsils. Histological findings of extracted tonsils, changes in clinical symptom before and after tonsillectomy, as well as prognosis were closely observed.

In accordance with Prof. HAYASHI of Department of Pathology, Kumamoto University, histological findings of tonsils were classified into the following types⁷²⁾.

Recurring type with adhesion around tonsil	Type I	} Group R
Recurring type with adhesion around tonsillar lacunae perse	Type II	
Severe but not-recurring (acute) type	Type III	} Group A
Mild but not-recurring (acute) type	Type IV	

Applying the above method of classification to 12 cases of tonsillectomy operated on nephritic patients, the following results were obtained (see table XI).

In more than one half of the cases, Type I was proved, most of which indicated chronic nephritis.

Six extracted tonsils out of 7 which were classified as type I were cultured. In 3 cases out of the 6 extracted tonsils cultured, streptococci with small hemolytic ring were found. In two instances out of the above 3, no streptococcus was so far found at throat culture which had been carried out before and after tonsillectomy. The remaining one was the case of exacerbation of chronic nephritis not apparently initiated by acute glomerulonephritis in which, while at the time of exacerbation, group A streptococci of unknown type had been found, the streptococci had disappeared as a result of administration of antibiotics, and after then streptococci with small hemolytic ring was frequently found. In another case, staphylococci were found. Histological findings of tonsils in which streptococci were found showed types I and II. Though after tonsillectomy in four cases increase of hematuria and in five cases increase of albuminuria were proved, no interrelation was found between those increases and isolation of streptococci from the extracted tonsil. There were also some in which after tonsillectomy CRP and erythrocyte precipitation

test showed temporarily aggravated result, but they recovered with 3 weeks, and no fractuation of ASLO was caused by tonsillectomy.

10) Impact of tonsillectomy upon nephritis

After tonsillectomy, test of urinary protein, of urinary sediments, of blood pressure, of renal function and so forth were continued for a long time. But in none of 12 cases of tonsillectomy, any improvement of clinical symptoms was resulted from the tonsillectomy.

11) Sensitivity of isolated streptococci for antiviotics

We examined sensitivity for antiviotics of 22 strains of streptococci isolated from throats of nephritic patients and from control group as well. As shown in table (XII), all strains proved to have sensitivity for erythromycin, achromycin and chloramphenicol. Against oxytetracyclin and penicillin, a few proved to have resistance at low concentration thereof. Against streptomycin, more than half strains proved to have resistance of various degrees. For sulfaisoxazol, most of all strains did not have sensitivity. As is shown in a case of H.F., though organisms originally isolated had sensitivity, after administration of sulfa-drug sensitivity was lost.

12) Impact of antibiotics upon streptococci in throat (Table XIII)

Administering various antibiotics upon patients from whose throat streptococci was isolated, we observed their impact upon organisms, and obtained the following results. Organisms disappeared when penicillin, erythromycin and chloramphenicol were administered. But administration of sulfa-drug did not bring about the disappearance of organisms. Those facts are in consistence with the results of sensitivity test of isolated streptococci dealt with in the preceding chapter. Despite the fact that isolated organisms disappeared as a result of administration of antibiotics (penicillin, erythromycin and chloramphenicol), the disappearance took place with regard to typical streptococci

TABLE XIII THROAT CULTURE, ASLO AND SERUM COMPLEMENT IN 3 CASES

TABLE XIV SERUM COMPLEMENT AND R.A.T.

	Complement	Case of Number	Measurement within 3 months after the Onset of Nephritis		Measurement over 3 months after the Onset of nephritis		R. A. T.	
			Normal	Decrease	Normal	Decrease	(+)	(-)
Acute	Healed Group	5		2			2	
Nephritis	Active Group	2						2
Postnephritic Albuminuria		14			3			
Subacute Stage of Nephritis		4			1			2
Chronic Nephritis initiated by	Exacerbation	(-)	10		3			2
Acute Nephritis		(+)	6		2			1
Chronic Nephritis not initiated by	Exacerbation	(-)	5				1	
Acute Nephritis		(+)	3					
				1 (Exacerbation)				
Interstitial Nephritis		1			1			
Nephrotic Syndrome		8			4	2		2
Total		58		3	14	2	3	9

only. And even during administration of antibiotics sometimes streptococci with small hemolytic ring were isolated, which were of unknown group.

13) Serum Complement

As is shown in table XIV, in two case measured at the time of onset of acute nephritis and in a case measured at the time of exacerbation of chronic nephritis, decrease of complement had been observed clearly since exacerbation, and after a month of decrease, the complement became normal. In the group of

nephrotic syndrome, inspite of decrease of complement observed in two case, no other cases marked the decrease thereof. In no case of chronic nephritic patients, decrease in complement titre was observed. And this did not change even the measurement was done after tonsillectomy.

14) Virus complement fixation reaction
(Table XV)

The test of virus complement fixation reaction of Influenza A and B, adenovirus, ECHO virus etc. was conducted in 13 cases. Out of

TABLE XV THROAT CULTURE, ASLO, VIRUS COMPLEMENT FIXATION TEST IN TWO NEPHRITIC PATIENTS

the 13 in two cases, in which patients had caught by pharyngitis during Influenza B epidemic and had not been administered with antibiotics, no streptococcal infection was proved but rise of titre of Influenza B virus complement fixation reaction was found. I will discuss in detail on these two cases in the following chapter.

15) Study on preceding infection in each group of nephritis

i) Healed group of acute nephritis (5 patients)

In this group, disappearance of albuminuria from which we could conclude that nephritis healed was observed either at the time of discharge or thereafter. Pharyngitis as preceding infection was observed in 4 cases among the 5. And in the remaining one, F.O.'s case, though pharyngitis was not proved, increase of ASLO was observed. Period between preceding infection and onset of nephritis was from a week to 3 weeks. In both cases of Y.S. and A.O., as a result of throat culture at the time of admission, Group A type 12 was isolated and ASLO measured 250 Todd units. When penicillin was injected streptococci disappeared in both cases for a time, but while A.O.'s case streptococci were never isolated thereafter, in Y.S.'s case atypical streptococci were sometimes

isolated which were of unknown group. In case of F.O. at throat culture at the time of admission, atypical streptococci (of unknown group) were isolated, — this may be because he was admitted more than 3 months after the onset of nephritis, — and ASLO titre measured from 250 to 500 Todd units. In M.T.'s case, because the patient was admitted 40 days after the onset of nephritis and had been administered with erythromycin and oral penicillin before admission, at throat culture no streptococcus was isolated, and ASLO titre measured 650–1250 Todd units. In H.A.'s case, since throat culture was not carried out and ASLO titre also recorded less than 125 Todd units, it was impossible to prove whether he had been infected by streptococci. As to complement titre, A.O.'s case showed the following results.

0.1 c.c. ; 23 days after the onset of nephritis

0.2 c.c. or more ; 26 days after the same.

0.04 c.c. ; 47 days after the same

From these results it was learned that the decrease of serum complement continued for 1 month following the onset, and then resuming the normal value by the 7th day. As to CRP, A.O.'s, F.O.'s and M.T.'s cases showed negative. In cases of F.O. and M.T., though both did not reveal rheumatoid arthritis symptoms,

the results of RAT were two positives to three positives. In brief, in this group of 5 cases the cases in which preceding infection of group A type 12 streptococci was proved numbered only 2. In one case, the infection of atypical β -hemolytic streptococci and the increase of ASLO were observed, and in another the increase of ASLO but no isolation of streptococci (probably because of adequate administration of antibiotics) were the results, whereas in a third case due to lack of sufficient materials, streptococcal infection was not clear.

ii) Active group of acute nephritis (2 cases)

Cases collected in this group were those recognized as the active form of acute nephritis in view of clinical findings and renal biopsy findings after the onset of acute nephritis. In A.F.'s case, though preceding infection was not clear, streptococci were isolated at the time of admission, i.e., two days after the onset of nephritis, and ASLO at that time showed 250 Todd units. The patient hasn't been in hospital long enough to heal completely. Although Y.O.'s case also indicated preceding infection and 2 years and a half had passed by the time of admission since the onset, still sulfosalicylic acid test showed two to three positives regarding urinary sediments, red blood cell counted 1 to 2 to several per one sight field; white blood cell, 1 per several sight fields; tubular epithelia, several per all sight fields; and urinary cast was negative. In the 4th week after the admission, biopsy of right kidney was executed. As a result thereof, glomeruli counted 3 or 4, which, though slight nuclear proliferation of endothelia was observable, mostly revealed normal picture, chronic picture including hyalinization being hardly observable. The flattening of proximal convoluted tubular epithelia, the defoliation of epithelial cell and the existence of albumin-like substance in tubular lumina were also observed. But there were no other findings except those above mentioned. Histological findings of renal biopsy showed recovery stage of acute glomerulonephritis of mild form. Also in the case of Y.O., no streptococcus was isolated from the throat culture at the time of admission, yet ASLO showed 500 to 625 Todd units by the unknown cause. Thus, out of the two cases con-

sisting of this group, in one case the infection of streptococcus was proved, while in another, despite the collapse of fairly long period after the onset, high ASLO titre, mild form of inflammatory picture in glomeruli at renal biopsy findings, and albuminuria were characteristic.

iii) Group of post-nephritic albuminuria (14 cases)

The 14 cases classified into this group are those in which, more than half a year after the onset of acute nephritis, albuminuria persisted slightly and at times sulfosalicylic acid test showed the negative result, exercises resulted in the increase of albuminuria, and taking bath also increased urinary protein. None or only a few of red blood cell was found in urinary sediments. Inflammatory reaction was not proved by renal biopsy, in glomeruli, the fact of which indicated the convalescence of nephritis.

As is shown in table II, except 5 cases with unclear infection, all developed nephritis within one or three weeks after the attack of pharyngitis (among which, S.F.'s case will be dealt with later). Out of three cases of early admission (M.H. O.K. C.H.), as to C.H., throat culture was not carried out, and ASLO was 166 Todd units., when measured only once at the time of admission.

O.K.'s case which had been administered with penicillin before admission, did not isolate streptococci at throat culture at the time of admission, but later atypical streptococci were isolated, and ASLO titre in this case was unable to be measured. In case of C.H., group A type 12 streptococci were isolated at the time of admission and ASLO titre recorded 250 Todd units. In other cases, (i.e., N.T., Ko.Ko., S.H., Ka.Ka., M.H., Y.S., F.O., K.S. and E.M.) in all of which patients were admitted because albuminuria did not disappear long after the acute stage of nephritis had been through, ASLO was less than 166 Todd units.

As for the result obtained from throat culture at admission of this group, in two cases of Y.S. and M.H., group G streptococci and un-identifiable atypical streptococci were isolated respectively. In the case of S.H., though no streptococci were isolated at throat culture at

the time of admission, un-identifiable atypical streptococci were isolated from his extracted tonsil. In an interesting case of S.F. who, at the age of 8, had developed scarlet fever after the attack of pharyngitis and one week thereafter again developed nephritis, whereupon she was treated in hospital for three months. After three months, she was discharged as completely cured, and the result of urinary examination was always protein negative thereafter. Nevertheless, in the end of February, 1961, that is twenty years after the afore mentioned discharge, she caught pharyngitis during type B Influenza epidemic with fever. At around 5 days thereafter, when she visited me, she was in condition of facial edema, positive urinary protein, negative microscopic hematuria, and high blood pressure. Examinations revealed that blood pressure was 150/90; urinary protein positive; red blood cells in urinary sediment (1-2/several sight fields); white blood cells (1-2/several sight fields); renal epithelia (1-2/many sight fields); granular casts (1/several sight fields); pharynx congested; tonsil hypertrophied; streptococci at throat culture, negative; and no antibiotics had been administered before. About two months later, biopsy of right kidney was executed. Among 10 glomeruli, fibrosis was observable, in one glomerulus, while in the 8 others, the increase of nuclei. The exudation of albuminlike substance in capsular spaces, the flattening of epithelia of proximal convoluted tubuli, and the enlarged picture of lumina were observed, but except these findings, no abnormality was observable. In this case of S.F., renal function resumed normalcy later on, but slight albuminuria has been still proved. The results of measurement of virus complement fixation reaction in this case are as follows. 30 days after the infection, only Type B Influenza proved positive at 64 times of dilution of serum, while 50 days after the infection, it became positive at 32 times of dilution. From the above facts, it is assumed that the invasion by Type B Influenza might have taken place at the end of February. This is a case in which the development of nephritis may be considered to have been caused by the infection of Influenza virus

without streptococcal infection. Thus in the group of postnephritic albuminuria, there are a case of C.H. of early admission in which group A type 12 streptococci, a case administered with antibiotics prior to admission in which no streptococcus was isolated, a case in which no throat culture was engaged but ASLO titre was normal, and the other 10 cases in which no increase of ASLO titre was recorded and in which while streptococci were isolated in part, its relation to the disease is unclear and its relation to present symptom also is unthinkable. Only in a case of S.F. in which 20 years after healing nephritis nephritic symptom reappeared after the infection of pharyngitis, no streptococcus was isolated. As to the cases of M.H., Ke.Ka. and Ka.Ka. serum complement titre was normal. None of all 12 instances of measurement of CRP was positive, and RAT was all negative in five instances of measurement.

iv) The group in subacute stage of nephritis (4 cases)

The four cases classified into this group are all those in which nephritis was developed one or three weeks after the infection of pharyngitis. None among them was admitted at the early stage of development, and in all of the four, ASLO titre was less than 166 Todd units, while only in H.M.'s case atypical streptococci were isolated from throat culture. In case of Si. Ok., the patient died five months after the onset of nephritis. In three cases other than that of Si. Ok., diagnosis of subacute nephritis was rendered as a result of renal biopsy.

Taking as an example the result of left renal biopsy of H.M.'s case, in 31 glomeruli counted therein, the thickening of glomerular basement membrane, the increase of mesangium, the exudation of albumin-like substance in capsular spaces were observed. In 5 glomeruli out of the 31, the increase of epithelia of Bowman's capsule forming crescent were found and in 2 glomeruli, necrotizing were observed. While ischemia and swelling of capillary which are usually observable at the acute stage (of nephritis) were not conspicuous, fibrosis which appears usually in chronic picture of nephritis was not recognized in this case. Although the

slight flattening of epithelia of proximal convoluted tubuli and the widening of lumina were observable, no conspicuous degenerative picture was found.

In a part of lumina, red blood cell casts and albumin-like casts were found. Though a part of arteriole revealed something like fibrotic proliferation picture, no other conspicuous changes were recognizable in general. Cell infiltration was also observable locally in interstitium. Histological diagnosis were (1) subacute diffuse glomerulonephritis with crescent formation and (2) focal interstitial inflammation. CRP at the time of admission was negative in all of the 3 cases. RAT rendered negative results in both cases to which the test was applied. In case of Si.Ki., in which tonsillectomy was carried out, no streptococcus was isolated from the extracted tonsil and no change in clinical findings was found thereafter. Studying thus on the 4 cases in the group in subacute stage of nephritis, the following facts were characteristic, i.e. in all cases there was a long interval between the times of attack and of admission; no increase of ASLO titre was observable; and except a case in which atypical streptococcus was isolated, no β -hemolytic streptococcus was isolated from throat culture.

v) Non-exacerbated group of chronic nephritis initiated by acute nephritis (10 cases)

Ten cases included in this group are all those which, starting with the onset of nephritis, developed into chronic nephritis. Among the 10 cases, two cases were admitted at the early stage after the onset of nephritis, (i.e. the cases of To.Ha. and Si.Ya.). In case of To.Ha., the patient was attacked by pharyngitis with the rise of body temperature 39.5°C . 10 days after nephritis developed and 2 weeks thereafter he was admitted to our hospital. Group A type 12 streptococci were isolated, in throat culture of the time of admission, with ASLO titre showing 250 Todd units. In case of Si.Ya., nephritis developed 3 weeks after the attack of pharyngitis, and 3 weeks after the development of nephritis, he was admitted. The patient had received treatment on his pharyngitis from a medical practitioner before admission. Therefore when he was admitted, no streptococcus was isolated, and ASLO titre

also remained within normal value. After the admission, his tonsil inflamed whereupon tonsillectomy was executed. But *nistreptococcus* was isolated from the extracted tonsil and no improvement was also found in clinical findings thereafter. In other cases the patients were admitted 1 to 20 years after the onset of nephritis. Some of patients (Ka.As. R.M. and H.O.) were already in uremic stage and no β -hemolytic streptococcus was isolated from throat culture at the time of admission. The increase of ASLO titre was not proved in them. From these facts it seems that there is no connection between the infection of β -hemolytic streptococci and present clinical symptoms in these cases.

vi) Exacerbated group of chronic nephritis initiated by acute nephritis (6 cases)

All of cases included in this group were those which had experienced the attack of acute nephritis in their past illnesses and which brought about exacerbation of nephritis due to another infection thereafter. In the case of Iw.Ma. nephritis had developed one year prior to the admission and had improved without hospitalization. One week before the admission, the patient had high fever and when visited us, he complained of dullness and was given the diagnosis of exacerbation of nephritis. After the admission, though he was given the diagnosis of acute pharyngitis by otorhinologist, no β -hemolytic streptococcus was isolated and ASLO titre also counted 125 Todd units.

In the case of Te.Ko., pharyngitis lasted for a week, with fever of 39°C and 2 weeks thereafter developed nephritis, whereupon the patient was admitted to our hospital, i.e. 3 weeks after the development of nephritis. At the time of admission, though many β -hemolytic streptococci were isolated from throat culture, all were un-identifiable group ASLO titre showed 71 Todd units. After a while, symptoms showed some improvement whereupon tonsillectomy was conducted. From the extracted tonsil, a great many group B streptococci were isolated. After tonsillectomy, this case resulted in macroscopic hematuria for a time. But the patient was dismissed while albuminuria was still observable.

A year and a half later, however, the patient,

as a result of infection after subcutaneous injection, exacerbated nephritis with high fever and macroscopic hematuria, and was admitted to our hospital again. But the patient, being scared of renal biopsy, dismissed himself from hospital, whereby no tests could be conducted.

In case of Gi.Ta., two years before admission the patient had caught pharyngitis, several days thereafter suffering from nephritis, but continued to work because nephritis improved before long. However, 5 days before the admission, the patient again suffered from pharyngitis resuming albuminuria. Since symptoms did not improve, the patient visited us. At the time of admission, no β -hemolytic streptococcus, was isolated from throat culture, and ASLO titre was 12 Todd units which never fluctuated since then 4 months after the admission he suffered from pharyngitis again, two days whereafter hematuria aggravated and macroscopic hematuria lasted for several days.

At this time, however, no increase of ASLO titre was found and no β -hemolytic streptococcus was isolated. When tonsillectomy was conducted on this patient later, by which macroscopic hematuria again appeared. No β -hemolytic streptococcus was isolated in tonsil culture. Findings of renal biopsy in this case two months after the admission are as follows: glomeruli were a little uneven in size, raging from those which had become fibrosis to those quite normal. As for capillary loops, there were those which showed mutual adhesion and adhesion to Bowman's capsule

Basal membrane was slightly thickened, and proliferation of mesangium fiber was observed, but no nuclear proliferation and ischemia were observed and capillaries were open. Characteristic finding was the proliferation of Bowman's capsular epithelia, some of which formed the so-called crescent. Epithelia of proximal convoluted tubuli which belonged to nephron with noticeable lesion of glomeruli showed remarkable hyalin-droplet degeneration, the flattening of epithelia, and the enlargement of lumina. Bleeding picture is hardly observed, and except two tubuli which were filled with red blood cells almost no cast was recognized in lumina. As for blood vessels, increase of nuclei was observable, raging from

vas afferens to juxtaglomerular apparatus and as for arterioles, slight thickening was recognizable. As for interstitium, although proliferation of part of connective tissue and infiltration of small round cells were observable, those changes were generally slight.

In case of Ma.Ta. who had developed nephritis at the age of 14 and had been hospitalized as a result of nephritis symptom consulted us ten days after developing pharyngitis at the age of 23, the patient (who is now 28 years old), was this time attacked by tonsillitis one month before the admission with the rise of fever of $37^{\circ}.5^{\circ}\text{C}$, and 3 weeks thereafter the patient was diagnosed to nephritis. In this case also, no β -hemolytic streptococcus was isolated at the time of admission and ASLO was 12 Todd units then. Renal biopsy conducted one month after the admission, revealed the followings; i.e. slight increase of mesangium of glomerular capillary loops; hyalin-like thickening of basal membrane; thickening of Bowman's capsule; enlargement of lumina with regard to tubuli; flattening atrophy of epithelial cells; hyalin-droplet degeneration and fatty degeneration in part thereof. No urinary cast was recognized to have been filled. As for blood vessels hyalin-like thickening of intima of vas afferens and arterioles was recognized. As to interstitium, except slight increase of connective tissue, no other changes were observable.

In case of Te.Ma., nephritis had developed after getting pharyngitis 7 months prior to the admission, which improved there-after. Two days before the admission, however, the patient again caught pharyngitis with fever of $38^{\circ}.5^{\circ}\text{C}$ and was admitted to our hospital due to macroscopic hematuria. At the time of admission, no β -hemolytic streptococcus was isolated and ASLO also showed no higher than 100 Todd units.

Finally, in case of T.N. acute nephritis had developed when the patient was 5 or 6 years old, from which he recovered later. Since then, for about 24 or 25 years, he had never took out measurement of blood pressure; nor urinalysis and reached to the age of 31. Then, he caught pharyngitis with fever of $37^{\circ}.6^{\circ}\text{C}$, several days thereafter feeling grave dullness, he received clinical examinations. He was

found to be in the stage of heavy albuminuria, of microscopic hematuria, and of hypertension, whereupon he was admitted. Though at the time of admission his tonsil showed enlargement and congestion, no β -hemolytic streptococcus was isolated from throat culture and ASLO was between 50 and 100 Todd units. Complement fixation reaction to Influenza B at the time of admission was not recognized even in 4 times serum dilution. But three months after the admission the reaction was recognized in 16 times serum dilution, from which it is inferred that the infection of Influenza B motivated the exacerbation of nephritis.

In this case, renal function was very poor (renal clearance; renal blood flow 232 c.c./m., glomerular filtration ratio 30.1 c.c./m., filtration fraction 209%, non-protein nitrogen 41.1 mg/dl). Renal biopsy also revealed that 4 glomeruli out of 9 transformed into perfect fibrosis, nephron resulted in organization. All remaining glomeruli were imperfect and those which seemed to be susceptible counted only 2.

vii) Group of non-exacerbation of chronic nephritis not initiated by acute nephritis (5 cases)

5 cases classified in this group are those in which no acute nephritis in past illnesses nor subjective symptoms were recorded but which revealed albuminuria at health examination and were admitted to our hospital to receive close examination. In all cases no β -hemolytic streptococcus was isolated from throat culture at the time of admission, and ASLO titres was 12 Todd units (Ke.Hi), 100 Todd units (No.Sa.'s case), 12 Todd units (Tu.Ue.'s case), 50 Todd units (Yo.Wa.'s case) and 166 Todd units (Ya.Ko.'s case) respectively.

In case of T.U., at the time of health examination one year before the admission, albuminuria and microscopic hematuria had been pointed out. At the time of admission, clinical findings were the same, and moderate decrease of renal plasma flow, renal blood flow and glomerular filtration ratio were recognized through renal clearance. Renal biopsy at the time of admission, (conducted upon right kidney, glomeruli counted 27) revealed the following changes: i.e., as to glomeruli hyalin-like

thickening of capillary tufts, their mutual adhesion, nuclear proliferation and ischemia in general and adhesion to Bowman's capsule in part. As for tubuli mainly in the part of proximal convoluted tubuli, hyalin-droplet degeneration of cells, flattening thereof, enlargement of lumina, casts were recognized. Although in a part of vas afferens thickening of intima were observable, no conspicuous change was recognizable in artery, arteriole and interstitium.

In case of Ya.Ko., though the patient showed no symptom of rheumatoid arthritis, RAT indicated 3 positives.

viii) Group of exacerbation of chronic nephritis not initiated by acute nephritis (3 cases).

Cases included under this heading are also those which were found albuminuria by chance, and aggravated nephritis later through infection. In case of To.On., albuminuria was pointed out when he received health examination to subscribe to life insurance policy 6 months before the admission. But the patient, being unconscious of any symptom of the disease particularly, did not take any further treatment. Then 3 weeks before the admission, he caught pharyngitis and ten days thereafter, manifestation of edema and hematuria was suspected of exacerbation of nephritis. At the time of admission, group A un-identifiable type of streptococci were isolated every day, ASLO showing 166 Todd units. Ten days after the admission (i.e. about one month after pharyngitis) ASLO rose up to 330 Todd units which lasted for a month, and then declined. Serum complement disappeared at the time of admission (i.e. two weeks after developing nephritis), then gradually recovered, and became almost normal one month after the attack of nephritis. In this case, after using penicillin β -hemolytic streptococci disappeared promptly but even after then, despite continued use of antibiotics, atypical β -hemolytic streptococci kept to be isolated, which were un-identifiable. As a result of tonsillectomy conducted 5 months after the admission, un-identifiable atypical β -hemolytic streptococci were isolated from the extracted tonsil. Renal clearance of this case indicated that even after recovering the acute from stage, both renal blood flow

and glomerular filtration ratio moderately declined. And renal biopsy soon after the admission, revealed; proliferation of cells, swelling of glomerular tufts, slight exudation of albumin-like substance into capsular space, slightly open capillary lumina, and passage of red blood cells. Severe congestion was not recognized. Fibrosis of one glomerulus was observed, which in not recent but old change. As for interstitium, increase of connective tissue was observed in this part, and light edema was also recognized in general.

ix) Group of chronic interstitial nephritis initiated by acute onset (1 case)

This group consists of only one case of N.H. The patient in this case developed pharyngitis with fever of 38°C., which declined to normal after 2 days. However, ten days thereafter, the patient suffered from nephritis with macroscopic hematuria and was admitted to a hospital. In spite of the hospitalization, macroscopic hematuria and heavy albuminuria dragged on. Since no improvement resulted from the hospitalization, the patient moved to our hospital after a month. The diagnosis of "interstitial nephritis" was given as a result of renal biopsy conducted 2 months after the development of nephritis in which conspicuous cell infiltration mainly consisting of small round cells, polymorph-nuclear white blood cells, plasma cells and fibrocytes was found in interstitials of renal cortex. But the renal biopsy also revealed the following findings, i.e., changes in glomeruli were rather scarce, and except recognizable formation of hyalin-like thrombosis in part of capillary loops, nuclear proliferation was slight, no bleeding in capsular space was recognized, tubuli revealed the stage of devastation and of atrophy. Degeneration was remarkable and blood filled lumina here and there, and slight proliferation of cells was observed in tunica media of arteriole. At the time of admission atypical hemolytic streptococci were isolated from throat culture, which was un-identifiable. After that, penicillin was used sufficiently, as a result of which no β -hemolytic streptococcus has come to be isolated from throat culture. ASLO indicated 166 Todd units at the time of admission, which lasted for 2 months and then declined. Hematuria

improved 3 months after the development, resulting in 400 times per one sight field, and 2 or 3 red blood cells per one sight field. And one year after the admission, (i.e. at the time of dismissal) protein in urine showed through sulfosalicylic acid test two to three positives.

x) Group of nephrotic syndrome (8 cases)

Out of 8 cases classified in this group, two cases of Ma.No. and Tsu.Sh. suffered from acute nephritis after the attack of pharyngitis and since then gradually proceeded to reveal symptoms of nephrotic syndrome. The case of Sh.Is. developed pyelonephritis from pyelitis and had come to add nephrotic syndrome. In the case of Ma.Mo., while the patient was being treated on tuberculosis in sanatorium, albuminuria, microscopic hematuria and hypertension were recognized, and had come to show nephrotic syndrome. In case of Mi.Ya., nephrotic syndrome developed from toxemia of pregnancy. In case of Wa.Im. and Ya.Fu. nephrotic syndrome developed from his overwork with the results of albuminuria and edema. And in the case of By.Ha., despite no motive was conceivable, nephrosis with anasarca developed. In cases included in this group no β -hemolytic streptococcus was isolated from throat culture at the time of admission. ASLO titre showed less than 100 Todd units in all cases and β -hemolytic streptococcal infection was observable in no cases. As to the serum complement, in case of Ry.Ha., it indicated disappearance and never resumed even after treatment, but in cases of Mi.Ya., Ma.Mo. and Wa.Im., it was normal.

DISCUSSION

Since Schick's report¹⁾ on *Str. scarlatinae* was published, acute diffuse glomerulonephritis has been considered to be motivated by the streptococcal infection in upper respiratory tract. Furthermore, when epidemic hemorrhagic nephritis was at its peak recently, many of group A β -hemolytic streptococci (types 12, 4 and 25 especially) were isolated from the swabb of pharynx of its patients¹⁰⁻³⁰⁾. As a result thereof these types were named nephritogenic types or strains¹⁰⁾. However, there is another report which tells the fact that nephritis followed the infection of β -hemolytic streptococci of the types other than those

nephritogenic strains³²⁾. And also, there is a report⁴⁰⁾ of no streptococcal infection proved preceding to the development of nephritis despite of various inspections. And again there is a report⁴¹⁾ on a fact that nephritis outbreak following the viral infection. In case of acute glomerulonephritic patients, even though β -hemolytic streptococcal infection was observable in upper respiratory tract, no organisms were isolated from kidney, of which fact, therefore, does not satisfy Koch's three principles. In the case of streptococcal infection, especially in the case of scarlet fever, since administration of antibiotics can not prevent nephritis from development, the impact of β -hemolytic streptococci upon acute nephritis is not sufficiently expounded. From the facts that there is a latent stage between the streptococcal infection and the outbreak of nephritis, that ASLO titre rises⁵⁴⁾, that serum complement temporarily decreases³³⁾³⁴⁾⁷³⁾, and that mechanism of the development of Masugi's nephritis³⁵⁾³⁶⁾ resembles histological findings of kidney, theory of allergy is generally attributed thereto. Nevertheless in case of rheumatic fever which is also considered to follow streptococcal infection, anti-allergic drugs proves remarkable effect, yet in case of nephritis except the group of nephrotic syndrome, no effect is proved in this regard.

We studied on the pathogenesis of nephritis, mainly on its relation with β -hemolytic streptococcal infection, taking nephritic patients admitted to Maekawa's Clinic of Kyoto University Hospital as materials. The acute nephritic patients dealt with in this report are all adult patients who developed nephritis sporadically and none of whose family developed nephritis at the same time. From more than half of the patients, β -hemolytic streptococci were isolated, in half of which group A type 12 streptococci were proved and significant difference from control was admissible. In those patients, streptococcal infection is proved to have close relation with the development of acute nephritis. The fact that group A type 12 streptococci were isolated from nephritic patients alone while no patients in control groups isolated the same organisms is quite impressive and endorses Rammelkamp's theory

of nephritogenic type¹⁰⁾. But the fact that the route of streptococcal infection in these patients is not known and no subsequent development of nephritis took place among people with close contact with these patients tell us that the infectivity of β -hemolytic streptococci is weak, and that the point of whether nephritis did not develop from infection is entirely unclear. However, that these patients were not infected for long time is inferred from the fluctuation of ASLO titre at the time of outbreak of nephritis. In many nephritic patients, tonsillar findings are observable, of which relation to pathogenesis will be discussed later.

As to the latent period between the outbreak of pharyngitis and the development of nephritis, from a week to two were rather dominant. However, it is difficult to discuss on the pattern of antigen-antibody relation of acute nephritis as the streptococcal infection sequela from our clinical examination data alone. To do so, i.e., it seems to be necessary to conduct theoretically experimental study of antibody based upon the theory of tissue allergy (cellular phospholipid) as proposed by Prof. MAEKAWA³⁷⁾³⁸⁾. The fact that nephritogenic type among β -hemolytic streptococci is easily bacteriolysed by bacteriophage³⁶⁾ is interesting to the theory of bacteriophage of Prof. MAEKAWA. But in this research, we could not go so far as to touch upon this theory. As stated above, in spite of its unknown pathogenesis, precedence of upper respiratory infection of group A streptococci is recognizable as a motive of the development of acute nephritis. Though on one hand we have seen a case in which nephritis developed after the infection of Influenza B, as to the problem on mechanism of development of nephritis, we are not sure whether virus itself reacts upon kidney or whether nephritis develops as a result of allergy which can be considered to take place at the time of streptococcal infection.

We will now proceed to chronic nephritis, ELLIS⁷⁴⁾ classified nephritis into two categories, i.e., nephritis type I and nephritis type II. According to him, nephritis type I develops rapidly and preceding infection is observable in 84% of the cases. In 60% of these cases, patients are under 20 years old and the per-

centage to heal is high (82% healed perfectly). Nephritis type II develops unconsciously and preceding infection could be proved only in less than 5% of the cases. The ages of patients at the time of nephritic outbreak vary and the percentage of perfect convalescence is also under 5%. The author classified nephritic patients into two groups, the one which developed acute nephritis and the other in which initiation by acute nephritis is not clear. As for the patients in whom chronic nephritis developed from acute nephritis except those at the time of exacerbation, no streptococcal infection was observable. Also the direct relationship between streptococcal infection and the duration of inflammatory findings of glomeruli admitted by histological biopsy was recognized. Among the cases of chronic nephritis initiated by acute nephritis, we found cases which could be considered as exacerbation of nephritis resulted from the infection of Influenza B. These facts, when compared with rheumatic fever which usually develops from streptococcal infection and which differs from nephritis at the point that rheumatic fever becomes active by streptococcal reinfection, seem to tell us the difference of mechanism from each other. Because the latent stage between the exacerbation of nephritis and the infection of organisms other than streptococci is shorter than that in the case of usual streptococcal infection, the pathogenesis in this case seems to be different from that of the cases in which acute nephritis develops after streptococcal infection or is exacerbated by streptococcal infection. Therefore we have to look for another appropriate cause. As to the patients of chronic nephritis not initiated by acute nephritis, since no streptococcal infection was proved at the time of admission and nothing was known with regard to symptoms at the time of development of nephritis, the pathogenesis of chronic nephritis of this group and the reason why inflammation of glomeruli endures even several years after development of nephritis were unable to be clarified.

In all the cases of exacerbation of chronic nephritis, preceding infection was proved. But in case of chronic nephritis initiated by acute nephritis, streptococcal infection could not be

proved by inspection in nephritis exacerbated within a week after preceding infection. In two cases out of three of chronic nephritis not apparently initiated by acute nephritis, preceding streptococcal infection was recognized, in one of which exacerbation was resulted in 3 weeks after group A β -hemolytic streptococci infection and serum complement also decreased. From these facts it seems possible to conclude that the exacerbation takes place in this case through the same mechanism as when acute nephritis follows streptococcal infection. Though difference in pathogenesis of exacerbation is proved between the group of chronic nephritis initiated by acute nephritis and the group of chronic nephritis not apparently initiated by acute nephritis, the number of cases obtained is scarce. Also as to cases of chronic nephritis initiated by acute nephritis, exacerbation is resulted by the infection of organisms other than streptococcus, and as to cases of chronic nephritis not apparently initiated by acute nephritis, all that can be said is that there is a case in which streptococcal infection might cause exacerbation. Among the cases in the group of nephrotic syndrome, no one isolated streptococci, and there were cases in which infection was not proved at the time of development. From these facts, it seems that the relation between the development of nephritis and the streptococcal infection is not close. The case of interstitial nephritic patient counted only one. Therefore in this report, with regard to the group of nephrotic syndrome, except a fact that streptococcal infection was not observed, nothing can be said as to its pathogenesis. The relationship between tonsillectomy and nephritis. Opinions⁵⁹⁻⁷¹⁾ differ as to whether tonsillectomy has good impact upon nephritis or not. I conducted tonsillectomy upon nephritic patients who passed more than 3 months after the development of nephritis and administered antibiotics sufficiently when the patients passed through the acute stage. Although in some cases tonsillectomy thus conducted resulted in temporary worsening of urinary findings, in all cases, it proved neither bad effect upon nephritis nor improvement of renal function. Nevertheless, there is a case in which streptococci were iso-

lated from extracted tonsil. So, if to extract tonsil which indicates chronic inflammation is proved to be effective in lessening the chances of infection, thus prebnting the exacerbation of nephritis and if the conception of autoallergy is proved to be significant in mechanism of the development of nephritis, extraction of chronically inflammatory tonsil does not seem to be insignificant. The fact that the cases in which tonsillectomy resulted in temporary worsening of urinary findings did not necessarily isolate streptococci from the extracted tonsil culture may be considered to indicate that some other factors other than above stated streptococci were related to nephritis. Regrettable to say, however, the relationship between tonsillar tissue and nephritis could not be studied in detail in this report.

SUMMARY

1) In more than half of the cases which were admitted to our hospital within 3 months after developing acute nephritis, streptococcal infection was recognized. Among 7 cases in which streptococci were isolated; group A type 12 β -hemolytic streptococci were found in 4 cases; group A un-identifiable type in one case; un-identifiable group in one case, from which it can be deduced that infection of streptococci, especially that of group A type 12 has close relation to the development of nephritis. There is a case in which nephritic symptom was revealed by the alleged infection of Influenza B more than 10 years after convalescence of acute nephritis.

2) As to the case of chronic nephritis, except the time of exacerbation, no streptococcal infection was proved both in those initiated by acute nephritis and in those not apparently initiated by acute nephritis. Therefore, it seems that there is some other reason for its aggravation.

3) As to the exacerbated cases of chronic nephritis, though at the time of exacerbation of chronic nephritis initiated by acute nephritis, streptococcal infection could not be proved, in many cases nephritis developed within a week after infection. In one case, however,

exacerbation was recognized to be resulted from the alleged infection of Influenza B. Among exacerbation cases of chronic nephritis not apparently initiated by acute nephritis, in two cases out of three, preceding infection of streptococci was proved, while in the remaining one, latent stage lasted for 3 weeks. From these facts it may be concluded that the exacerbation of chronic nephritis results either from streptococcal infection or from infection of something other than streptococci, and mechanism of development of nephritis in these two categories is different.

4) As to the group of nephrotic syndrome, the cases in which preceding infection was recognized were few and no streptococci were isolated. It seems that the relationship between the development of this disease and streptococcal infection is not quite close. There is only one case of interstitial nephritis in which though preceding infection was recognized, no streptococcal infection was proved.

5) Neither aggravation nor improvement of nephritis resulted from tonsillectomy operated upon nephritic patients. Between the temporary worsening of urinary findings following the tonsillectomy and the isolation or non-isolation of streptococci from extracted tonsil culture, there seemed to be no interrelation whatsoever. Therefore, it seems that tonsil itself gives some impact upon kidney.

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REFERENCES

- 1) Schick, B., *Jahrb. f. Kinderh.* **65**: 132, 1907.
- 2) Wahrer, C. F., *J. A. M. A.* **5**: 1410, 1908.
- 3) Brown, L., In discussion, Epidemic Nephritis (Meeting in Medical Society of London, Nov. 13, 1916) *Brit. M. J.*, **2**: 723, 1916.
- 4) Brown, W. L., *J. Roy. Army M. Corps.* **25**: 75, 1915.
- 5) Evans, G., *M. Press.* **206**: 68, 1941.
- 6) Peace, P. G., *Brit. M. J.* **1**: 17, 1926.
- 7) Addis, T., *Glomerular Nephritis. Diagnosis and Treatment.* Mc Millan N. Y. 1948.

- 8) Klein, J., *Acta Med. Scandinav.* **129**: 156, 1947.
- 9) Pratas, S., *Amatus Lusitanus*, **3**: 539, 1944.
- 10) Rammelkamp, C. H. and Weaver, R. C., *J. Clin. Inv.* **32**: 345, 1953.
- 11) Mc Carty, M., *Streptococcal Infections*, Columbia Univ. Press., 1954.
- 12) Stetson, C. A. and Rammelkamp, C. H. et al, *Medicine* **34**: 431, 1955.
- 13) Reed, R. W., *Canad. M. A. J.* **68**: 448, 1953.
- 14) Wertheim, A. R., *J. Clin. Inv.* **23**: 359, 1953.
- 15) Siegel, A. C. et al, *Pediatrics*. **15**: 33, 1955.
- 16) Wilmers, H. I. et al, *Lancet* **2**: 17, 1954.
- 17) Earle, D. P. and Seegal, D., *J. Chron. Dis.* **5**: 1, 1957.
- 18) Stetson, C. A. and Rammelkamp, C. H. et al, *Medicine* **34**: 431, 1955.
- 19) Bennicke, T. et al, *Acta Med. Scand.* **139**: 253, 1951.
- 20) Rammelkamp, C. H., *J. Chron. Dis.* **5**: 1, 28, 1957.
- 21) Reinstein, C. R., *J. Ped.* **47**: 25, 1955.
- 22) Kleinman, H., *Minesota Med.* **37**: 479, 1954.
- 23) Updyke, E. L., *Science* **121**: 171, 1955.
- 24) Kodama, T., *Nippon Iji Shimpo* **1660**: 3, 1956.
- 25) Kodama, T., *J. J. A. Inf. D.* **31**: 4, 283, 1957.
- 26) Kodama, T., *J. Jap. Bac.* **12**: 7, 511, 1957.
- 27) Nakamura, *Nippon Rinsho* **14**: 11, 111, 1956.
- 28) Kasuga, et al, *J. Jap. Bac.* **12**: 10, 816, 1957.
- 29) Omori, G. et al, *Osaka City Med. J.* **4**: 1, 7, 1957.
- 30) Noguchi et al, *Shizuoka Eiken Nempo* **5**: 58, 1957.
- 31) Saslow, M. S. and Streifeld, M. M., *A. M. A. J. of Dis. Child* **91**: 555, 1956.
- 32) Goldsmith, H. J., *Lancet* **7048**: Sept. 27, 1958.
- 33) Fischel, E. E., *J. Chron. Dis.* **5**: 1, 34, 1957.
- 34) Uchida, *J. Jap. Society Int. Med. J.* **45**: 628, 1956.
- 35) Masugi, M., *Beit. z. Path. Anat. u. z. Allg. Path.* **91**: 82, 1933.
- 36) Masugi, M., *Ibid.* **92**: 429, 1934.
- 37) Maekawa, M., *Saishin Igaku* **8**: 422, 1953.
- 38) Maekawa, M., *Shinryo* **6**: 716, 1953.
- 39) Rammelkamp, C. H., *J. Chron. Dis.* **5**: 1, 28, 1957.
- 40) Bates, R. C., *Amer. J. Med.* **23**: 4, 510, 1957.
- 41) Michon, P., *La Press Med.* **68**: 9, 309, Feb. 20, 1960.
- 42) Brown, J. H., *Monogr. Rockefeller Inst. Med. Res.* No. 9, 1919.
- 43) Ando, K., *Jap. Bac. J.*, 1922.
- 44) Swift, H. F., *J. Exp. Med.* **73**: 127, 1943.
- 45) Swift, H. F., "Textbook of Bacteriology" edited by Dubos.
- 46) Lancefield, R. C., *J. Exp. Med.* **47**: 481, 1928; **57**: 571, 1933; **61**: 335, 1935; **47**: 91, 1928; **47**: 469, 1928; **71**: 521, 1940; **79**: 99, 1944; **84**: 449, 1946; **78**: 127, 1943.
- 47) Lancefield, R. C., *J. Exp. Med.* **47**: 769, 1928.
- 48) Ranz, L. A. and Randall, E., *Proc. Soc. Exp. Biol. and Med.* **59**, 22; 1945.
- 49) Ranz, L. A. and Randall, E., *Proc. Soc. Exp. Biol. and Med.* **70**: 414, 1949.
- 50) Anderson, H. C. and Mc Carty, M., *A. J. M.* **8**: 445, 1950.
- 51) Ogata, T., Monograph.
- 52) Committee on Standard Serological Procedures in Influenza Studies: *J. Immun.* **65**: 347, 1950.
- 53) Singer, J. M., and Plotz, C. M. *Am. J. Med.* **21**: 888, 1956.
- 54) Nagai, H., *Syonika-kiyo.* **3**: 4, 610, 1957.
- 55) Tobimatsu, G., *J. Jap. Bac.* **11**: 503, 1956.
- 56) Rantz, L. A., Randall, E. and Rantz, H. H., *Am. J. Med.* **5**: 3, 1948.
- 57) Rantz, L. A., Di Caprio, J. M. and Randall, E., *Am. J. Med. Soc.* **224**: 194, 1952.
- 58) Tomioka, H. et al, *J. Jap. Assoc. Inf. Dis.* **35**: 591, 1961.
- 59) Wyllie, W. G. et al, *Lancet* **1**: 1281, 1926.
- 60) Kono, G. et al, *Nippon Ijishimpo* **1799**: 15, 1958.
- 61) Tateno, K. et al, *J. Jap. Ped.* **62**: 926, 1958.
- 62) Itani, K. et al, *J. Jap. Ped.* **62**: 927, 1958.
- 63) Kobayashi, K. et al, *J. Jap. Ped.* **62**: 927, 1958.
- 64) Noro, Y. et al, *J. Jap. Ped.* **62**: 927, 1958.
- 65) Riml, O. et al, *Dtsch. Klin. Med.* **177**: 71, 1935.
- 66) Dorscheid, A. et al, *Dtsch. Med. Wschr.* **83**: 1937, 1958.
- 67) Chireskin, D. G. et al, *Zbl. f. d. g. K. H. K.* **71**: 201, 1959.
- 68) Addis, T., *J. A. M. A.* **85**: 163, 1925.
- 69) Irie, H. et al, *Syonika Rinsho.* **11**: 609, 1958.
- 70) Rubin, M. I., Nelson, Textbook of Pediatrics, 1959.
- 71) Frisk, A. et al, *Acta Paediatrica.* **33**: 349, 1945/46.
- 72) Noda, E., *Jap. Arch. Int. Med.* **7**: 569, 1960.
- 73) Lange, K., *Arch. Int. Med.* **88**: 433, 1951.
- 74) Ellis, A., *Lancet.* **1**: 1, 1942.