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Acute glomerulonephritis in Trinidad: serological typing of group A streptococci

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INTRODUCTION

The recent epidemic of acute glomerulonephritis in Trinidad is the third to be recorded there in the past 20 years (Symonds, 1960, Poon-King *et al.* 1967). The number of persons who suffered from the disease in the years 1964–6 probably exceeded 2000; between September 1964 and April 1966, 720 cases of acute nephritis were admitted to San Fernando General Hospital (Poon-King *et al.* 1967) and a further 528 to the Paediatric Department alone of Port of Spain General Hospital.

The first evidence that epidemic nephritis in Trinidad was a poststreptococcal disease, and that the primary streptococcal lesion was more often of the skin than of the upper respiratory tract, was obtained in San Fernando (Simon *et al.* 1965). In March 1965, 21 hospital patients with acute nephritis were examined; 18 of them had skin sepsis, and group A streptococci were isolated from the skin, nose or throat of 16 patients. Potter, Moran, Poon-King & Earle, (1967) have since examined the serological characters of 25 strains of group A streptococci isolated at that time, and have described a new M-type of group A streptococcus (provisional type 55) thought to have nephritogenic properties.

In September 1965, one of us (D. C. J. B.) arrived in Trinidad as a member of the staff of the Trinidad Regional Virus Laboratory, University of the West Indies, and began a study of the bacteriology of nephritis and skin sepsis. His work in Trinidad was carried out at first in the Trinidad Regional Virus Laboratory and later in the laboratories of the Port of Spain General Hospital.

We now report observations on the typing of the group A streptococci that were isolated from cases of nephritis, and of skin sepsis uncomplicated by nephritis, in the first year of this study and during the previous 6 months. The part played by streptococci of type 49 (the Red Lake streptococcus) as a cause of nephritis in Trinidad has already been described briefly (Maxted, Fraser & Parker, 1967).

MATERIALS

The population of Trinidad is concentrated chiefly in the western part of the island (see Fig. 1). Port of Spain and San Fernando have large general hospitals, which constitute the main part of the hospital service for the whole island. Each

can be regarded as serving about 400,000 persons. Nearly all the patients with nephritis on whom we made bacteriological observations had been admitted to one of these hospitals, but a few were in the small district hospital at Arima, about 20 miles east of Port of Spain.

Acute glomerulonephritis

We report the results of typing cultures of group A streptococci from a total of 117 cases of acute glomerulonephritis.

From the Port of Spain and Arima hospitals, in the period September 1965 to August 1966, we collected 133 cases in which swabs were obtained, and for whom the presence of oedema, albuminuria and haematuria was recorded; when the

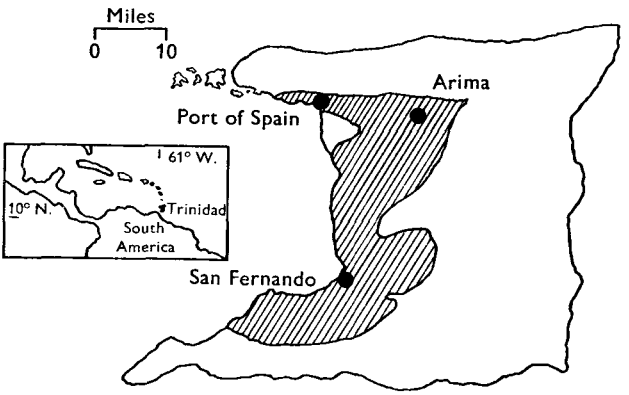

Fig. 1. Map of Trinidad. Shaded area: heavily populated savannah country. Inset: map showing position of Trinidad in relation to south and central America.

haematuria was detected only on microscopic examination, the number of red blood cells exceeded 10 per high-power field in the deposit after centrifugation. These patients formed about one-half of those on whom a diagnosis of acute glomerulonephritis was made during the period. Group A streptococci were isolated from 69 of the 133 patients.

From the San Fernando hospital within the same period we obtained streptococci from 38 patients who were subsequently confirmed as cases of acute glomerulonephritis.

The remaining ten cases had occurred in the Port of Spain area between May and August 1965, i.e. before the main part of the investigation was begun.

Uncomplicated skin sepsis in school children

Streptococci were isolated from the skin lesions, and also from nose and throat swabs, of children attending a number of schools. These included cultures obtained in the earlier part of 1965 in three brief school surveys. We carried out three further surveys between September 1965 and October 1966 as part of a general study of the epidemiology of skin sepsis, which will be described in a later publication. Brief details of these investigations follow: in surveys 1–5, nose, throat

and lesion swabs were collected from all children with skin sores, but in survey 6, nose and throat swabs were also collected from healthy children.

1. *Aranguez Schools.* Single visit to two small country schools near Port of Spain in April 1965.

2 and 3. *Princes Town and Mount Pleasant Schools.* Country schools near San Fernando. Swabbing on two occasions in June and July 1965 in connexion with a trial of the antibiotic treatment of skin sepsis.

4. *St Agnes Anglican School, Port of Spain.* Large urban school; single swabbing in November 1965.

5. *Mucurapo Girls' School, Port of Spain.* Urban school; six visits in 2 months, November 1965–January 1966.

6. *St Joseph Presbyterian School.* Small rural school 7 miles east of Port of Spain. Nose and throat swabs from all children weekly, and separate swabs of each skin lesion; April 1966 to April 1967, but only first 27 weeks reported here.

Survey of distribution of serotypes of group A streptococci in Britain 1964–65

A collection of 445 cultures of group A streptococci, isolated in 17 public health laboratories from all classes of streptococcal diseases, forming part of an international survey of the distribution of serotypes of group A streptococci (Parker, 1967), was included for comparison, because these cultures were typed with the same sera as the Trinidad streptococci.

BACTERIOLOGICAL METHODS

Cultural

The Port of Spain and Arima hospitals could be visited as required, and nephritis cases were seen and swabbed as soon as possible after admission. Visits to San Fernando were made no more often than once a week.

Plates of culture medium were always taken to the hospital ward or school, and swabs were inoculated on the medium immediately after the specimen had been taken. The specimens from the San Fernando hospital include some taken by the hospital staff and kept in transport medium (Holmes & Lermit, 1955) until the next visit to the hospital was made.

Cotton-wool swabs were used for the collection of all specimens; they were moistened with sterile physiological saline before sampling the nose or skin lesion.

The culture medium used was 5 % horse-blood agar containing crystal violet in a concentration of 10^{-6}, which was found to be optimal for the batch of dye being used. Cultures were incubated overnight at 37° C. in air.

Representative colonies of β-haemolytic streptococci were subcultured, and grouping was performed in Trinidad by the precipitin method after extraction with acid (Lancefield, 1933) or digestion with *Streptomyces* enzyme (Maxted, 1948).

At least one strain of group A streptococci from each positive culture was sent to Colindale for typing. A loop was charged from a pure culture on a blood agar plate. This inoculum was rubbed on to a sterile filter-paper disk, which was allowed to dry in air. The disk was then placed in a gelatin capsule which had previously

been sterilized by ultra-violet light. On receipt in London, each disk was transferred to a tube of nutrient broth containing 10% horse blood, which was then incubated. Less than 1% of the strains failed to grow.

Typing methods

A combined typing system was used (Williams & Maxted, 1953), in which the streptococci were first examined for T-antigens by slide agglutination and were subsequently tested by the precipitin method with a limited number of M-antisera determined according to the T-typing pattern of the strain.

Agglutination typing

Trypsinized suspensions of streptococci were tested by slide agglutination with antisera for the following T-types: 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19, 22, 23, 25, 27, 28, 44 and 47, and for the provisional T-types Imp. 19 and B 3264. Antisera of the series 5, 11, 12, 27 and 44 had been specially absorbed by the method of Pakula (1951; see McLean, 1953). During the course of the investigation, a method for the cross-absorption of T-antisera for types 14 and 49 was introduced (Maxted *et al.* 1967), and specially absorbed sera for these types became available. All streptococci which had been agglutinated by the routine T/14 serum were re-examined with the specially absorbed T/14 and T/49 sera.

The above antisera are the ones used in our routine typing of streptococci isolated in Britain, and our experience had been that they usually gave clear-cut results. With the Trinidad streptococci, many cross-reactions were seen; the most common patterns of cross-reaction were between type 49 and members of the 8, 25, Imp. 19 and of the 3, 13, B 3264 agglutination-complex, but a number of other unusual combinations were seen. Further investigation showed that these cross-reacting strains did not absorb agglutinins from all the antisera by which they were agglutinated. It was concluded that they possessed additional trypsin-resistant agglutinating antigens not represented in the current T-typing system. The antibodies to these cross-reacting antigens could be removed by further absorption of the T-typing antisera with Trinidad streptococci which were agglutinated by the sera but did not remove the type-specific antibody from them.

Absorptions of this kind were carried out on the antisera for T-types 3, 13, B 3264, 25, Imp. 19, 9, 12, and 49, and these sera were used for the examination of any cultures which gave unusual cross-reactions.

Precipitin typing

Capillary precipitin tests (Swift, Wilson & Lancefield, 1943) were carried out on acid extracts of organisms grown in Todd-Hewitt broth. The following antisera were used throughout the investigation: M-antisera for types 1, 2, 3, 5, 6, 9, 11, 12, 14, 15, 17, 18, 19, 22, 23, 24, 25, 26, 29, 30, 31, 33, 36, 37, 39, 41, 43, 46, 47, 48, 50 and 51; R-antisera for types 3 and 28. In addition, M-antisera for type 49 and for provisional types 52 and 55 became available during the investigation, and were used as will be described.

While this work was in progress, the value of adding Neopeptone to Oxoid

Todd-Hewitt broth also became apparent, particularly in increasing the yield of M-antigens for types 41 and 49 (see Maxted *et al.* 1967; Top, Wannamaker, Maxted & Anthony, 1967). All except some of the earlier cultures were grown in the improved medium, and early cultures having T-agglutination patterns suggesting that they might belong to either of these types were retested after growth in it.

RESULTS

The 1964–6 *epidemic of nephritis in Trinidad*

The course of the outbreak may be followed in the fluctuation of the rate of admission of patients with nephritis to the two main hospitals in Trinidad. The number of children with nephritis admitted each month to the Paediatric Department of the Port of Spain General Hospital is shown in Fig. 2. This curve shows a

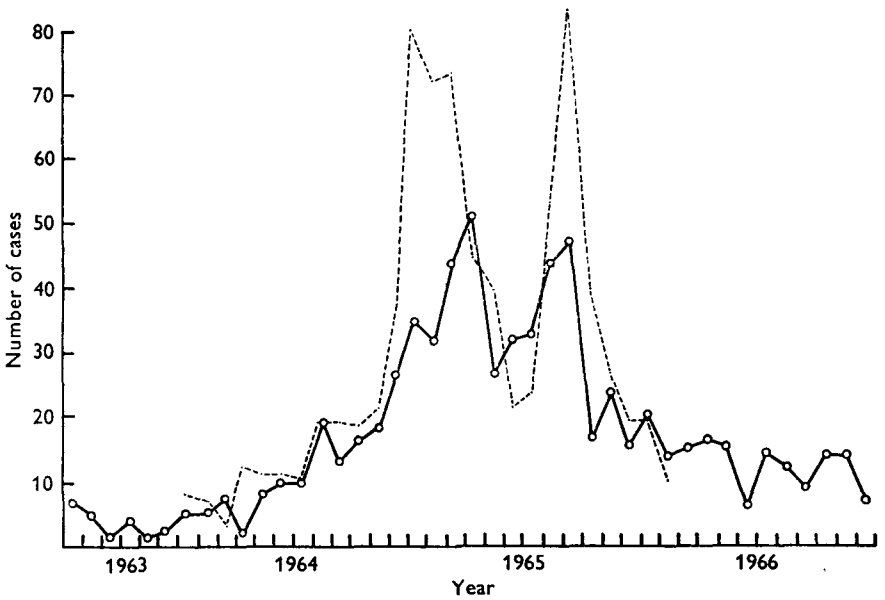

Fig. 2. Number of cases of nephritis admitted each month to two hospitals in Trinidad. O — O, Port of Spain General Hospital (Paediatric Department only). – – –, San Fernando General Hospital (Poon-King, 1968).

striking similarity to that for all admissions with nephritis to the San Fernando General Hospital, which is also shown in the figure (T. Poon-King, personal communication; Poon-King *et al.* 1967). The records of both hospitals show an outbreak extending from mid-1964 until early 1966, and in both there is a striking double peak in the admission rates, with an intervening interval of several months.

After the 1959–60 outbreak (Symonds, 1960) relatively few cases of nephritis were seen in either hospital for several years, and in 1963 admission rates to both were between three and five cases a month. In both hospitals there was some increase in early 1964, and a striking acceleration in the latter months of this year. This was particularly obvious in San Fernando, where the peak of the first wave

of the epidemic occurred in January 1965. The rise continued more gradually in Port of Spain, and the highest rate of admission here was in April 1965.

Admissions to both hospitals fell after April 1965, but rose again in the following August. The second wave of admissions reached its peak simultaneously in the north and the south in September 1965. By the end of the year admissions to both hospitals had fallen to the mid-1964 level.

The first bacteriological observations were made in March 1965, at the height of the first wave of nephritis cases in San Fernando, by Dr Elizabeth Potter, of Northwestern University, Chicago. Our main investigation began in September 1965, at the peak of the second wave, and extended until August 1966, when the main epidemic was over.

Table 1. *Isolation of group A streptococci from patients with acute nephritis admitted to the Port of Spain and Arima hospitals, September* 1964 *to August* 1965

	Total no. patients examined	No. patients with group A streptococci			
		Total	in skin lesion	in nose swab	in throat swab
With skin sores	101	67	64	17	7
Without skin sores	32	2	0	2	0
Total	133	69	64	19	7

Isolation of group A streptococci from cases of nephritis

Group A streptococci were isolated from 69 of the 133 patients in the Port of Spain and Arima hospitals (52 %), as shown in Table 1. Unhealed skin sores were observed on 101 of the patients on admission to hospital (76 %).

Group A streptococci were isolated from 67 of the 101 patients with skin sores (66 %). The streptococcus was present in the sore in 64, in the nose in 17, and in the throat in only seven patients. The organism was isolated only from a skin lesion in 48, from a skin lesion and the respiratory tract in 16, and from the respiratory tract only in three patients.

Very few isolations of group A streptococci were made from patients who did not have skin sores. In two out of 32 patients (6 %) the organism was isolated from the nose, but no streptococci were found in any of the throat swabs. The fact that group A streptococci were so seldom found in the respiratory tract in the absence of a skin lesion supports the view that nephritis did not often follow a primary respiratory tract infection in this epidemic.

Unpublished studies by one of us (D. C. J. B.) of uncomplicated skin sores in school children suggest that lesions not treated with antibiotics have a 'half-life' of about 10 days. It was to be expected, therefore, that in a proportion of patients with nephritis the skin lesion would have healed before admission to hospital.

We also isolated group A streptococci from 38 patients in the San Fernando hospital in whom a diagnosis of acute nephritis was subsequently confirmed. Since swabs were obtained at weekly intervals, some of them were collected after the patients had been in hospital for several days. The organism was isolated from a skin lesion in 28 of these patients.

Typing of group A streptococci from case of nephritis

Group A streptococci were isolated from 172 swabs from 117 patients with nephritis: this total of 117 includes the 10 patients mentioned on page 658 from the Port of Spain area from whom streptococci were isolated between May and August 1965. Two or more positive swabs were obtained from 39 patients; the streptococci isolated from different sites had the same T-typing pattern in 32 patients, but seven patients yielded serologically distinguishable streptococci from different swabs. In addition, two different streptococci were isolated from the same swab on one occasion. We therefore had 125 serologically distinct streptococci from patients with nephritis.

Table 2 summarizes the T-typing patterns of these distinct strains, and also of the 445 cultures from all classes of streptococcal illness in Britain, which had been typed with the same antisera.

Four distinct T-typing patterns together accounted for 97% of the Trinidad nephritis strains. The most common of these was agglutination by the routine T/14 antiserum, and by the specially absorbed T-antiserum for type 49. The evidence that this antigen is characteristic of the Red Lake streptococcus, which has been found in association with nephritis following skin sepsis, has already been presented by Maxted and his colleagues (1967). Although this organism formed 38% of the strains isolated from cases of nephritis in Trinidad, members of the 8, 25, Imp. 19 T-complex (29%) and of the 3, 13, B 3264 T-complex (21%) appeared not much less frequently. Only two cultures were agglutinated by the T/12 antiserum. It therefore appeared that type 12, which is the type most frequently associated with nephritis following streptococcal infection of the respiratory tract, was of little importance as a cause of nephritis in Trinidad.

The most remarkable finding, however, was our almost total failure to detect any of the M-antigens commonly found in British group A streptococci. With the regular set of precipitating sera used in our laboratory, an M-antigen was detected in only one of the 125 cultures—a member of M-type 18. With the same set of sera, M-antigens were detected in 32% of the British survey strains. This difference is apparent even when we compare strains with similar T-typing patterns. For example, members of the 3, 13, B 3264 complex isolated in Britain include about one-third which have the M-antigen 3, but this antigen has never been identified in Trinidad.

The Trinidad nephritis streptococci resemble in T-typing pattern and in apparent absence of M-antigen the streptococci isolated in Europe and North America from cases of superficial skin sepsis. The association of members of the 3, 13, B 3264 and of the 8, 25, Imp. 19 T-complex with impetigo was noted in Britain some years ago (Parker, Tomlinson & Williams, 1955), but nephritis secondary to skin sepsis was not observed. At Red Lake, Minnesota, organisms with the 3, 13, B 3264 T-pattern were predominant in skin sores at a time when nephritis was absent (Anthony, Perlman & Wannamaker, 1967*b*). In Alabama, on the other hand, organisms with the agglutination pattern 25/Imp. 19 were the largest group to be found in association with impetigo and nephritis, and

Table 2. *Typing of group A streptococci from cases of nephritis in Trinidad*

(The 172 cultures isolated from 117 patients included 125 distinct strains recognizable by T-agglutination. The results of typing 445 cultures isolated from all classes of streptococcal disease in Great Britain are included for comparison.)

Agglutinated by T-antiserum for types	Trinidad: No. distinct strains from cases of nephritis				Great Britain (all streptococcal diseases)
	Port of Spain, May–Aug. 1965	Port of Spain and Arima, Sept. 1965–Aug. 1966	San Fernando, Sept. 1965–Aug. 1966	Total	
1	0	0	0	0	31 (7%)
2	0	0	0	0	4 (<1%)
3, 13, B 3264	3	17	6	26 (21%)	63 (14%)
4, 4/28	0	9	2	11 (9%)	89 (20%)
5, 27, 44	0	1	0	1 (<1%)	21 (5%)
6	0	0	0	0	15 (3%)
9	0	1	0	1 (<1%)	8 (2%)
11	0	0	0	0	13 (4%)
12	1	1	0	2 (2%)	132 (30%)
14 (14)	0	0	0	0	2 (<1%)
14 (49)	2	27	18	47 (38%)	
18	1	0	0	1 (<1%)	0
22	0	0	0	0	12 (3%)
8, 25, Imp. 19	4	20	12	36 (29%)	27 (6%)
28	0	0	0	0	10 (2%)
Other or untypable	0	0	0	0	18 (4%)
Total no. distinct strains	11	76	38	125	445
No. cultures M-positive*	1	0	0	1	142 (32%)

* Before introduction of M-antisera for Types 49 and 55.

Table 3. *Distribution of T-agglutination patterns among group A streptococci isolated from skin lesions, and from the nose and throat swabs of children with skin lesions, in five Trinidad schools*

	No. of distinct strains						
	From cases of uncomplicated skin sepsis						From acute nephritis
Agglutinated by T-antiserum for types	Aranguez, May 1965	Mount Pleasant, July–Aug. 1965	Princess Town, July–Aug. 1965	St Agnes, Nov. 1965	Mucurapo, Nov. 1965–Jan. 1966	Total	
3, 13, B 3264	4	3	20	11	14	52 (27%)	26 (21%)
4 or 4/28	0	1	1	1	2	5 (3%)	11 (9%)
5, 27, 44	1	1	8	1	1	12 (6%)	1 (<1%)
9	0	1	1	3	6	11 (6%)	1 (<1%)
11	1	0	0	1	8	10 (5%)	0
12	3	1	1	7	14	26 (13%)	2 (2%)
8, 25, Imp. 19	2	0	14	6	15	37 (19%)	36 (29%)
49	2	7	7	8	5	29 (15%)	47 (38%)
Other	1	0	0	6	4	11 (6%)	1 (<1%)
Total no. distinct strains	14	14	52	44	69	193	125

there outnumbered members of type 49 (Dillon, Moody, Maxted & Parker, 1967). The investigation at Red Lake (Top *et al.* 1967) provided an explanation of the apparent absence of M-antigen from skin streptococci, when it was found that the common 3, 13, B 3264 T-complex contained, in addition to M-types 3, 13, and 33, others such as M-type 41 and the two new provisional M-types 52 and 53.

It appeared, therefore, that our lack of success in finding M-antigens might be due to the presence there of hitherto undescribed M-types, or of types for which we had not succeeded in making a good precipitating antiserum. While the investigation was in progress, we were able for the first time to make a reasonably potent M-antiserum for type 49 (Maxted *et al.* 1967). All streptococci that had been agglutinated by the special T-antiserum for type 49 were tested with this serum, and nearly half of them (26 of 60) gave a precipitin reaction. Since this time we have made better M-antisera for this type, and nearly all the T/49 streptococci isolated in Trinidad in 1967 have been found to be M-positive.

Since type 49 streptococci formed little more than one-third of the strains isolated from cases of nephritis in Trinidad, we were encouraged to look among the other streptococci for possible nephritogenic strains. To narrow the field of search, we first compared the T-typing patterns of streptococci from cases of nephritis with those of streptococci from children with skin sepsis not complicated by nephritis.

Typing of group A streptococci from school children with skin sepsis

Five sets of cultures of group A streptococci, isolated from the skin lesions and from the nose and throat swabs of children with skin sepsis were collected between April 1965 and January 1966, while the nephritis outbreak was in progress. The result of T-typing these cultures is set out in Table 3, which shows the number of distinct strains with each T-typing pattern; as in Table 2, multiple isolations of the same strain from a patient appear only once. The distribution of T-typing patterns among strains from each school is shown separately, and the percentage-distribution of T-typing patterns in the whole series is compared with that found in the streptococci from cases of nephritis.

Streptococci of many different serotypes were present in each of the schools, and the variety of T-typing patterns was considerably greater than in the cultures from cases of nephritis. Several organisms poorly represented in the nephritis series (e.g. T/12, T/11, T/9 and the 5, 27, 44 T-complex) were found frequently among the school children, and were at times prevalent in individual schools.

Type 49 streptococci were found much less often in uncomplicated skin sepsis (15 %) than in nephritis (38 %) and this supported the view that they were nephritogenic. A similar comparison suggested that there might be a nephritogenic strain in the 8, 25, Imp. 19 complex, in that 29 % of nephritis strains, but only 19 % of strains from patients with uncomplicated skin sepsis had patterns in this complex. There was no such indication for the 3, 13, B 3264 strains.

Further investigation of the 8, 25, *Imp.*19 *T-agglutination complex*

In Trinidad, as in Alabama, most of the streptococci belonging to the 8, 25, Imp.19 complex were agglutinated only by the T-antisera 25 and Imp.19. A culture with the T-typing pattern 25/Imp. 19, isolated from the skin lesion of an undoubted case of nephritis admitted to San Fernando General Hospital in October 1965 [C. Chudee, No. 65/4127] was therefore used to prepare an M-antiserum in a rabbit. Precipitating antibody was formed promptly; the serum gave a strong reaction with an extract of the homologous strain, but not with extracts of the type strains of M-types 1 to 51. It also enhanced the bactericidal effect of fresh whole blood on the homologous strain, but not on members of other M-types. It therefore appeared to possess a new M-antigen.

The serum was incorporated in our routine set, and was used in the typing of many streptococci from Trinidad from March 1966 onwards, but no positive results were obtained. Interest in the serum therefore waned, because similar situations had been observed before on a number of occasions.

In 1967, Dr Potter sent us a culture of an organism described as 'Trinidad A', together with a sample of antiserum prepared with it. She told us that many of the organisms she had isolated in Trinidad in March 1965 appeared to belong to a new M-type of which the culture 'Trinidad A' was representative, and she made available to us the information she has since published about the new type (provisional M-type 55; Potter *et al.* 1967).

A comparison of the M-antigen of our strain no. 65/4127 and of 'Trinidad A' (provisional Type 55) showed that they were identical. We therefore re-examined nearly all of our early collection of 25/Imp. 19 strains from Trinidad with the serum prepared with culture no. 65/4127. It then became apparent that M-type 55 was indeed associated with a number of cases of nephritis, but only in the earlier part of the outbreak.

Table 4. *Number of cases of acute nephritis associated with group A streptococci of type* 49, *of provisional type* 55, *and with other types of group A streptococci*

No. of cases	Mar. 1965*	May–Aug. 1965	Sept.–Dec. 1965	Jan.–Apr. 1966	May–Aug. 1966	Total
Type 49	1	2	16	13	16	48
Type 55	11	3	9	0	0	23
Other types only	4	5	13	16	24	62
Total no. cases	16	10	38	29	40	133

* Data provided by Dr E. V. Potter

The re-examination of these cultures greatly clarified the picture of the epidemic. Table 4 was constructed by combining our results with those of Dr Potter, and shows the number of patients admitted to hospital with nephritis within certain periods of time from whom type 49, provisional type 55, and other types of group A streptococci, were isolated.

Dr Potter identified provisional type 55 among the group A streptococci from

11 of 16 patients with nephritis in San Fernando in March 1965, but found type 49 in only one patient. The few cultures isolated in Port of Spain between May and August 1965 included members of both types. In the period September to December 1965, type 49 was for the first time the most common type, but a considerable minority of provisional type 55 was present. From January 1966 onwards, no culture of provisional type 55 was isolated, and type 49 was by far the commonest type present.

A consideration of Table 4 in conjunction with Fig. 2 indicates that provisional type 55 predominated at the time of the first peak of the epidemic, and that it was progressively replaced by type 49 during the second wave of cases.

Re-examination of 25/Imp. 19 streptococci from the cases of uncomplicated skin sepsis revealed few streptococci of provisional type 55. Thirty-seven strains with this T-pattern were isolated from the children in the five school surveys (Table 3); 33 were re-examined and only two members of provisional type 55 were found. There were no members of this M-type among the many 25/Imp. 19 streptococci from other sources in Trinidad examined during 1966 and 1967.

While this work was in progress, Dillon, Reeves & Maxted (1968) re-examined the 25/Imp. 19 streptococci that had been isolated from cases of nephritis in Alabama, and found that many of them had the M-antigen 2. With rare exceptions, streptococci with this unusual combination of M- and T-antigens were found only in patients with nephritis and their close associates. This unexpected finding caused us to re-test with every available antiserum all the cultures from Trinidad that belonged to the 8, 25, Imp. 19 T-complex and gave no precipitin reaction with the M-antiserum type 55. Good antisera for M-types 2 and 25 were included, but no M-antiserum for type 8 was available. None of the cultures gave a precipitin reaction with any of the antisera.

Multiple infections with different types of streptococci

If both provisional type 55 and type 49 are responsible for nephritis in Trinidad, a cause was established for 71 out of 133 cases (54 %; see Table 4). What then are we to think of the significance of the streptococci isolated from the remaining 46 % of cases?

It is clearly unreasonable to assert that every strain of group A streptococci isolated from a patient with acute nephritis was the cause of this disease. We had observed that seven out of 39 patients from whom two or more isolations of streptococci had been made on admission to hospital (18 %) were carrying two serologically distinct streptococci at different sites. In populations with a high incidence of streptococcal infection, particularly when there are opportunities for heavy dispersion of streptococci from exposed skin lesions, the frequent transfer of infection by the skin-to-skin route might lead to a high rate of multiple infection.

Indeed, Anthony *et al.* (1967 *b*), who typed serial cultures of group A streptococci from the skin lesions of children in the Red Lake Indian Reservation, found that, when two or more isolations were made from the same patient, over half of the patients were found to be carrying more than one distinct type of streptococcus. These observations, however, were spread over several months, and it is

not possible to obtain from the published information any idea of the rate of 'change' of type.

We therefore studied the frequency of multiple infection at St Joseph Presbyterian School, where a continuous study of skin sepsis was in progress. This was a rural school in north Trinidad in which there was a relatively high rate of streptococcal infection. Weekly swabbing was carried out during term-time for 27 weeks from the beginning of April 1966. Nose and throat swabs were collected from each child, and each unhealed skin lesion was swabbed separately. In any one week, on average 26 % of the children had skin lesions, and group A streptococci were isolated from skin lesions of 15 % of the children. The average throat carrier-rate was 6 %, and the nasal carrier-rate was 4 %.

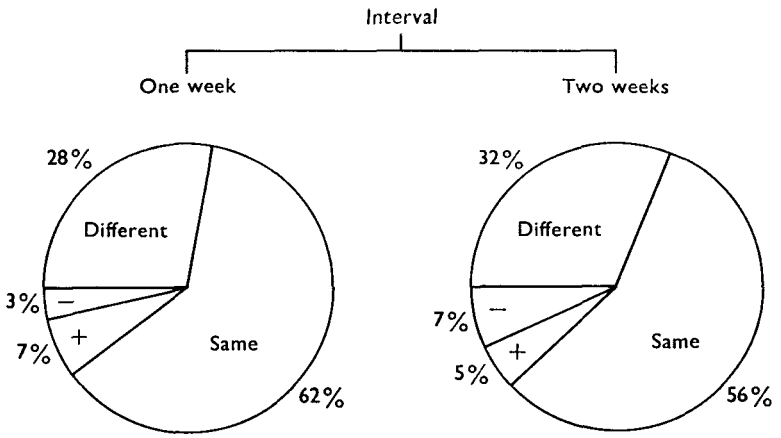

Fig. 3. Isolation of serologically distinct strains of group A streptococci from the same person on two occasions. Comparison of the T-typing patterns of the strains when group A streptococci were isolated on both occasions at an interval of (*a*) 1 week and (*b*) 2 weeks. Percentage of occasions on which: (1) strains with the same T-typing pattern were isolated on both occasions ... ('same'); (2) all strains isolated on the first occasion were different from those isolated on the second ('different'); (3) the original strain persisted, and a fresh one was added ('+'); (4) one of the two strains present on the first occasion was absent on the second ('−').

One colony from each positive culture was typed, and a comparison was made of the T-typing patterns of streptococci isolated from the same patient at different sites on the same day, and on successive weekly examination. During 27 weeks' observation, 84 % of the 98 children had one or more streptococcal infection; 56 % had two or more infections due to serologically distinct streptococci, 26 % had three or more infections, 9 % had four or more infections, and 2 % had five infections. Group A streptococci were isolated 108 times from two or more swabs collected from the same patient on the same day, and more than one type of streptococcus was found 24 times (22 %).

Next, all occasions on which group A streptococci were isolated from one patient on two successive weeks were examined (Fig. 3). The same type of streptococcus was found both times on 62 % of occasions; but on 28 % of occasions, the strain

found the first time was not found the second time, and an entirely different strain was present. In a further 7 % of occasions the original strain persisted but a new one was added, and in 3 % of occasions one of two strains had disappeared after a week. When the interval between positive swabs was 2 weeks, the percentage 'change' of type was slightly but not greatly increased.

These findings suggest that an attempt to identify the type of streptococcus responsible for the primary skin infection which initiated an attack of nephritis by the examination of swabs collected when the patient is admitted to hospital will give an incorrect answer in at least one case in three. This is probably an underestimate of the error, because it is based on the results of T-typing, and organisms considered alike on the basis of T-agglutination patterns may have different M-antigens.

The finding that the two types of streptococci suspected of nephritogenic properties could be isolated from only 54 % of cases is therefore consistent with the view that they were responsible for most of the cases of nephritis in the recent outbreak in Trinidad.

DISCUSSION

Since the early years of this century it has been recognized that acute glomerulonephritis may sometimes follow streptococcal infections of the skin (Dillon, 1967). Recently it has become apparent that in parts of north and central America most cases of glomerulonephritis are associated with impetiginous skin lesions.

The difficulties that have been encountered in recognizing nephritogenic strains of group A streptococci from skin lesions are attributable in part to the characters of the organisms, and in part to the frequency with which more than one serotype of streptococcus can be isolated from the lesions of one patient. In all investigations, however, there has been a notable absence of streptococci of M-type 12, which is responsible for most cases of nephritis which follow infections of the upper respiratory tract (Rammelkamp, Weaver & Dingle, 1952).

For some time it has been recognized that group A streptococci from skin lesions seldom have M-antigens that are detectable with the antisera used in routine typing (Parker *et al.* 1955; Barrow, 1955; Dillon *et al.* 1967). Top *et al.* (1967) have shown that this is not because 'skin' streptococci lack M-antigens, but because they belong to previously unrecognized or supposedly rare M-types.

It was not surprising, therefore, that the first nephritogenic 'skin' streptococcus to be identified should have been a member of a hitherto undescribed M-type, now known as type 49 (Updyke, Moore & Conroy, 1955). This type was first found in the Red Lake Indian Reservation, Minnesota, in 1953 (Kleinman, 1954), but was rarely recorded elsewhere during the next 10 years, because of a widespread lack of success in making sufficiently potent M-antisera for the type. There is now evidence that it is to be found, and has caused nephritis, over a wide geographical area (Maxted *et al.* 1967).

Two outbreaks of nephritis due to type 49 have occurred at Red Lake, separated by an interval of 13 years during which the disease was rarely seen

(Anthony *et al.* 1967*a*). Skin lesions were prevalent during the intervening period, but the group A streptococci isolated from them were of different types (Anthony *et al.* 1967*b*; Top *et al.* 1967).

In at least two other areas in which type 49 has been shown to be an important cause of nephritis, there is evidence that it is not the only nephritogenic streptococcus present in skin lesions. In Birmingham, Alabama, nephritis and streptococcal skin sores are prevalent each year in the summer and early autumn; over 80% of the cases of nephritis, and over 90% of skin sores occur in the months July to September. Members of M-type 49 make up about a quarter of the streptococci from the skin lesions of cases of nephritis (Dillon *et al.* 1967). Further investigations showed that a larger proportion (46%) were an unusual streptococcal strain which had the M-antigen 2, but with the agglutination pattern 25/Imp. 19. Streptococci with this antigenic structure were rarely found except in cases of nephritis or their family contacts (Dillon *et al.* 1968).

In Trinidad, major epidemics of nephritis occurred in 1952–4, in 1958–9, and in 1964–6. Little is known about the first epidemic, which was revealed by a retrospective examination of the records of the San Fernando General Hospital (Poon-King *et al.* 1967), but the two later outbreaks appear to have involved the whole island. Our initial observations, made in the second half of the 1964–6 epidemic, revealed that some 39% of the group A streptococci from cases of nephritis belonged to M-type 49. However, in a small collection of streptococci isolated several months earlier, Dr Potter found few members of M-type 49, but a predominance of cultures with the T-agglutination pattern 25/Imp. 19, which proved to have a new M-antigen of provisional type 55 (Potter *et al.* 1967). Retrospective examination revealed that a number of M-type 55 streptococci were present in the earlier part of our collection of cultures. A consideration of our results with those of Dr Potter suggested that in March 1965, at the height of the first wave of nephritis cases, provisional type 55 was the predominant strain, and that it became progressively less common between May and December 1965, and then disappeared; type 49 was seldom seen in April 1965, but exceeded provisional type 55 in frequency at the peak of the second wave, and was the only supposedly nephritogenic strain isolated in the stage of decline of the epidemic. From this, it appears that the double-peaked outbreak consisted of two partly overlapping epidemics due to different serotypes.

At the outset of the investigation in Trinidad, very few of the group A streptococci from cases of nephritis were typable with the available M-antisera, and T-typing was the only available means of characterizing them. This proved difficult, because cross-reactions were unusually frequent with the regular set of T-typing sera, and had to be removed by a series of additional absorptions. It then became apparent that the streptococci included strains with several different T-typing patterns. When the relationship between the T-antigens of types 14 and 49 had been elucidated, a highly specific T-agglutinating serum for type 49 was obtained and this type was found to be the most common streptococcus in our series of cultures from cases of nephritis. This finally was confirmed later when we succeeded in making a sufficiently good M-antiserum for type 49.

The remaining strains from nephritis cases showed a predominance of members of the 3, 13, B 3264 and the 8, 28, Imp. 19 agglutination complexes, which are known to contain members of several M-types, together with a large number of strains in which no M-antigen has yet been identified. Some guesswork was therefore necessary in deciding which of these strains should be selected for further investigation. The distribution of T-typing patterns among the streptococci from cases of nephritis and from children without nephritis led to a presumption that the 8, 25, Imp. 19 complex might contain a nephritogenic strain. By good fortune, we and the Northwestern University group both chose a member of provisional type 55 for serum production, and our results are therefore comparable. Between us, we succeeded in finding a probable cause for just over half of the cases of nephritis from which a streptococcus was isolated.

We had to consider the possibility that some of the other streptococci isolated from cases of nephritis were also nephritogenic. Very few of them, or indeed of the streptococci isolated from skin lesions unassociated with nephritis, or from other streptococcal diseases in Trinidad, had M-antigens identifiable with existing antisera. A careful search for M-antigen 2 in strains with the T-agglutination pattern 25/Imp. 19 failed to reveal a single culture resembling the Alabama strain with this antigenic structure. There is a suggestion in Table 4 that the proportion of cases of nephritis associated with one or other of the supposedly nephritogenic types was falling towards the end of the period of observation, and further investigations in 1967 confirmed this trend. Indeed, by mid-1967, when the incidence of nephritis had again risen above the immediate postepidemic level, type 49 had become uncommon, and the most frequently encountered strain had the T-agglutination pattern 25/Imp. 19, but appeared to have no recognizable M-antigen. An antiserum made with one such strain [V. Ramkissoon, no. 67/3890] was found to contain antibody to a hitherto unrecognized M-antigen (now designated provisional type 57). About two-thirds of the T-25/Imp. 19 cultures from nephritis cases in the second half of 1967 gave a precipitin reaction with this serum. These results will be published in detail later.

Patients with skin lesions frequently harbour two or more different strains of group A streptococci, and this makes it difficult to identify nephritogenic strains with certainty unless they are responsible for clear-cut localized epidemics, as at Red Lake. Our calculations (Fig. 3) of the probable rate of 'change' of type suggests that in Trinidad only about two out of every three of the streptococci isolated from cases of nephritis on admission to hospital are likely to have been the ones responsible for the nephritis. This estimate is only approximate, and is based on the assumption that the predominant streptococcus in each lesion was the only one present. This may not be so, though we found two different streptococci in the same lesion on only one out of 150 occasions on which we typed ten different colonies from the primary plate. Infection with multiple types was assumed when streptococci with different T-agglutination patterns were isolated from the same patient; therefore any lack of reproducibility in the typing would have exaggerated its frequency. Despite the manifest difficulty in T-typing the Trinidad strains, we do not think that this occurred often enough to influence

our general conclusions. Multiple streptococcal infection of skin lesions has been reported elsewhere, but it appears to have been exceptionally common in Trinidad. The fact that *Hippelates* flies are very commonly to be seen feeding from the skin lesions, and will deposit group A streptococci on a blood agar plate several hours after such a meal (Bassett, 1967), suggests that the passive transmission of the organism may be an important factor in causing multiple infections.

The periodicity of nephritis epidemics in Trinidad is not explicable on the basis of the present evidence. An epidemic in a small and relatively isolated community such as the Red Lake Indian Reservation might follow the introduction of a nephritogenic strain of streptococcus for which there is little type-specific immunity in the child population. Alternatively, a rise in the incidence of nephritis might be a consequence of an increase in the total number of skin infections, if nephritogenic 'skin' streptococci are constantly present in the population. This appears to be the situation in Alabama. In Trinidad, skin lesions due to group A streptococci are present at all seasons of the year, and affect at least 10% of the child population of the island at any one time. There have been long periods of relative freedom from nephritis, and large epidemics have occurred at intervals of about 6 years. However, the most recent epidemic appears to have consisted of two distinct outbreaks in rapid succession, which were observed at about the same time in the northern and southern parts of the island. It is remarkable that the two nephritogenic streptococci appear to have spread so rapidly and evenly through the relatively immobile child population of the villages of Trinidad. The regular intervals between the major outbreaks might suggest that type-specific immunity to nephritogenic strains plays some part in preventing epidemics, but it would be necessary to assume that immunity to type 49 and to provisional type 55 in the child population had fallen below a critical level at about the same time.

Investigations over limited periods of time in three geographical areas have produced evidence of nephritogenic strains in three different M-types of group A streptococci. Two of these strains have so far been incriminated only in a single area, though they have not yet been sought widely elsewhere. There is evidence, however, that M-types found frequently in skin lesions in one area are rare in others. For example, streptococci of M-types 41 and 52, with the T-agglutination pattern 3/13/B 3264, were very prevalent at Red Lake (Top *et al* 1967). We have searched extensively for them among streptococci with similar T-patterns from Trinidad and Britain, but have very rarely found them. The number of unidentified M-types among 'skin' streptococci is probably still quite considerable.

SUMMARY

The recent epidemic of acute glomerulonephritis in Trinidad had two peaks, separated by an interval of about 6 months.

Evidence is presented that there were in fact two successive but overlapping epidemics, the first due to streptococci of provisional M-type 55, and the second to streptococci of M-type 49.

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