

BETA-HEMOLYTIC STREPTOCOCCI GROUPING AND GROUP A TYPING IN RIBEIRÃO PRETO, S.P., BRAZIL, 1966-1970

Carlos SOLÉ-VERNIN ⁽¹⁾ and Max D. MOODY ⁽²⁾

SUMMARY

Nearly five thousand patients from the geographical area of Ribeirão Preto, São Paulo State, Brazil, were bacteriologically examined during the quinquennium 1966-1970, for the presence of beta-hemolytic streptococci. Half of the patients proceeded from modest socio-economic levels, the other half, from medium to high. The majority of the patients exhibited a sore throat, the remaining ones had other areas of infection of a possible streptococcal etiology. Four hundred and fifty six cases of Group A (*Streptococcus pyogenes*) infections were diagnosed. Epidemic proportions of the etiologic agent (in 1967, but specially in 1968 and 1969) indicate coincidence of epidemic periods for the diseases concerned. With regard to the seasonal distribution, the mean rates for the whole quinquennium were verified in decreasing order of values in Winter, Spring, Autumn and Summer. Typing of *Streptococcus pyogenes* by T-sera could classify a general high proportion of the strains (90%), the non-typability being generally small but reached its maximum in the period immediately following the epidemic (1970). T-1 showed the highest incidence rates among all Types in 1968 and 1969. Each of the other Types showed independent waves during the quinquennium. The presence of Type M-49 (Red Lake) was verified. New patterns of agglutination were found which were related but not identical to the ones found in Europe and other areas.

INTRODUCTION

The world nosologic statistics indicate that, in order of prevalence, the infections due to streptococci are among those which most afflict mankind. There are sore throats, skin conditions and other areas of streptococcal infections which may begin a few weeks after the cure of the infection itself, non-pyogenic sequels of an immunological basis, such as acute diffuse glomerulonephritis and the rheumatic fever. The first is benign as a general rule, but the second produces carditis with irreversible anatomic deformations which constitute the gravest consequence of the disease. The proportion of individuals infected by streptococcus who

suffer an evolution of rheumatic carditis is small. However, the primary infection is so extensively disseminated that the contingent of rheumatic cardiacs reaches a significant level.

The investigations here reported were carried out with two purposes: 1) to obtain epidemiologic basic data with regard to the etiologic agent in the area of Ribeirão Preto City, São Paulo State, Brazil; and, 2) to take steps in the organization of a laboratory established for Grouping of beta-hemolytic streptococci, and Group A Typing nearer to our geographic area.

Presented at the 4th Brazilian Congress of Microbiology, Niterói, Rio de Janeiro, Brasil, 1972

- (1) Associate Professor, Department of Parasitology, Microbiology and Immunology, Ribeirão Preto Medical School, University of São Paulo, São Paulo State, Brazil.
(2) Technical Director, Wellcome Reagents Division, Burroughs Wellcome Co., Research Triangle Park, North Carolina, USA. Former Chief, Streptococcus Unit, CDC, Atlanta, Ga, USA.

MATERIAL AND METHODS

1. *Strains* — More than one thousand beta-hemolytic streptococci strains isolated from 979 patients were classified (Table I).

other areas of infection; from February to December 1970, this was the only laboratory to contribute specimens. The other half of the specimens came from two laboratories of private practice (respectively Prof. O. Ba-

TABLE I

Number of specimens examined (one for each patient) and corresponding cases with beta-hemolytic streptococci by year and by Laboratory of origin. Ribeirão Preto, SP, Brazil

| Year | Laboratory of origin | | | | | | Total per annum | | |
|-------------|----------------------|-------|-----------|-------|-----------|-------|-----------------|-------|--------|
| | I | | II | | III | | Specimens | cases | |
| | specimens | cases | specimens | cases | specimens | cases | | N. | % |
| 1966 | 503 | 90 | 356 | 51 | 83 | 10 | 942 | 151 | (16.3) |
| 1967 | 363 | 61 | 289 | 42 | 0 | 0 | 652 | 103 | (15.8) |
| 1968 | 383 | 77 | 664 | 196 | 73 | 1 | 1.120 | 274 | (24.5) |
| 1969 | 491 | 112 | 842 | 186 | 93 | 14 | 1.426 | 312 | (21.9) |
| 1970 | 752 | 109 | 102 | 30 | 0 | 0 | 854 | 139 | (16.3) |
| Grand Total | 2.492 | 449 | 2.253 | 505 | 249 | 25 | 4.994 | 979 | (100%) |

Nearly all these patients had sore throat; the remaining (93, or 10%) had other areas of infection of a possible streptococcal etiology (Table III).

2. *Origin of the Specimens* — The specimens were supplied by three laboratories of bacteriology of Ribeirão Preto, only one specimen for each patient. Nearly half of the specimens were from patients of the General Hospital of Ribeirão Preto Medical School (*); its laboratory of bacteriology (installed in the Department of Parasitology, Microbiology and Immunology) had been under the responsibility of one of us (C.S.-V.) from 1961 to 1970 (Laboratory I, Table I). That Hospital serves patients with modest income levels of Ribeirão Preto and surrounding areas. It contributed specimens from sore throat and all the specimens from

racchini's (Laboratory II, Table I) and Dr. Moraes Rêgo's (Laboratory III, Table I)) which serve levels of better income.

For comparison purposes we included in this report the T-Typing at the present time of 56 Group A strains which had been isolated in 1961¹⁰.

3. *Requisition of Bacteriological Cultures* — The Clinicians were not notified that the results were also intended for the present study so that their requests could be fulfilled without any interference in their habits of requisition in certain clinical situations; the variable number of requisitions would probably express the relative occurrence of the infections studied.

4. *Culture Media* — The fundamental culture medium was the HPTH (Hitchens-Pike-Todd-Hewitt)⁹. This selective-enrichment medium was also used as a transport medium. After the swabs had been examined by the collaborating laboratories according

(*) To the Clinicians of which, and to many Clinicians in town, we owe the courtesy of the clinical diagnosis registered in this paper.

to their own technics, they were inoculated into the HPTH medium. Blood agar from an agar base of fresh ox heart infusion and rabbit's blood was used. The broth used was Todd-Hewitt's; however, its glucose was adjusted to 0.5% for a collection of possible Group D strains (See 12).

5. *Selection of Colonies* — One colony of each different macroscopic aspect was selected; beta-hemolysis was confirmed by pour plate.

Counting the duplicates, 1,193 colonies of beta-hemolytic streptococci were Grouped (Table II) and 526 Group A colonies were Typed (Tables III and IV). (See *Discussion*, 2).

TABLE II

Number of isolations of Groups A, B, C, D, F, G and NG, positive beta-hemolytic streptococci among 979 patients (sore throat and other localizations of infections; one specimen per patient) Ribeirão Preto, SP, Brazil, 1966-1970

| Group | No. of cases |
|-------|--------------|
| A | 456 |
| B | 42 |
| C | 132 |
| D | 78 |
| F | 0 |
| G | 269 |
| NG | 39 |
| TOTAL | 1016 |

Legend:

NG = Non-Groupable, i.e., no A, B, C, D, F or G group beta-hemolytic streptococci.

6. *Cases and Diagnosis* — A few patients had two or three different Groups (a case for each one of the Groups).

7. *Preservation of the Strains* — It was done by the processes of freezing the culture in normal rabbit blood⁹ (from -18°C to -20°C, with occasional failures due to lack of electricity).

8. *Recuperation of the Cultures* — From April 1970 on, those cultures were recuperated for antigenic classification. From more than 1,300 cultures preserved, 9% were lost.

9. *Polysaccharide Extraction for Grouping* — Extraction was done by Maxted's method, mass of culture from the purity plate. The lytic system was obtained according to recent recommendations of Maxted (W. R. Maxted, Personal Communication, 1970) (*). For the production of more extensive sporulation and greater lytic activity, we observed that incubation at 30°C is doubtless superior to higher temperatures.

For a certain number of strains, extraction was also done by Fuller's method⁸ (See 12).

10. *Grouping and Typing* — All Grouping and Typing were done in Ribeirão Preto; eleven of the cultures were also sent to the CDC (Atlanta, Ga) for verification (See 14). The extract of the cultures was initially submitted to precipitating sera in capillary tubes for the identification of Groups A, B, C, D and G (later on, for Group F, too; see 12). Technical details for Griffith's T-agglutination Typing sera were very well described⁶.

11. *Sera for Grouping and Typing* — Grouping sera were, for the most part, prepared and evaluated in Ribeirão Preto, according to methods and Type-Cultures furnished to one of us (C.S.-V.) by courtesy of Dr. Rebecca C. Lancefield, in 1961; the other part, at the CDC (Atlanta, Ga); T-Typing sera were totally prepared, absorbed and evaluated at the CDC (Atlanta, Ga).

12. *Observations with Groups D and F, and the NG (Non-Groupable) Strains* — For a small number of strains which could not be grouped as described initially (NG), a working hypothesis was that they could be Group D strains which, in a like manner to Lancefield's C1 strains, need to be grown in 0.5% glucose broth for the synthesis of the Group-specific polysaccharide. It was verifi-

(*) *Streptomyces albus* (ATCC 17900) kindly furnished by Dr. William Arthur Clark, Director of the American Type Culture Collection.

TABLE III

Grouping and Group A Typing of Beta-Hemolytic Streptococci from patients with specified Clinical Conditions, Ribeirão Preto, SP, Brazil, 1966-1970

| Clinical Condition | Total no. of cases | Groups | | | | | | Group A Types Diagnosis | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----------------------------|--------------------|--------|----|-----|----|-----|----|-------------------------|----|---|----|----|----|----|----|----|--------|----|----|----|----|-------|------|------|-------------|------------|---------|---------|---------------|---------------|--------|--------|----------------|---|
| | | A | B | C | D | G | NG | NT | 1 | 3 | 4 | 6 | 9 | 11 | 12 | 13 | 14(49) | 22 | 23 | 25 | 28 | 11/12 | 3/13 | 6/23 | 8/25/Imp.19 | 3/13/B3264 | B3264/9 | 5/27/44 | 3/13/B3264/12 | 5/11/12/27/44 | 3/5/12 | 8/13/9 | 8/25/Imp.19/14 | |
| Sore Throat (*) | 886 | 369 | 42 | 129 | 76 | 268 | 39 | 32 | 75 | 2 | 20 | 27 | 11 | 17 | 50 | 5 | 10 | 22 | 2 | 24 | 14 | 2 | 5 | 3 | 25 | 10 | 6 | 5 | 1 | 4 | 0 | 1 | 1 | |
| Other Respiratory Condition | 27 | 24 | 0 | 2 | 0 | 1 | 0 | 5 | 8 | 0 | 2 | 2 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Abscess | 23 | 23 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 0 | 3 | 2 | 1 | 1 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Skin Condition | 14 | 12 | 0 | 1 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urinary Infection | 8 | 7 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Otitis | 7 | 7 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vaginitis | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Boil | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Peritonitis | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fistula | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Without Reference | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T o t a l | 979 | 456 | 42 | 132 | 78 | 269 | 39 | 45 | 94 | 2 | 23 | 36 | 14 | 22 | 54 | 6 | 16 | 25 | 3 | 27 | 15 | 3 | 6 | 3 | 28 | 16 | 8 | 6 | 2 | 4 | 1 | 1 | 1 | |

Legend:

NG: Non-Groupable (Not A, B, C, D, F, G)

NT: Non-Typable

(*) All Di- and Tri-Group Cases and all Group A Di-Type Cases are from Sore Throat

TABLE IV

Typing of *Streptococcus pyogenes* isolated from a Few Clinical Conditions, specially Sore Throat. Ribeirão Preto, SP, Brazil

| Type | 1966 | 1967 | 1968 (*) | 1939 (*) | 1970 | Total |
|--|------------|------------|------------|------------|------------|-------------|
| 1 | 2 | 1 | 49 | 42 | 0 | 94 |
| 3 | 0 | 0 | 0(+1) | 0 | 1 | 1(+1) |
| 4 | 2 | 1 | 4 | 15(+1) | 0 | 22(+1) |
| 6 | 0 | 1 | 19 | 11 | 5 | 36 |
| 9 | 4 | 2 | 1(+1) | 6 | 0 | 13(+1) |
| 11 | 4 | 1 | 5 | 12 | 0 | 22 |
| 12 | 8 | 15 | 6 | 21 | 4 | 54 |
| 13 | 1 | 0 | 4 | 0(+1) | 0 | 5(+1) |
| 14(49) | 6 | 3 | 4 | 1 | 2 | 16 |
| 22 | 2 | 0 | 2 | 15(+1) | 5 | 24(+1) |
| 23 | 0 | 0 | 1 | 1 | 1 | 3 |
| 25 | 1 | 1 | 11(+1) | 12 | 1 | 26(+1) |
| 28 | 3 | 2 | 5 | 3 | 2 | 15 |
| 11/12 | 1 | 0 | 1 | 0 | 1 | 3 |
| 3/13 | 1 | 1 | 1 | 2(+1) | 0 | 5(+1) |
| 6/23 | 2 | 0 | 1 | 0 | 0 | 3 |
| 8/25/Imp.19 | 1 | 3 | 5 | 18(+1) | 0 | 27(+1) |
| 3/13/B3264 | 3 | 5 | 1(+1) | 1 | 5 | 15(+1) |
| B3264/9 | 0 | 1 | 4 | 3 | 0 | 8 |
| 5/27/44 | 0 | 1 | 1 | 0 | 4 | 6 |
| 3/13/B3264/12 | 0 | 0 | 2 | 0 | 0 | 2 |
| 5/11/12/27/44 | 0 | 0 | 0 | 0 | 4 | 4 |
| 3/5/12 | 0 | 0 | 0 | 1 | 0 | 1 |
| 3/13/9 | 0 | 0 | 0 | 1 | 0 | 1 |
| 8/25/Imp.19/14 | 0 | 0 | 0 | 0(+1) | 0 | 0(+1) |
| Cases: | | | | | | |
| Uni-Type | 41 (95%) | 38 (83%) | 127(90%) | 165(97%) | 35 (64%) | 406(89%) |
| Di-Type | 0 (0%) | 0 (0%) | 2(1%) | 3(2%) | 0 (0%) | 5(1%) |
| Non-Typable | 2 (5%) | 8 (17%) | 13(9%) | 2(1%) | 20 (36%) | 45(10%) |
| Total | 43(100%) | 46(100%) | 142(100%) | 170(100%) | 55(100%) | 456(100%) |
| Proportions of Different Type Patterns Isolated (**) | 15/25(60%) | 14/25(56%) | 21/25(84%) | 19/25(76%) | 12/25(48%) | 25/25(100%) |

(*) 5 Di-Type Cases in parenthesis (see *Results and Discussion*, 2.).

(**) 25 Different Type Patterns were isolated during the whole quinquennium.

ed that, firstly, if they were grown on blood-agar plates and extracted by Fuller's method, the reactions maintained themselves negative with D serum and with normal rabbit serum as control; two other cultures (behaving as Lancefield's strain D76) served as positive controls. However, many of those strains, if grown in 0.5% glucose broth and extracted

by Maxted's method (purity verified), as with Lancefield's strain C1 now used as a positive control, gave positive precipitation with D serum (and negative with normal rabbit serum as another control).

Subsequently, the last strains of this NG series, which remained NG, were submitted to Group F serum, and to a control of normal

serum; extract by Maxted's method from a purity plate growth; however, all were negative with Group F serum. At last, those strains which were consistently NG were re-examined by microscopy (Gram stain) and by pour plate, as a final confirmation of beta-hemolytic streptococci diagnosis.

13. *NT (Non-Typable) Strains* — The Group A cultures which were finally NT were re-grouped, as a final confirmation of Group A diagnosis.

14. *Observation with Type 14(49)* — As we lacked both anti-T and anti-M sera to identify Type 49 (Red Lake), and as with the use of the anti-T set sera of ours, Type 49 strains agglutinate with T-14 serum

as the homologous T-14 strain^{5,6}, eleven from the sixteen (68%) of the strains classified as T-14 in Ribeirão Preto were sent to the CDC (Atlanta, Ga) to verify whether among them there would be any which were actually M-49 (See *Discussion*, 8). Strains M-49 are included in the T-14 of ours, which, for this reason, should be called Type 14(49).

RESULTS

Almost half of the beta-hemolytic streptococci isolates from a few clinical conditions, but specially sore throat, were Group A; only a very small number were Non-Groupable (*i.e.*, Not Groups A, B, C, D, F or G) (Table II). The incidence of Group A show-

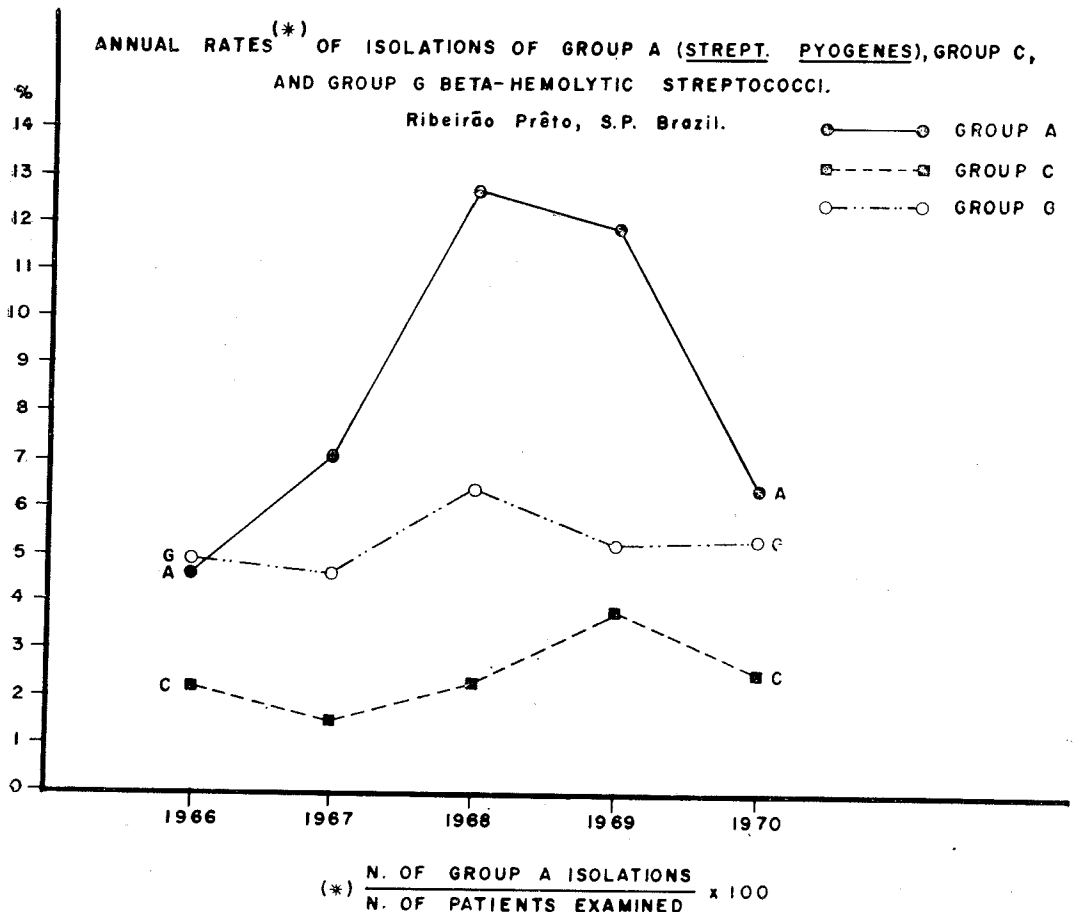


Fig. 1 — Annual rates of isolations of group A (*Streptococcus pyogenes*), group C, and group G beta-hemolytic Streptococci, Ribeirão Preto, SP, Brazil.

ed epidemic acmes in the years 1968 and 1969 (Fig. 1). In spite of differences of incidence from year to year, the seasonal mean rates for Group A were observed, in order of decreasing value, in Winter, Spring, Autumn and Summer (Fig. 2). Typing of Group A revealed a rich series of epidemiological informations. No correlation between Types and clinical conditions could be established on account of limited number of observations, except with regard to sore throat;

Type I was the overall predominant Type, followed by Type 12, some types being very rare (Table III). The annual acmes of incidence of Group A were definitely due to a certain type, the predominant type varying from year to year (Fig. 3). Twenty-five different type patterns were isolated during the quinquennium; however, they were present chiefly in the epidemic years; some patterns, not previously described, were found (Table IV).

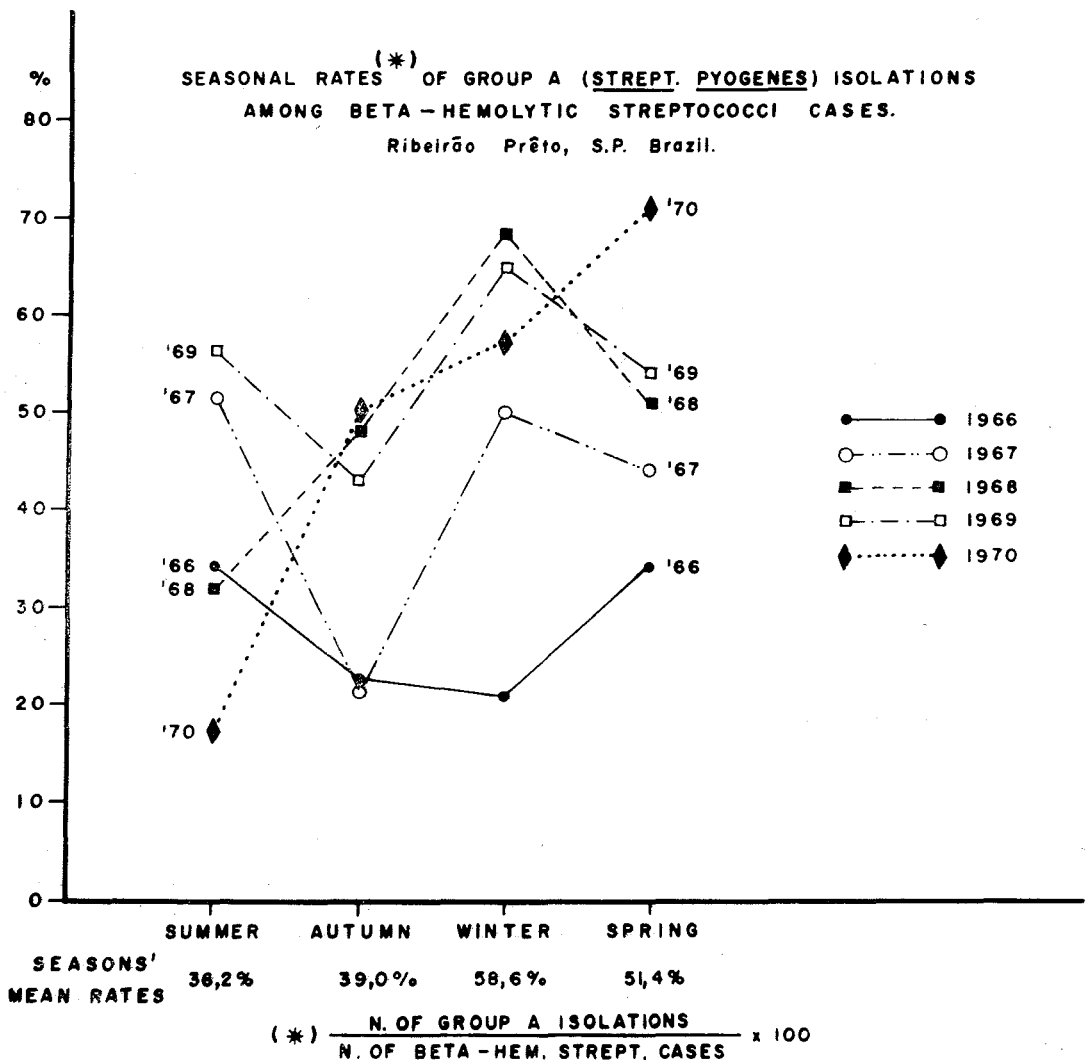


Fig. 2 — Seasonal rates of group A (*Streptococcus pyogenes*) isolations among beta-hemolytic Streptococci cases. Ribeirão Preto, SP, Brazil.

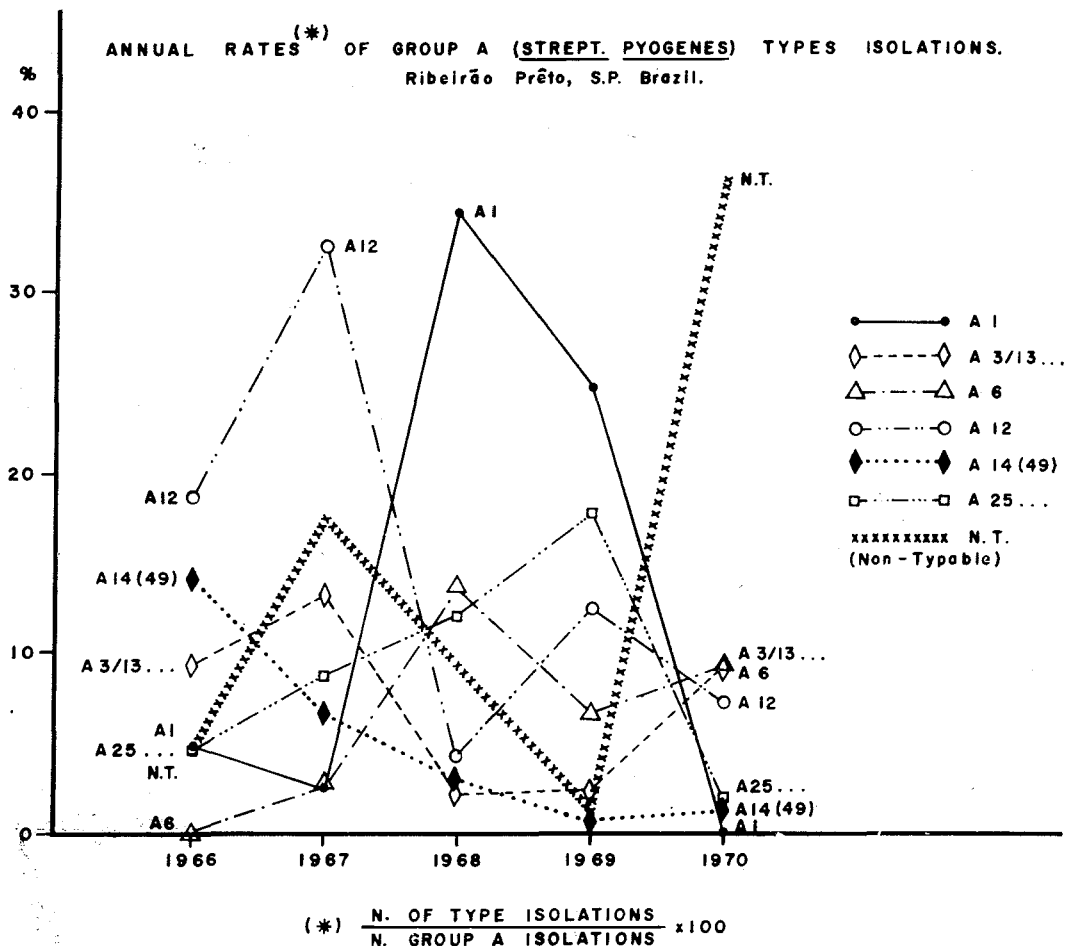


Fig. 3 — Annual isolation rates of group A (*Streptococcus pyogenes*) types. Ribeirão Preto, SP, Brazil.

DISCUSSION

1. *Healthy Carriers and Cases of Disease* — The general proportions of isolations of *Streptococcus pyogenes* with the use of enrichment medium, even in epidemic periods (12-13%, Fig. 1), was below that of healthy carriers (23% among rural school children of Ribeirão Preto in 1961¹⁰). It appears, therefore, that *Streptococcus pyogenes* in healthy carriers who, by chance, get sick with acute anginas of a non-streptococcal etiology, either disappear from the throat or diminish to such small numbers — at any rate, it becomes much more difficult to detect, even adopting enrichment media.

2. *Multi-infected Cases* — The fact that Di- and Tri-Group cases, and also Di-Type cases were found, was a result of a search that was done on a possible diversity of macroscopic aspects of colonies; and to the antigenic classification of, at least, one colony of each different aspect. It is noteworthy to mention that this diversity of strains showed up only in patients with sore throat. In other areas only one strain was found (Table III). From 1966 to 1970 there were respectively for each year, 11, 7, 14, 0 and 3 multi-Group cases. From these 35 multi-Group cases (3.5% among the 979 cases with beta-hemolytic streptococci, Table I) only two were Tri-Group. In 1968-1969 there was absolute

predominance of Group A in the period of greatest epidemic prevalence (Fig. 1). The tendency to co-exist with other Groups is the least for Group A: only 4% of the 369 cases of Group A sore throat were Multi-Group. Following it, in decreasing order came Groups G, C, D and B. The NG have the greatest tendency to co-exist with the Groups: 26% of the 369 cases of sore throat with NG were multi-Group.

Five Di-Type cases were found, two in 1968 (T-9 + T-25, and T-3 + T-3/13/B3264) and three in 1969 (T-4 + T-22, T-13 + T-3/13, and T-8/25/Imp.19 + T-8/25/Imp.19/14). The second 1968 case, and the second and third 1969 cases could be considered antigenic variants of a same complex type pattern.

For the 456 cases of Group A infections, we had 461 type diagnosis, i.e.: 406 Mono-Type cases (406 diagnosis), 5 Di-Type cases (10 diagnosis) and 45 NT cases (45 diagnosis) (Tables III and IV).

3. *Group D Strains* — Among the 78 Group D strains recognized, 65 (83%) were so by the general method adopted (See *Material and Methods*, 12.). The remaining 13 (17%), however, could be recognized only after being grown in 0.5% glucose broth. On the other hand, it is interesting to note that these 13 strains represent 33% of a total of 40 NG tested in that way; the remaining 27 of this series of 40 maintained themselves as NG since none of them reacted with Group F serum.

4. *Frequency of NG* — Almost all the beta-hemolytic streptococci from sore throat were groupable in Groups A, B, C, D and G, the remainder few belonging surely to rare Groups, but not to Group F. However, in the case of other areas of infection, all the strains found were grouped, with predominance of Group A (Table III).

5. *Immediate Reason of Non-Typability* — Among the 84 cultures which couldn't be typed, 48 (57%) presented irreducible roughness. The other 36 (43%), although smooth, didn't react with any of the pools.

6. *Advantage of Repetition of Typing in New Conditions* — From the 84 strains NT

just mentioned, another colony was inoculated into broth and incubated at 22-25°C. In this way, 25 (30%) became typable.

7. *Types Encountered after Adoption of New Conditions* — There was a great variety of types encountered (12 Types among 25 cultures). The commonest type was T-11 (with six strains), and T-22 (with five strains), followed by T-4 (three strains), and T-9, T-28 and T-6, with two strains each; one typable strain was presented by the following Types: 12, 13, 14, 25 and 3/13/B3264. However, according to Padula (Padula, J., Personal Communication, 1969) strains from skin conditions more frequently than others, required a lesser temperature of incubation to be typable.

8. *Presence of Type 49* — Three of the eleven (27%) of Ribeirão Preto's T-14 strains revealed themselves as being M-49 (See *Material and Methods*, 14). It is a nephritogenic Type of wide geographical distribution⁵; they were isolated, two from oropharynx, and one from a skin condition at the knee. It is noteworthy to mention that nowadays there is a T-serum for agglutination specially prepared to be specific for Type 49⁵.

9. *New Patterns of Agglutination* — As a rule, antigenic patterns are observed with mono-specific sera which take part of a pool. However, some Group A strains isolated years ago (1967) from a rural area near Ribeirão Preto involved in an epidemic with nephritis² were identified (*) as being formed by new combination of antigens, with the following variants of a presumably complex pattern: 6/23, 6/22/23, 6/17/23/47 and 6/15/17/23/47. These are different but related to the patterns encountered in Europe and other geographical areas (Parker, M. T., Personal Communication, 1967). In the present investigation new patterns were found: B3264/9, 3/13/B3264/12, 3/5/12, 3/13/9 and 8/25/Imp. 19/14 (Table IV); and 3/28/9 of 1961 (See 15.).

10. *Adequacy of the Set of Agglutinating Sera for Typing* — The level of typability

(*) By courtesy of Dr. M.T. Parker (Colindale, London).

depends on the epidemiological phase investigated and also on the geographical area^{1,8,11}. The set adopted showed itself adequate to the epidemic phases recognized in this investigation. The incorporation of other sera, however, would appear to be worth of investigation for our 1970 strains.

11. *Epidemiological Interpretation of the Quinquennium, Ribeirão Preto, 1966-1970* — To understand the epidemiology of streptococcal diseases, typing of Group A strains is indispensable^{1, 7, 8, 11}. Griffith's T-agglutination method has undergone a process of continuous improvement and attains the distinct advantage of Typing a high proportion of strains^{7, 21}.

As we had absolute predominance of sore throat specimens (Table III), this investigation refers to the particular etiologic responsibility of *Streptococcus pyogenes* in these infections.

In 1968-1969 the following facts could be detected: 1) increase in the number of solicitations of bacteriological cultures by the Clinicians, probably reflecting incidence of greater number of suspected cases (Table I; See *Materials and Methods*, 3.); 2) the proportion of specimens yielding Group A doubled or trebled (Fig. 1); 3) the proportions of isolations of certain Type(s) increased (Fig. 3); 4) there was a greater variety of types encountered (Table IV); 5) there were present the majority of the 25 patterns of agglutination found in this study (Table IV); 6) these years were also the time of appearance of the new agglutination patterns (Table IV); and 7) it was when Di-Type cases showed up (Table IV).

When we found beta-hemolytic streptococci in sore throat, the *Streptococcus pyogenes* could be detected in 24-36% of the cases in inter-epidemic years; these proportions ascend to 40-51% in the epidemic period.

When we found beta-hemolytic streptococci in other areas of infection of possible streptococcal etiology, the *Streptococcus pyogenes* could be detected in 80% of the cases in the inter-epidemic years; this proportion ascends to 100% in the epidemic period.

The epidemic proportions of *Streptococcus pyogenes* both in sore throat and other areas

of infection indicate, therefore, general coincidence of epidemic periods.

12. *Seasonal Distribution* (Fig. 2) — Most of the cases of Group A were observed in Winter; subsequently in Spring, next in Autumn, at last in Summer. In 1970, the seasonal distribution followed these general lines, with the difference, however, that the epidemic acme deviated from Winter to Spring, but it happened that the usual low level of Summer was specially accentuated. In 1966, a complete subversion of the usual curve of incidence was observed: the lowest incidence was verified just in Winter; in Summer the incidence was as high as in Spring, the highest of the year; in addition, again 1966 had the lowest general incidence of the quinquennium. It would probably be enlightening if we had at our disposal similar epidemiological data for the previous year.

13. *Outbreaks of Group A Types* (Fig. 3) — The following general facts were observed: 1) the increase of incidence of a certain Type, after reaching its acme, falls to a minimum (herd Type-immunity?); 2) the epidemic wave of T-1 repeated itself for two consecutive years, the waves of other Types happened particularly in one year; 3) the nephritogenic Types didn't show epidemics as accentuated as T-1 (are they less communicable?), T-12 being the most epidemic, its acme was verified in 1967, a year before the great epidemics of T-1; 4) each Type produced its own epidemic waves, so that the epidemic waves succeeded each other accordingly: T-14(49) had its highest incidence in 1966; the T-3/13/B3264 complex (A 3/13... in Fig. 3), in 1967; T-6, in 1968; the T-8/25/Imp.19 complex (A 25... in Fig. 3), in 1969; the year 1970 had small acmes of T-3/13/B3264 complex, T-6 and T-12. 5) the most characteristically epidemic Type was T-1; next came the nephritogenic Types. 6) the Types which appeared in low levels gave like endemic fluctuations.

14. *The year 1970* — That year represents the period immediately post-epidemic and had interesting characteristics of itself. During that year most of the specimens came from Hospital patients, (See *Material and Methods*, 2.) many patients were examined (more than in 1967, Table I) the proportion

of beta-hemolytic streptococci isolated was similar to that of the inter-epidemic period (1966-1967). In addition, it was verified (Fig. 1, Fig. 3, Table IV): 1) the greatest proportion of NT of the quinquennium; 2) the least variety of types; 3) the types which appeared included the rare ones of the previous years; 4) an abrupt fall to zero for T-1 and great falls for T-25..., T-12 and T-14 (49).

15. *Healthy Carriers' Types in 1961* — Seventy-five of 330 rural school children of Ribeirão Preto were healthy carriers of Group A¹⁰. The strains were preserved till 1971, when 56 which survived were T-Typed. The proportion of typable cultures was 59%, distributed according to this frequency (number of strains in parentheses): T-4 (4); T-6 (3); T-9 (2); T-3/13/B3264 (6); for T-11, T-12, T-23, T-25, T-3/13, T-13/28/9 and T-Imp.19, one strain each. In 1961 a collection of 37 from the 75 strains were sent to the CDC (Atlanta, Ga) to be M-typed^(*); with M-sera from 1 to 47, except 9, 11, 27, 34 and 35, only three (8%) could be M-typed: two were M-6 and one was M-41.

These three strains, fortunately enough, could be verified by the T-sera adopted in Ribeirão Preto in 1971, being T-6 for the M-6, and NT for the M-41, as would be expected.

RESUMO

Grupagem de estreptococos beta-hemolíticos e tipagem do grupo A em Ribeirão Preto, S.P., Brasil, 1966-1970.

Cerca de cinco mil pacientes do quinquênio 1966-1970, e da área geográfica de Ribeirão Preto, S.P., Brasil, foram examinados bacteriologicamente tendo em vista o isolamento de estreptococos beta-hemolíticos. A metade dos pacientes proveio de níveis sócio-econômicos modestos, e a outra metade, de médio a alto. A maioria dos pacientes tinha angina aguda, e os restantes, infecções de outras áreas de possível etiologia estreptocócica. Quatrocentos e cinquenta e seis casos de infecções pelo Grupo A (*Streptococcus*

pyogenes) foram diagnosticados. Proporções epidêmicas do agente etiológico (em 1967, mas especialmente em 1968 e 1969) indicam coincidência de períodos epidêmicos para as doenças em apreço. Com relação à distribuição estacional, as médias verificadas para o quinquênio corresponderam em ordem decrescente de frequência ao Inverno, Primavera, Outono e Verão. A tipagem do *Streptococcus pyogenes* por meio de aglutinação com soros T pôde classificar uma alta proporção geral de amostras (90%), a não-tipabilidade sendo geralmente pequena mas atingindo seu máximo no período imediatamente após os surtos epidêmicos (1970). T-1 mostrou os índices de maior incidência sobre todos os outros Tipos em 1968 e 1969. Cada um dos outros Tipos mostrou ondas epidêmicas próprias durante o quinquênio. A presença do Tipo M-49 ("Red Lake") pôde ser verificada. Novos padrões ("patterns") de aglutinação foram encontrados, os quais são relacionados mas não idênticos aos encontrados na Europa e outras áreas.

ACKNOWLEDGEMENTS

This research was possible in part from a "São Paulo State Research Foundation" (*) grant. The support of Prof. José Oliveira de Almeida, Chief of the Department, to the Streptococcus Laboratory is recognized. We also thank Prof. O. Baracchini and Dr. S. F. de Moraes Rêgo for permission to re-examine their routine throat swabs; Dr. Euclides C. de Lima Filho, for assistance in the statistical presentation; Mr. Dácio Burjato Jr. and Mrs. Mirandolina T. M. Junqueira, for technical assistance.

REFERENCES

1. BERGNER-RABINOWITZ, S.; SKLUT, O.; HAIMOWICI, E. & DAVIES, A. M. — Streptococcal Types in Israel Hospitals. A Four-Year Study. *Israel J. Med. Sci.* 2:423-435, 1966.
2. FAVERO, M.; NOGUEIRA, J. L.; SOLÉ-VERNIN, C. & SOUZA, S. L. de — Surto de Glomerulonefrite Difusa Aguda em Comunidade Rural. *Hospital (Rio)* 72:1190-1202, 1967.

(*) By courtesy of Dr. Elaine L. Updyke (CDC, Atlanta, Ga).

(*) Fundação de Amparo à Pesquisa do Estado de São Paulo.

3. FULLER, A. T. — The Formamide Method for the Extraction of Polysaccharides from Hemolytic Streptococci. *Brit. J. Exp. Path.* 19:130-139, 1938.
4. MAXTED, W. R. — Preparation of streptococcal extracts for Lancefield Grouping. *Lancet* 2:255-256, 1948.
5. MAXTED, W. R.; FRASER, C. A. M. & PARKER, M. T. — *Streptococcus pyogenes*, Type 49, A Nephritogenic Streptococcus with a Wide Geographical Distribution. *Lancet* 1:641-644, 1967.
6. MOODY, M. D.; PADULA, J.; LIZANA, D. & HALL, C. T. — Epidemiologic Characterization of Group A Streptococci by T-Agglutination and M-Precipitation Tests in the Public Health Laboratory. *Health Lab. Sci.* 2:149-162, 1965.
7. PARKER, M. T. — The Definition of *Str. pyogenes* serotypes by Agglutination and Precipitation. *Zent. Bakt.* 196:708-714, 1964.
8. PARKER, M. T. — International Survey of the Distribution of Serotypes of *Streptococcus pyogenes* (Group A Streptococci). *Bull. Wld. Hlth. Org.* 37:513-527, 1967.
9. SOLÉ-VERNIN, C. — *Streptococcus pyogenes* carriers detection: Preservation of original throat specimens and their enrichment. *Hospital (Rio)* 65:765-784, 1964.
10. SOLÉ-VERNIN, C. — Groups A, C and G Streptococci and Antistreptolysin O Serum Level from Healthy Rural School-Children of Ribeirão Preto, SP, Brazil. *Hospital (Rio)* 66:331-348, 1964.
11. WANNAMAKER, L. W. — The Epidemiology of Streptococcal Infections, in *Streptococcal Infections*. M. McCarty ed., New York, Columbia University Press, Chap. 12, pp. 157-175, 1954.
12. WILSON, E.; ZIMMERMAN, R. A. & MOODY, M. D. — Value of T-agglutination typing of Group A Streptococci in epidemiologic investigations. *Health Lab. Sci.* 5:199-207, 1968.

Recebido para publicação em 7/12/1972.