

## SCARLATINA IMMUNITY IN HONG KONG

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## INTRODUCTION

SCARLET fever is generally considered to occur extremely infrequently in tropical countries (Rogers, 1908; Castellani, 1919). This is certainly the case in Hong Kong. We are informed by the Director of Medical and Sanitary Services that during the decade 1923-32 only twenty-four cases were reported, of whom thirteen were Europeans and eleven Asiatics (Wellington, 1933).

Hong Kong, situated in latitude 22° N., lies just inside the tropics, and has a very definite seasonal change. During the summer, from about April till October, the mean temperature is over 80° F. with but little daily range, and the mean relative humidity is over 80 per cent. During the winter months, thanks to the north-east monsoon, the climate is considerably cooler and drier. From December till March the daily mean temperature is often below 60° F. with a mean relative humidity of 70 per cent. The population, computed to be well over 500,000, consists mainly of southern Chinese living for the most part under overcrowded conditions. The European population is about 20,000, including probably several hundred children.

In an attempt to obtain data that might throw some light on the factors responsible for this freedom from scarlet fever, the Dick reactions of various age groups of a sample Hong Kong Chinese population were ascertained, together with the frequency among them of haemolytic streptococcal carriers.

## DICK REACTIONS

Relatively few observations appear to have been recorded concerning the Dick test in warm latitudes. Smits (1927) working in Sumatra tested 450 adult Javanese (among whom scarlet fever is said not to occur) and recorded positive Dick reactions in 25 per cent. Among 150 children under twelve years of age he obtained 35 per cent. of positive reactors. Parr and Avery (1927) in Grand Lebanon, where scarlet fever is very rare, examined a small group of people of Arabic extraction. Of twenty-seven tested two gave positive reactions. Among forty-seven refugee children, nationality not stated, six were positive. A number of observations have been made outside the tropics on communities free from scarlet fever. Thus, Asbelew and Margo (1932) tested 103 inhabitants of the island of Kolgujew and found no positive reactors, whereas among a sample of the same race who had crossed to the mainland of Nowaja Semlja, 30.3 per cent. were Dick-positive. Georgiewski and Sembjanowa, quoted by Asbelew and Margo, found that in Taschkent where the natives were apparently immune

to scarlet fever only 1.3–23.3 per cent. of the children gave positive Dick reactions compared with 54 per cent. among European children. Minkewitsch, also quoted by Asbelew and Margo, found that Turkomans, who very seldom developed scarlet fever, yielded a low percentage of positive reactors. In China, a Dick survey was undertaken by Lai (1931) in Shanghai which is some 800 miles north of Hong Kong and where scarlet fever is said to be relatively common. Of 1396 school children and students tested 50.8 per cent. were positive. Lai notes a lower incidence of positive reactors among students from south China. During the progress of the present investigation we learnt that a survey was being conducted among school children near Canton by Dr K. L. Hsii (1934), who kindly sent us his preliminary results: of 684 tested 26.9 per cent. were positive.

The present investigation was conducted in Hong Kong. The Dick testing was commenced in October, 1933, and completed the following April. The toxin used was a "standard" scarlet fever toxin, X 48, prepared from the Dochez N.Y. 5 strain, kindly supplied by Dr R. A. O'Brien, C.B.E., of the Wellcome Physiological Laboratories. It was received undiluted, and diluted as required to 1/1000 in the isotonic borate buffer solution described by Glenny, Pope and Waddington (1928). The dilutions were made up in relatively small batches, each batch being tested before use on a known positive reactor. For the routine testing 0.2 c.c. of the diluted toxin was injected intradermally into the flexor surface of the forearm. In each of the first 250 subjects tested a control dose of diluted toxin inactivated by heating at 95° C. for 2 hours was injected into the opposite arm. As the subjects were often reluctant to receive the injections and as confusion with pseudo-reactions was seldom experienced, the control doses were not persisted in during the later part of the investigation. A clean needle was used for each subject. The results were read 20–24 hours after the injections. The criterion taken for a positive reaction was a definite area of erythema at least 1 cm. in diameter. The positive reactions were quite easy to read on a Chinese skin. The subjects tested were for the most part hospital patients, either in the wards or out-patient departments. Patients acutely ill or suffering from apparent throat conditions were excluded. Some difficulty was experienced in obtaining an adequate number of the younger age groups as school children were not available. Facilities were, however, obtained for testing a group of seventy-five young girls in an orphanage and a number of university students. The numbers of each sex tested were approximately equal, and the results did not show any significant difference between them.

The results of the Dick tests are set forth in Table I. It will be seen that the figures are similar to those reported by Zingher (1924) for a sample of 7700 persons in the United States and by Heller (1927) for 1112 persons tested in Germany. The new-born infants examined were born in a maternity hospital. Their mothers were also tested. Nine of the mothers giving birth to negative reacting infants were themselves positive. The one positive infant was from a

positive reacting mother. Cooke (1927) and Tomcsik (1929) examined the sera of infants for the presence of antitoxin with negative results, irrespective of whether the mothers were Dick-positive. The former author concluded that the negative reactions were due to lack of sensitisation. The allergic or anaphylactic conception of the Dick reaction is discussed at length by Okell (1932), who, however, concludes against its acceptance.

Table I. *Dick reactions of Hong Kong Chinese*

Age	Number tested	Number positive	Percentage
New-born (1-7 days)	39	1	2.6
11-12 months	16	9	56.3
1-4 years	41	29	70.7
5-9 "	66	27	40.9
10-19 "	151	37	24.5
20-29 "	237	40	16.9
30-39 "	164	19	11.6
40-49 "	89	6	6.7
50-59 "	71	5	7.0
60-69 "	38	2	5.3
70-89 "	9	1	—
Total...	921	176	19.1

## FREQUENCY OF HAEMOLYTIC STREPTOCOCCAL CARRIERS

The reported frequency of haemolytic streptococci in normal throats in temperate climates varies from 6 (Williams, Nevin and Gurley, 1921) to 61 per cent. (Pilot and Davis, 1919). Topley and Wilson (1929), examining 1731 swabs taken at monthly intervals from 100 normal people, found the organisms in 13.23 per cent. Bourn, Carpenter and McComb (1933) found them in 9.3 per cent. of 1460 healthy people in Boston.

In the tropics comparatively few studies appear to have been made. Coburn (1931) states that haemolytic streptococci appear but rarely in the throats of Puerto Ricans. In this connection it is significant that Coburn and Pauli (1932) state that scarlet fever is almost unknown in Puerto Rico, but that inhabitants of that island contract the disease on arrival in New York. The same authors quote Vieira to the effect that in the Brazilian port of Santos scarlet fever is rare but common in the adjacent hill station of São Paulo. The Graces (1931) reported the presence of haemolytic streptococci in 15 per cent. of 126 normal throats examined in British Guiana and the West Indies. Milam and Smillie (1931) found the organisms in only 3 per cent. of the inhabitants of the isolated Virgin Island in the West Indies; they also noted a seasonal variation in the frequency, which was 4 per cent. in winter and 1.7 per cent. in summer. These authors also encountered similar findings in Labrador. Concerning the frequency of haemolytic streptococci in isolated Arctic communities it is of interest to note that Wells (1933) found the organisms in 13 per cent. of throat cultures from 300 Central and Polar Eskimos, while Paul and Freese (1933) working in an isolated community in Spitzbergen found them in only 0.3 per cent. of 545 cultures from 140 inhabitants.

The present investigation was commenced in October, 1933, and concluded September, 1934. Most of the individuals Dick tested were swabbed for the determination of the frequency of haemolytic streptococci. Some 700 additional individuals were also investigated for this purpose. For the most part they were hospital out-patients, and, as in the case of the Dick tests, care was exercised to exclude individuals acutely ill or suffering from apparent throat conditions. As a routine, the swabbing was performed once a week, about forty being done at a time. In this way about 160 individuals were examined each month of the calendar year, with the exceptions of December and July when no swabbing was done. The technique adopted was as follows. Swabs of the West pattern protected by glass tubes were employed. After each had been well rubbed over the tonsils and the mucosa of the nasopharynx, the swabs were brought back to the laboratory and immediately seeded on to 5 per cent. ox-blood agar plates. One plate was used for each case, the swab being dabbed on to the centre and the inoculum well distributed with a sterile glass spreader. After 24 hours' incubation the plates were examined with a binocular microscope for small colonies producing definite zones of haemolysis, such colonies were picked off into tubes containing 3 c.c. of 16 per cent. rabbit-serum broth. These subcultures were then incubated for 8–10 hours. The test for haemolysis was performed by adding to each tube 0.5 c.c. of 5 per cent. washed rabbit red cells. Rabbit cells were employed because they were the only ones readily obtainable under sterile conditions. Before adding the red cells, films were made to check the morphology of the organisms and subcultures were put up. The results of the haemolytic tests were read after 1 hour in the water-bath at 37° C. and again after remaining in the ice-chest overnight. Only those tubes showing complete haemolysis were considered positive. There was generally a close correlation between the production of a wide zone of haemolysis on the plate and complete haemolysis in the tube. It is realised that a greater number of strains might have been isolated by mouse inoculation and by anaerobic incubation, but these procedures were not convenient to carry out.

Table II records the frequency of haemolytic streptococci in cultures from each monthly sample of apparently normal people. The mean temperature for each month is also appended. For these temperature data we wish to acknowledge our indebtedness to the Director of the Royal Observatory, Hong Kong. It will be seen that the cooler months coincide with a rise in the frequency of positive cultures. This seasonal variation although suggestive would need further investigation for the determination of its significance.

In Table III the frequency of haemolytic streptococci is classified according to age. It is apparent that the organisms were isolated more frequently from the earlier age groups. Bourn, Carpenter and McComb in their Baltimore investigation also recorded a higher frequency of haemolytic streptococci in children than in adults. Inspection of Table III will reveal an observed difference in streptococcal frequency of 4.51 per cent. between the age groups 0–19 and 20–39. The standard deviation of this difference is 1.56 per cent. The

difference is therefore 2.89 times its standard deviation and consequently probably significant.

Table II. *Monthly frequency of haemolytic streptococci in nasopharyngeal flora of random samples of Hong Kong Chinese*

Month	Mean temp. °F	Number examined	Number positive	Percentage
October	77.7	169	9	5.33
November	70.3	129	9	6.98
January	56.7	232	27	11.64
February	61.1	164	18	10.97
March	64.1	161	16	9.94
April	68.8	150	10	6.67
May	77.5	124	6	4.84
June	81.5	168	2	1.19
August	80.9	148	5	3.38
September	82.3	155	4	2.58
Total...	—	1600	106	6.625

Table III. *Frequency of haemolytic streptococci in nasopharyngeal flora of Hong Kong Chinese of different age groups*

Age	Number examined	Number positive	Percentage
0-9	188	17	9.04
10-19	221	25	11.31
20-29	504	30	5.95
30-39	329	18	5.47
40-49	197	12	6.09
50-59	114	4	3.51
60-69	39	0	—
70-89	8	0	—
Total...	1600	106	6.625

A number of the strains of haemolytic streptococci isolated were tested for production of erythrogenic toxin. Cultures were grown in trypsin digest broth for 4 days, killed by exposure to 60° C. for half-an-hour, and filtered through Chamberland L 3 candles. The filtrates were diluted in the borax buffer solution already referred to. Four dilutions were prepared from each filtrate, namely 1/100, 1/250, 1/500 and 1/1000. All the filtrates were tested by the intradermal injection of 0.1 c.c. into one or other of two susceptible subjects. The criterion taken for a positive reaction was a definite area of erythema at least 1 cm. in diameter. The results are expressed in Table IV in terms of skin test doses in 1 c.c. of undiluted toxin. It will be seen that of thirty-three strains tested twenty produced toxins giving positive skin reactions in dilutions of

Table IV. *Toxigenicity of strains of haemolytic streptococci from normal throats*

Skin test doses per c.c.	Number of strains
10,000	1
5,000	1
2,500	5
1,000	13
Nil, or less than 1,000	13

1/100 or more. Several of the remaining strains produced faintly erythrogenic reactions in dilutions of 1/100. As a control, a filtrate was prepared from a Dochez strain of scarlatinal *Streptococcus*. This was made at the same time and under identical conditions as the others. This filtrate diluted 1/1000 gave a considerably more vigorous reaction than any of those from the throat strains.

Tillet and Garner (1933) found that strains of haemolytic streptococci derived from human infections had the property of digesting clotted human fibrin, while most strains derived from animal sources failed to do so. Hare and Colebrook (1934), applying this test to strains of saprophytic haemolytic streptococci isolated from the vagina of afebrile parturient women, reported that the majority of such strains caused no fibrinolysis. We submitted twelve of the strains isolated from normal throats to this test. The technique described by Hare and Colebrook was adopted. Three of the strains completely digested the human fibrin within 30 min. incubation, five more did so after 3 hours, while two of the strains produced partial digestion after several hours. These results probably justify the conclusion that several of the throat strains are true haemolytic streptococci of potential pathogenicity to human beings.

#### DISCUSSION

The results of the Dick tests indicate that among the Hong Kong Chinese there exists a definite skin susceptibility to the erythrogenic streptococcal toxin, and that this susceptibility becomes less frequent with advancing age. In seeking an explanation of this in relation to the general freedom from scarlet fever it must be borne in mind that scarlet fever is essentially a clinical entity. Its relation to streptococcal sore throat is universally recognised to be close. We are informed by local medical practitioners that epidemic sore throat is not uncommon in Hong Kong during the winter months. As yet, however, we have had no opportunity of investigating this condition. It is appreciated that the careful examination of strains of streptococci isolated from such complaints is a desirable step in the approach to the general problem. In view of the frequency of haemolytic streptococci in the normal throat flora it seems not unreasonable to assume that the acquirement of Dick-negativeness is an immunity reaction proceeding along the lines commonly held to obtain in temperate lands, *i.e.* active immunisation by subclinical infections with toxigenic streptococci. This assumption is compatible with the similarity of the age distribution of Dick reactions in Hong Kong compared with those recorded elsewhere.

Other observers have attempted to explain local freedom from scarlet fever. Thus Smits (1927) in his paper on Dick reactions in Sumatra suggests that the loss of susceptibility to Dick toxin proceeds as a non-specific process. He attributes the freedom from scarlet fever to the absence of scarlatinal strains of streptococci. Doull, quoted by Asbelew and Margo (1932), contends that scarlet fever is an accident in the process of immunisation and is more common in cold countries owing to the resistance of the mucosa being lowered by

catarrhal conditions. In Hong Kong, however, catarrhal conditions are by no means uncommon. Smits also suggests that in Sumatra the constant exposure of the skin to the sun may be not without effect in rapid development of scarlatinal immunity. In Hong Kong this consideration may be ruled out, as exposure of the skin of the body is exceptional.

That local freedom from scarlet fever may be due to racial factors is rendered unlikely by the widespread immunity reported throughout the tropics. The cause of topical immunity awaits discovery.

#### SUMMARY

1. In Hong Kong, where scarlet fever is extremely rare, the Dick reactions of a random sample of 921 Chinese were ascertained. Positive reactions were obtained in 19.1 per cent. The distribution of positive reactors in different age groups is recorded; it bears a general similarity to those recorded by various observers in Europe and the U.S.A.

2. A haemolytic streptococcal carrier rate of 6.6 per cent. was determined by nasopharyngeal swabbing of 1600 Hong Kong Chinese. The frequency of haemolytic streptococci was higher during the cooler months of the year and among the younger age groups.

3. Erythrogenic toxin production and fibrinolytic activity were demonstrated in a number of the strains of streptococci isolated from apparently normal throats.

4. The significance of these findings is discussed.

ACKNOWLEDGMENTS. We wish to acknowledge our indebtedness to Dr R. A. O'Brien, C.B.E., and Dr H. J. Parish, of the Wellcome Physiological Research Laboratories, for helpful advice and for kindly supplying us with Dick toxin, and to the staffs of the Hong Kong Government Civil and Tung Wah Hospitals for facilitating our access to the wards and out-patient departments.

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(*MS. received for publication* 12. XII. 1934.—Ed.)