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The role of cutaneous diphtheria in the acquisition of immunity

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INTRODUCTION

Several workers have described the carriage of *Corynebacterium diphtheriae* organisms in the cutaneous ulcers of troops stationed in the tropics during war time. Craig (1919) attributed the origin of these 'desert', 'septic', or Veldt sores to the Klebs–Loeffler bacillus, whilst other workers such as Benstead (1936) and Liebow, MacLean, Bumstead & Welt (1946) observed that the diphtheria organisms were mere secondary invaders on lesions of the skin, which had been previously caused by some trivial injury. Liebow and his colleagues, working in the Pacific Islands, presented data indicating 'the existence of a tremendous reservoir of diphtheria amongst the natives in the tropics which is largely cutaneous, which affects chiefly young children and which accounts in large measure for the remarkably accelerated acquisition of a state in which they do not react to the Schick test'. Similar inferences have been drawn by workers such as Marples & Bacon (1956) and P. J. Collard (personal communication). The present investigation carried out amongst a non-immunized population in a semi-rural area of Ceylon, confirms the findings of the above workers and suggests that cutaneous diphtheria may be the main method whereby natural immunization is acquired in tropical countries.

METHODS

The field survey was carried out in four villages situated in the Western Province of Ceylon. The villages cover an extent of about 14 square miles and have a population of about 43,000 (figures from the Medical Officer of Health, Kotte, Ceylon). Before this investigation, no active immunization against diphtheria had been carried out in this area on a large scale.

The Schick test

The Schick test toxin ('Wellcome' brand) was flown from England in small batches and stored at 4° C. Each batch was used within 2 months of arrival. Tests were read on the fourth day after injection, thus allowing time for any pseudo-reactions which may have developed to disappear.

Isolation of C. diphtheriae *from carriers*

Swabs were taken from all the children who were Schick tested to determine the carrier rate of *C. diphtheriae*. One swab was taken from both tonsillar areas

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of the throat, another was introduced into each nostril in turn and the anterior nares were swabbed, a third was rubbed against both knees and the inner malleoli of both ankle joints. Swabs were also taken from any septic lesions such as otitis and conjunctivitis and from any cutaneous ulcers present. Each swab thus taken was immediately inoculated on Downie's blood tellurite plates (Cruickshank, 1965*a*). After 24 hr. incubation at 37° C., any colonies morphologically resembling *C. diphtheriae* were subcultured on blood agar plates and Loeffler's serum slopes. When pure cultures were obtained, they were confirmed to be *C. diphtheriae* by cellular morphology, colony appearance on tellurite media, fermentation tests in serum sugars, reduction of nitrates to nitrites and the inability to hydrolyse urea.

In testing for acid production in serum sugars, 1 % peptone water containing 20 % ox serum, 1 % of each sugar and 10 % Andrade's indicator was used. The ability to reduce nitrates was tested on Cook's plates (Cook, 1950). Christensen's medium (Christensen, 1946) was used for the testing of urease production.

Tests for toxigenicity

Tests for toxigenicity were carried out by both *in vivo* and *in vitro* methods. The *in vivo* tests were carried out according to the methods described by Cruickshank (1965*b*). The *in vitro* tests were done by using Elek's agar diffusion technique (Elek, 1948) as modified by M. Glasset (personal communication). Glasset reduced the concentration of New Zealand agar in the basal medium from 1·5 to 0·7% and correspondingly reduced the concentration of antitoxin in the filter paper strip from 1000 units to 50 units per ml. This technique not only accelerates the production of lines of precipitate due to the toxin-antitoxin complex but also prevents the formation of other lines of precipitate which are not specifically due to the toxin and antitoxin. All the cultures tested gave identical results when tested by both methods.

RESULTS

The percentages of Schick negative children, the percentages of *C. diphtheriae* carriers and the percentages showing cutaneous ulcers in each age group are

Table 1. *Results of Schick survey and carrier survey*

Age groups	Total no. tested	Percentage Schick negative	Percentage *C. diphtheriae* carriers	Percentage occurrence of cutaneous ulcers
0–3 months	20	95	0	0
3–6 months	28	78·5	10·7	3·4
6 months–1 yr.	97	22·7	7·2	7·2
1–1½ years	85	23·5	8·2	14·1
1½–2 years	57	33·3	12·3	21·1
2–2½ years	44	34·1	15·9	13·6
2½–3 years	48	64·5	4·1	22·8
3–3½ years	43	60·5	16·3	18·6
3½–4 years	40	62·5	10	22·5
4–5 years	59	64·4	8·5	10·2
5–6 years	85	64·7	15·3	18·8
6–7 years	58	76	10·3	17·2

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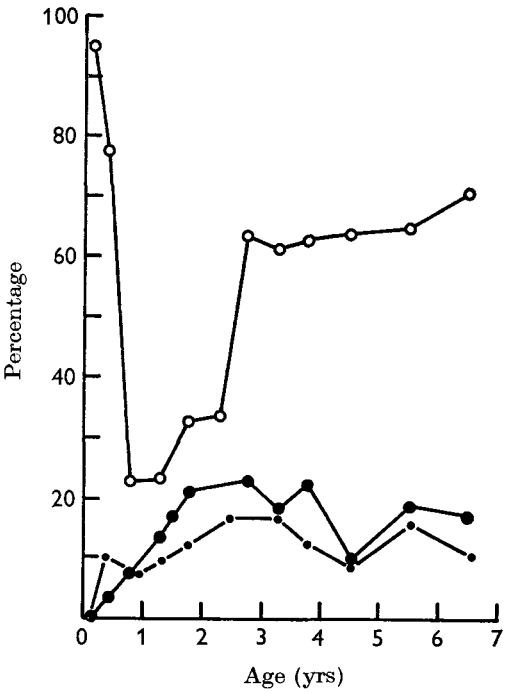

Fig. 1. Results of Schick survey and carrier survey. Schick negative children ○——○; incidence of cutaneous ulcers ●——●; total isolations of *C. diphtheriae* •——•

Table 2. *A summary of* C. diphtheriae *isolations and carrier rates*

Strains isolated	
70 mitis type	
2 gravis type	
1 intermedius type	
73	

Sites of isolation

Throat	24 (3 toxigenic)
Cutaneous ulcers	40 (9 toxigenic)
Intact skin	6
Nose	2
Ear	1 (1 toxigenic)
	73* (13 toxigenic)

* Includes isolations from five children who carried the organism in more than one site.

Carrier rates

Number of children tested	664
Number of children carrying *C. diphtheriae*	68 (10·2 %)
Number of children with cutaneous ulcers	98
Number of isolates of *C. diphtheriae* from ulcers	40 (40·8 %)
Cutaneous ulcer carriage rate of *C. diphtheriae*	6·0 %
Throat carriage rate of *C. diphtheriae*	3·9 %

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tabulated in Table 1. Although about 700 children were tested altogether, only the results of 664 have been included in the series for various reasons such as not being available when the results were read, uncertainty of the age, etc. The same results are also depicted in Fig. 1. A summary of the *C. diphtheriae* isolations is given in Table 2. Included among the group classified as ulcers was a culture isolated from a swab taken from an infected smallpox vaccination site. Five children harboured the organisms in more than one site.

DISCUSSION

The pattern of immunity in the different age groups as measured by the Schick test is typical of that found in any non-immunized population where the rate of exposure to the disease is high. The findings agree partly with those of a few surveys carried out in India (Robinson & Lalitha Bai, 1964; Das, 1934; Paricha, Banerjee & Wordsworth, 1939). It is seen in Fig. 1 that 65 % of the children are Schick negative by the age of 5 years.

The incidence of diphtheria in Ceylon as judged by the notifications of clinical cases is not very high. For example, only 465 cases of diphtheria were reported from the whole of Ceylon in 1964 (Assistant Epidemiologist, Ceylon 1967) from a population of about eleven million. The carrier rate of *C. diphtheriae* as assessed by Gulasekeram, Gunaratna & Somasunderan in 1956 from a group of school children below the age of eleven was only 2·2 %. Making allowance for the fact that all cases of diphtheria are not reported, the incidence of the disease and the carrier rate mentioned above are not sufficiently high to justify a development of 65 % Schick negativity by the age of 5 years. Unless grossly apparent in other sites, clinical diphtheria is only diagnosed from lesions in the throat. Similarly in the survey mentioned above, the carrier rate was assessed only from throat and nasal swabs. The present work reveals the high incidence of diphtheria organisms in the cutaneous ulcers of these children. Whereas only 3·9 % of the children carried *C. diphtheriae* in their throats or noses, 6·0 % of them had the organisms in ulcers; 40·8 % of the ulcers examined in these children yielded *C. diphtheriae* organisms. A higher percentage of toxigenic organisms was found among the cultures isolated from the ulcers than from those isolated from other sources. Not only is there a relationship between the percentage of total diphtheria isolations and the percentage of ulcers found in each age group, but there is also a relationship between the latter and the Schick conversion rate in each age group (see fig. 1).

Although 69 % of the toxigenic *C. diphtheriae* cultures were isolated from children between 1 and 3½ years where the rate of Schick conversion is the highest, the majority of isolations represent non-toxigenic cultures. The question arises whether non-toxigenic diphtheria organisms contribute in any way towards the acquisition of immunity to diphtheria. 'Non-toxigenic' organisms cannot obviously produce an antitoxic immunity unless they produce minute quantities of toxin which cannot be detected by routine laboratory techniques, as suggested by Marples & Bacon (1956). On the other hand, the non-toxigenic diphtheria organisms may have been derived from strains which were originally toxigenic. Before the intro-

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duction of antibiotic therapy, several workers reported the isolation of non-toxigenic *C. diphtheriae* organisms from convalescent patients who were originally infected with toxigenic cultures. Okell (1929) while discussing the association of virulent and avirulent strains in patients, reports several instances where toxigenic and non-toxigenic organisms existed in the same patient at the same time, or where the isolation of the original toxigenic organism was followed by the isolation of a non-toxigenic. The toxigenic and non-toxigenic organisms found in the same patient were shown to belong to the same serological type in several cases. Liebow and his colleagues in their study of cutaneous diphtheria in the tropics (Liebow *et al.* 1946) noticed that as the ulcers got older the toxigenic strains were replaced by non-toxigenic strains. Although the work of Anderson & Cowles (1958) showed that such a conversion from toxigenicity to non-toxigenicity may occur *in vivo* through the mediation of antibodies against bacteriophages, the present writers could not detect any bacteriophage-neutralizing antibodies in the sera of fifty Schick negative children tested.

A third possibility is that the non-toxigenic organisms are not descendants of the original toxigenic organisms, but that they belong to a different group which is better adapted to lead a parasitic existence upon its host. The toxigenic organisms are probably more fastidious in their requirements and need the devitalized tissue produced by their toxin for continual existence. In fact it is seen from this survey that ten out of thirteen of the toxigenic organisms isolated were from sites where there was pus or serum. Only three toxigenic organisms have been isolated from apparently healthy throats, while none were isolated from intact skin or from the nose. The fact that the non-toxigenic organisms isolated appeared to belong to a bacteriophage pattern which is different from that of the toxigenic organisms also supports this view.

Frobisher & Parsons (1943) have shown that non-toxigenic diphtheria organisms produce an antibacterial immunity in rabbits whereby the injected animals become more resistant to doses of toxigenic *C. diphtheriae* which are invariably fatal to normal animals. A similar antibacterial immunity may occur in humans, thus protecting them from the severity of the infections due to subsequent attacks by toxigenic organisms.

Cutaneous diphtheria may present a source of danger to non-immunized adults who come into the tropics for the first time from other countries where the carrier rate is low. This has been demonstrated during the two world wars by Craig (1919), Benstead (1936) and Liebow and his colleagues (1946.) But amongst the local child population which is exposed to cutaneous diphtheria while partially immune owing to the presence of maternal antibody, it does not appear to present such a serious problem. At least one of the dangers of faucial diphtheria, that of laryngeal obstruction, does not present itself. Being limited to the surface area of an ulcer, the organisms cannot spread over a large area and hence probably cannot produce much toxin. As the area for absorption of toxin is also small, it is likely that less toxin enters the system. Hence in the absence of artificial immunization, this 'live vaccination' may provide a method of acquiring a natural immunity against the occurrence of serious cases of diphtheria.

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SUMMARY

A field survey conducted amongst children in a semi-rural area of Ceylon has revealed a high rate of Schick negative conversion early in life. A high carrier rate of *C. diphtheriae* in cutaneous ulcers which probably accounts for this early Schick conversion has been detected. The role of cutaneous diphtheria and that of non-toxigenic organisms in the acquisition of natural immunity to the disease is discussed.

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