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Naso-pharyngeal carriage of *Haemophilus influenzae* type B

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INTRODUCTION

Pittman (1931) showed that the bacterial species *Haemophilus influenzae* is divisible into S (capsulate) and R (non-capsulate) strains. She also distinguished among the capsulate strains six sero-types, *a*, *b*, *c*, *d*, *e* and *f* (1931 and later unpublished work).

Haemophilus influenzae is commonly present in the upper respiratory tracts of healthy children and adults (Blackburn *et al.* 1930; Straker, Hill & Lovell, 1939; Masters, Brumfitt, Mendez & Likar, 1958); and also in the sputum of patients with bronchial diseases (Mulder, 1940; May 1953, 1954; Allibone, Allison & Zinnemann, 1956). By routine use of appropriate media it can be found in many other situations (Rogers, Zinnemann & Foster, 1960). Most strains from all the sources mentioned so far are non-capsulate.

In contrast, the strains which cause meningitis, pneumonia, epiglottitis, suppurative arthritis and certain other acute infections are nearly always capsulate and of Pittman's type *b* (Pittman, 1931; Alexander, Ellis & Leidy, 1942; Thilenius & Carter, 1959).

The available information suggests that under normal conditions *H. influenzae* type *b* is carried in the upper respiratory tract by about 3 % of children under 5 years old and about 1 % of older children and adults (Dawson & Zinnemann, 1952; Masters *et al.* 1958). But Good, Fousek, Grossmann & Boisvert (1943) found carriers of such strains in the homes of 3 out of 5 children with illnesses due to *H. influenzae* type *b*; whereas they found none in the homes of 9 'control' children (3 carrying type *b* strains without associated symptoms and 6 from whom no such organisms had been grown).

Haemophilus influenzae was much the commonest cause of meningitis seen at the University College Hospital of the West Indies, Jamaica, in the years 1958–60 (Turk & Wynter, 1961). All the strains isolated belonged to type *b*. All the patients with haemophilus meningitis were children, most of them between the ages of 3 months and 2 years (cf. Smith, 1954; Ouyang & Ting, 1957; Karelitz, Desposito, Spinner & Isenberg, 1960). Partly because of this prominence of *H. influenzae* type *b* as a cause of meningitis in Jamaica, naso-pharyngeal carriage-rates for the whole species were studied in various samples of the island's population. The methods and findings of that survey have been reported in full elsewhere (Turk,

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1962). The present paper is primarily concerned with the frequencies of isolation of type *b* strains, and especially with the high concentrations found in some of the samples.

METHODS

Specimens

A single specimen was collected from each subject on any one occasion, by passing a fine, flexible wire swab along the floor of one nostril to the naso-pharynx. The swab was used within $2\frac{1}{2}$ hr. of collection to inoculate the two media described below.

Primary cultures

(*a*) Half of a horse-blood agar plate was inoculated and spread so as to give progressive thinning of the inoculum from the periphery to the half-way line. After the other half-plate had been similarly inoculated with another swab, *Staphylococcus aureus* was streaked across the plate at right angles to the half-way line, so as to pass through the middle of both inocula. After 24 hr. incubation the plates were examined for the presence of satellite colonies alongside the staphylococcal streaks.

(*b*) Each swab was also used to inoculate half a plate of a selective medium. This was made by adding 5 % of citrated horse blood to melted Veal Infusion Agar (Difco), heating the mixture in a boiling water-bath for 10 min., cooling to about 50 °C., and adding 1 % of supplement A (Difco) before pouring the plates. The supplement, as well as being an additional source of X- and V-factors, contained crystal violet, of which the final concentration was 1:714,000. This delayed the growth of many respiratory-tract organisms—notably staphylococci and neisseriae—while allowing *H. influenzae* to form colonies of up to 2 mm. in diameter after 24 hr. incubation at 37 °C.

Recognition of capsulate strains

Colonies on either of the primary media which resembled those of *H. influenzae* were subcultured to Levinthal's agar medium as modified by Alexander (1958). After 18 hr. incubation, the Levinthal's agar plates were examined by strong, obliquely transmitted light for the greenish iridescence of capsulate strains described by Pittman (1931). Capsulate strains were also distinguishable on this medium by their greater opacity, more mucoid surface appearance and tendency to confluence of colonies.

Typing of capsulate strains

All strains considered to be capsulate were typed by slide-agglutination, using sera bought from Burroughs, Wellcome and Co. in England; and by 'capsule-swelling' tests, using either sera made and kindly supplied by Miss Grace Leidy of the Presbyterian Hospital, New York, or those bought from Hyland Laboratories, Los Angeles. Sera from the two American sources usually gave convincing type-specific 'capsule-swelling' reactions with organisms from 18 hr. Levinthal's

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agar cultures, though for a few strains it was necessary to use much younger cultures. The Burroughs, Wellcome sera did not give satisfactory 'capsule-swelling' reactions with over night cultures.

RESULTS

Non-Jamaican children

These were members of white families from Britain only temporarily resident in Jamaica. Specimens were collected from:

(*a*) 58 children, aged between 4 months and 13 years, swabbed within a few days of their arrival from Britain.

(*b*) 32 of the same children re-examined 7 months later.

(*c*) 37 other children, aged between 8 months and 11 years, who had lived in the island for periods exceeding 7 months.

Type *b* strains were recovered from one 3-year-old boy in (*a*) and from one 4-year-old and one 9-year-old girl in (*c*), giving an overall recovery rate of 2·4 per 100 swabs. The corresponding rate for all *H. influenzae* strains was 60·6 per 100 swabs.

Jamaican children giving 'normal' results

Nearly all of the children referred to under this and subsequent headings were at least partly of negro descent, most of them predominantly so; but a few pure Chinese children were included in some of the samples. Specimens were collected from:

(*a*) 100 babies, aged between 3 months and 2 years, attending a child welfare clinic.

(*b*) 200 school children, aged between 5 years and 9 years, representing 4 large schools with a total attendance of about 3000 pupils.

(*c*) 92 children, aged between 4 months and 14 years, living in 23 homes on a lower-income-group housing estate.

(*d*) 81 of these 92 children re-examined 4 months later, together with 5 other children from the same homes who had not been available on the first occasion.

Type *b* strains were recovered from 2 of the 100 babies in (*a*), from 7 of the 200 school children in (*b*), from 3 of the 92 children in (*c*) and from 2 of the 86 in (*d*), giving an overall recovery rate of 2·9 per 100 swabs. The highest concentration consisted of 4 strains found among the 60 representatives of one of the schools in (*b*).

The recovery rates for all *H. influenzae* strains were lower in each of these samples and in all age groups than those found among the non-Jamaican children, the overall figure being 40 per 100 swabs.

Families of meningitis patients

Swabs were obtained, in each case within 2 days of the patient's admission to hospital, from the families of 6 children with haemophilus meningitis. All available members of each family who lived in the same home as the patient were included—the 'home' being a house, a single room or a shack, according to the

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economic status of the family. As shown in Table 1, *H. influenzae* type *b* was recovered from 5 of the 6 families, from 2 of the 8 parents and from 7 of the 14 siblings.

Table 1. *Families of meningitis patients*

Family number	Meningitis patient	Members of families: Found to carry *H. influenzae* type *b*	Members of families: Not found to carry H. *influenzae* type *b*
1	Male 2 years	F 4 years	F 5 months
		F 6 years	M 8 years F 10 years Mother
2	Female 3 years	—	M 1 year Mother Father
3	Male 5 months	F 2 years	F 5 years M 7 years Mother Father
4	Male 11 weeks	M 3 years Mother	F 4 years
5	Female 11 months	F 10 years	Mother
6	Female 6 months	F 2 years M 4 years Mother	—

M, male child (patient's brother or half-brother); F, female child (patient's sister).

An orphan home

(*a*) The senior house contained about 170 children, nearly all between the ages of 5 years and 15 years. Two visits were made at an interval of 4 months, and 329 swabs were collected. No type *b* strains were isolated. Recovery rates for all *H. influenzae* strains were 49·3 and 67·7 per 100 swabs on the two occasions.

(*b*) The junior house contained up to 50 children, aged between 1 year and 4 years, who had very little contact with those in the senior house. 4 visits were made, with the results shown in Table 2. Type *b* strains were considerably more common than in any of the preceding groups other than the families of meningitis patients. Recovery rates for all *H. influenzae* strains were also high.

(*c*) The nursery contained up to 15 babies not yet old enough to walk, and kept in separate cots in a room to which older children were not admitted. They were, however, cared for by the same staff as the children of the junior house, and several of them were promoted to the junior house during the period of observation. They gave such remarkable results when first examined that they were swabbed repeatedly. From the results shown in Table 3 it can be seen that:

(i) On each of the first 6 visits *H. influenzae* type *b* was isolated from between 70 and 38 % of the children present.

(ii) No child was proved to enter the nursery carrying a type *b* strain.

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(iii) All the children who were in the nursery at the beginning of the period of observation carried such a strain at some time.

(iv) Only 3 of those admitted later failed to yield such a strain at some time, and 2 of these were only present during the latter part of the period.

(v) No child was observed to carry a type *b* strain for more than 3 months, though some left the nursery while still carrying such strains.

Table 2. *The orphan home junior house*

Date	Number of children	Number found to carry *H. influenzae* type *b*	Number found to carry *H. influenzae* all strains
17. viii. 60	49	5	33
31. viii. 60	49	5 (3)	36
27. ix. 60	48	8 (5)	39
28. xi. 60	46	6 (2)	39
Total	192	24	147

The figures in parentheses indicate the number of those found to carry type *b* strains who had also yielded such strains on previous occasions.

Table 3. *The orphan home nursery*

(August 1960 to March 1961)

Ref. no. of child	Age (months)	Sex	Date of admission	Results from swabs collected on 29 Aug.	31 Aug.	27 Sept.	12 Oct.	27 Oct.	28 Nov.	26 Jan.	2 Mar.
1	7	F	April	*b*	*b*	+	*b*	*b*	*b*+	.	.
2	12	F	April	*b*	+	(*b*)	.	.	(*b*)	.	.
3	7	M	April	*b*	*b*	*b*	*b*	*b*+	+	+	+
4	8	F	April	*b*	*b*	+	*b*	*b*+	*b*	.	.
5	4	F	May	*b*	*b*	*b*	*b*	.	.	.	.
6	9	M	June	−	−	+	*b*	.	(*b*)	.	.
7	2	F	June	+	+	*b*	*b*	*b*+	*b*	*b*	.
8	5	M	July	*b*	*b*	*b*	*b*	*b*	*b*	+	+
9	5	F	July	*b*	*b*	+	+	+	*b*	+	+
10	9	M	July	+	+	*b*+	+	+	+	*b*+	+
11	4	M	8 Sept.	.	.	−	−	−	*b*	*b*	−
12	3	F	9 Sept.	.	.	−	+	−	*b*+	.	.
13	?1	M	12 Sept.	.	.	−	−	+	+	+	+
14	4	M	14 Sept.	.	.	−	*b*	?	*b*	+	+
15	8	F	*	.	.	.	?	*b*+	*b*+	+	+
16	?8	M	*	.	.	.	−	−	+	*b*	*b*+
17	3	M	21 Nov.	.	.	.	.	.	−	+	*b*+
18	12	M	30 Nov.	.	.	.	.	.	.	+	+
19	5	F	24 Dec.	.	.	.	.	.	.	+	+

The age given for each child is that when first examined.

−, no *H. influenzae* grown; +, non-capsulate strains grown; *b*, type *b* grown; *b*+, type *b* and non-capsulate strains grown; (*b*) type *b* grown but the child was no longer in the nursery; ?, cultures overgrown by *Proteus* sp., with probable underlying *H. influenzae* which could not be obtained in pure culture; slide-agglutination and capsule-swelling tests for type *b* in the mixed growth negative; ., not present on this date; *, in the home before the period of observation began, but absent during August and early September.

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(vi) At the last visit only two of the more recent arrivals yielded type *b* strains, whereas no such strains were isolated from 7 children then present who had carried them on previous occasions.

No case of meningitis or other illness attributable to *H. influenzae* occurred in the nursery immediately before, during or immediately after the period of these studies. Haemophilus meningitis had occurred there in the past, but the last case was in May 1959.

The study of this very interesting situation could not be continued because I left the island in April 1961.

A day-nursery and play-school

Children from low-income homes were left at this institution while their parents were at work. Its population therefore varied, but some children attended fairly regularly over long periods.

Table 4. *The day-nursery and play-school*

	October 1960						March 1961					
		Number found to carry *H. influenzae*						Number found to carry *H. influenzae*				
		Capsulate types						Capsulate types				
Grade	Number of children examined	*a*	*b*	*e*	*f*	All strains	Number of children examined	*a*	*b*	*e*	*f*	All strains
A	10	0	0	0	0	5	10	0	0	0	0	3
B	15	0	0	0	0	9	15	0	1	1	1	13
C	16	0	3	1	0	10	13	0	0	3	0	9
D	10	0	2	0	0	8	12	0	1	4	2	10
Play-school	34	1	4	0	0	18	0					
Total	85	1	9	1	0	50 (59 %)	50	0	2	8	3	35 (70 %)

The nursery children were graded according to age and activity and accommodated in 4 adjoining rooms of one building, as follows:

A, under or just over 1 year old, kept in cots;

B, aged 1 and 2 years;

C, aged 2 and 3 years;

D, aged 3 years.

These grades mixed very little, except that C and D sometimes played together out of doors; but the staff moved freely between the 4 rooms.

The play-school was in a separate building with different staff. It contained children aged 2–4 years, many of whom had formerly been in the nursery or had siblings there.

In October 1960 *H. influenzae* type *b* was isolated from 5 of the children in grades C and D (see Table 4). 13 female nursery staff were therefore swabbed 2 days later, but no type *b* strains were found. The nursery (but not the play-school) was

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re-visited in March 1961, but of the 50 children present at that time only 22 had been examined in October 1960. These included only 2 of the 5 who had then yielded type *b* strains; neither of these did so again. However, both of the carriers of type *b* strains who were found in March had been present in October.

For the sake of simplicity, no reference has been made to the few capsulate strains of types other than *b* which were found in the groups of children already discussed. But at the second visit to the nursery 11 such strains were found (8 of type *e* and 3 of type *f*) among the 40 children of grades B, C and D, as compared with only one (of type *e*) on the first occasion. No capsulate strains were found among the babies of grade A on either occasion.

No illness attributable to *H. influenzae* was recognized among the nursery or play-school children during the relevant period.

The author's family

This consisted of 4 children, aged from 5 to 11 years, my wife and myself. All were swabbed in June 1960 and failed to yield *H. influenzae*. But in November 1960, when I was in frequent contact with the orphan home babies and their cultures, a swab from my own naso-pharynx yielded a type *b* strain, as did 3 others at approximately 3 week intervals until the beginning of January 1961. No *H. influenzae* was grown from the next swab, which was not collected until the end of February but a final specimen in the middle of March again yielded a type *b* strain. Meanwhile, the other members of the family, swabbed in November 1960 and in January and March 1961, failed to yield any type *b* strains despite my persistent carriage of such an organism and normal close family contacts.

DISCUSSION

The finding of a 41% *H. influenzae* type *b* carrier-rate among the families of patients with haemophilus meningitis, as compared with rates of 2·4 and 2·9% among the 'normal' groups, confirms the report from Yale (Good *et al.* 1943) that carriers are common in such families. But the meaning of this fact is far from clear. It is somewhat reminiscent of the situation described by Glover (1920) in connexion with meningococcal meningitis; but the two diseases are quite different, in that no epidemic of haemophilus meningitis has ever been reported. Seasonal fluctuations in incidence have been observed (Neal, Jackson & Appelbaum, 1934; Ouyang & Ting, 1957), and also groups of synchronous but geographically remote cases (Turk & Wynter, 1961); but there have been very few published reports of multiple cases occurring in the same household or institution, or otherwise demonstrably connected (Kline, 1962). Furthermore, the Yale workers' and my own findings do not permit the deduction that an abnormally high carriage-rate for *H. influenzae* type *b* precedes and gives rise to a case of meningitis. It could be that the affected child in the early stages of the disease is a potent source of infection for others in the household. It is unlikely that any one will ever have the good fortune to show which of these interpretations is correct by swabbing a family just before a case of haemophilus meningitis

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occurs. But it may be possible to obtain retrospective information about the sequence of events by repeated measurements of type-specific antibodies in the blood of members of the family after the case has occurred. We are at present attempting to do this in Newcastle, using a serological procedure which had not been developed at the time of the Jamaican studies.

Ounsted (1950, 1951) found that children with haemophilus meningitis more often had 2 or more older siblings under the age of 12 years than did children of similar age admitted to hospital for other reasons, including meningococcal meningitis. He suggested that 'the *H. influenzae* organism requires one or two passages through partially immune contacts before a strain is evolved which can pass the meningeal barrier'. There are other possible explanations of his findings, as he admitted, and the good nutritional state of patients with haemophilus meningitis seen at the University College Hospital of the West Indies, as compared with most of those with other conditions, may indicate some influence of social status (Turk & Wynter, 1961). The sequence of events in the orphan home nursery, as shown in Table 3, would seem to have provided the ideal setting for Ounsted's evolutionary process, together with an exceptionally good supply of likely victims; and yet no case of meningitis occurred. It may be that only some strains of *H. influenzae* type *b* are even potentially pathogenic. The possibility must also be considered that the organism in the nursery was not in fact transmitted from child to child; for the babies were kept in well-spaced cots, and came into much closer contact with their attendants than with one another. Unfortunately permission to swab the staff could not be obtained. But if one of these had been found to carry *H. influenzae* type *b*, this would not have proved her to be the source of the babies' infection; for I was carrying such a strain during most of the time that I was visiting the nursery, and probably acquired it there, but I was clearly not the source from which 7 out of 10 children had been infected before my first visit there. Nor is it necessary to postulate a carrier among the staff to explain the rather high frequency of type *b* strains in the junior house of the orphan home; for although only the staff moved to and fro between the 2 groups of children, carriers of type *b* strains were promoted from the nursery to the junior house in September and October 1960, and this may also have happened earlier.

It seems unlikely that the high concentrations of capsulate strains found in grades B, C and D of the day-nursery were due to transmission by members of the staff; for no carriers were found among the staff in October 1960; when type *b* strains were common in grades C and D, and no capsulate strains were isolated on either occasion from the babies in grade A, who were attended by the same staff. It is rather surprising that the babies escaped infection, since several of them had older siblings in the other grades.

In the orphan home nursery there was always a time-lag before any new arrival became demonstrably infected with *H. influenzae* type *b* (see Table 3). Several of these new arrivals were too old for maternal antibodies to be invoked as an explanation of the delay, and it has been shown that very young babies can carry type *b* strains (Donald & Coker, 1957—quoted more fully later). The time-lag suggests that even in this nursery transmission of *H. influenzae* type *b* did not

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occur readily. The failure of my own family to be demonstrably infected by my organism points in the same direction, but acquired immunity may have played some part there.

Development of immunity may also be the reason why some of the children in the orphan home nursery yielded type *b* strains on several occasions and then ceased to do so, but no serological investigations were carried out because none of the procedures then available seemed likely to give significant results. In almost every case the type *b* strain was succeeded by a non-capsulate strain (see table 3), a finding which accords well with the ideas of May (1958 and personal communications). He suggests that R (non-capsulate) strains found in chronic bronchitis may be partially degraded S (capsulate) strains that have lost their smooth colonial morphology during prolonged sojourn in the human body, but have retained traces of type specificity (detectable by gel-diffusion techniques) and some virulence.

Donald & Coker (1957) described an outbreak of a pertussis-like illness, associated with pneumonia in some cases, affecting 19 out of 30 infants in a nursery for premature babies. They recovered *H. influenzae* from the naso-pharynges of 21 infants, but not from any of the adult attendants. 8 of the 21 strains belonged to type *b*; it is not clear whether the rest were untypable or died before typing sera were obtained. The authors concluded that *H. influenzae* played a part in the pathogenesis of the respiratory illness, making the assumption that so high a carrier rate for this organism was abnormal. But is this true? Such a rate has not been reported by other workers, but it has seldom been looked for in appropriate communities. The findings in Jamaica indicate that a high carrier rate for *H. influenzae* type *b* in a group of small children is compatible with normal health and does not necessarily result in respiratory tract disease or meningitis. The circumstances in which this organism becomes pathogenic are as yet unknown.

SUMMARY

H. influenzae type *b*, the sero-type which causes meningitis, was isolated from 3 (2·4 %) of 127 naso-pharyngeal swabs from white non-Jamaican children resident in Jamaica, and from 14 (2·9 %) of 473 such swabs from a mixed group of Jamaican children. Much higher frequencies were found in households in which cases of haemophilus meningitis had recently occurred (41 %), in the nursery of an orphan home (up to 70 %) and in a day-nursery. In the orphan home nursery the high frequency persisted over a number of months, but no case of meningitis or other relevant disease occurred. A high concentration of this organism is therefore compatible with normal health, and the significance of high concentrations in the homes of meningitis patients is not certain.

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