pus from three of these joints produced a pure culture of Staph. aureus resistant to penicillin, streptomycin, and tetracycline.

We are grateful to Dr. H. S. Barber for allowing us to publish details of the two patients who were under his care. We thank the members of the medical and nursing staff of the Manchester Royal Infirmary who have helped in the diagnosis and care of these difficult cases, and the members of the laboratory staff for much technical assistance.

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# **BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT IN PADDINGTON FAMILIES**, 1952–4

BY

# P. L. MASTERS, M.D., D.C.H.

# W. BRUMFITT, M.D., M.R.C.P.

# R. L. MENDEZ,\* M.B., B.S.

AND

### M. LIKAR, M.D.

From Paddington Green Children's Hospital and the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London

This paper presents the bacteriological findings of a survey of respiratory disease among families in the Paddington area. The regular visits to the families provided a unique opportunity to sample the upper respiratory flora of the family members and to correlate the information obtained with the clinical and epidemiological findings. Previous workers have studied adults and children separately (Straker, Hill, and Lovell, 1939) or a particular organism (Cunliffe, 1949; Holmes and Williams, 1954; Breese and Disney, 1956), but this seems to be the first long-term investigation of the upper respiratory flora of families.

The families were recruited from those who brought a child to Paddington Green Children's Hospital for medical advice. They were included in the survey only if they consisted of father, mother, and three children (at least one of which was under 5 years of age) and lived as a separate and independent household. Bv confining the survey to families of the same size and similar structure it was hoped to be able to assess more accurately the effect of different domestic conditions, such as housing, nutrition, cleanliness, and maternal care, on the introduction and spread of respiratory disease.

Brimblecombe et al. (1958) have described the methods and epidemiological results of the survey, in which were recorded the incidence and spread of respiratory pathogens in families in relation to environmental factors such as crowding and climate. The purpose of this paper is to give the bacteriological findings in greater detail. These are based on the results of over 9,000 nose and throat swabs taken fortnightly from 72 families, most of whom were swabbed for more than a

year, and of 700 pairs taken daily or three times a week from 10 families, each swabbed for four weeks.

We aimed to develop and test simple techniques for isolating and identifying respiratory pathogens and for increasing their yield by enrichment. We also wished to determine the isolation rates of each main pathogen in the nose and throat for different age groups and different seasons, and to gain information on the carriage and spread of specific bacterial types in the survey families.

# Techniques

# Standard Methods

Pernasal and throat swabs with dry cotton-wool tips were taken from each available member of a family every fortnight. The pernasal swabs were made with thin flexible wire so that they could be introduced well into the nose without discomfort. The throat swabs were rubbed over both tonsils and the posterior pharyngeal wall.

Within two hours of being taken the swabs were streaked on to 5% blood agar (B.A.) and the same medium with the addition of 1:500,000 crystal violet (B.V.). The swab was used to inoculate a small portion of the plate, and this inoculum was spread with a wire loop in the standard fashion over half the B.A. plate and over a quarter of the B.V. plate. The "plating' was carried out by the same two operators throughout the survey. The plates were incubated aerobically at 37° C. and inspected after 18 hours. It was found that the size of the bacterial colonies and the mucoid character of colonies of pneumococci were enhanced by placing large trays of water at the bottom of the incubator to keep the atmosphere moist.

The bacterial species identifiable on these two culture media included staphylococci, haemolytic streptococci, pneumococci, Haemophilus influenzae, and coliform organisms. Meningococci were searched for during the first two months of the survey without success, perhaps because the plates were incubated for only 18 hours.

In addition to the two media described, Hoyle's tellurite agar was inoculated in the first two months of the survey, but was abandoned because no diphtheria bacilli were isolated. During most of the second year of the survey quarter-plates of nutrient agar containing 5% Fildes peptic digest of blood and 1: 500,000 crystal violet (F.V.) were inoculated in addition to the other two media to improve the isolation of haemophili.

The plates were inspected in a good light with the aid of a watchmaker's lens, which was found more convenient for identifying and subculturing colonies of pneumococci, etc., than the dissecting microscope favoured by the Commission on Acute Respiratory Diseases (1948).

The number of each suspected bacterial pathogen present on the plate was estimated roughly as follows : confluent growth was recorded as + + +, over 20 colonies but not confluent as ++, and fewer than 20 colonies as +. Further identification of each species was made by the methods described below.

#### Pneumococci

Samples of each kind of smooth or flat colony showing green zones on either the B.A. or B.V. plate were streaked in parallel rows on a B.A. plate. Before incubation a strip of blotting paper 1 by 8 cm. on which had been dried eight 20-c.mm. drops of 1:2,000 "optochin" was laid on the plate at right angles to the inoculated streaks. After

overnight incubation subcultures showing a zone of inhibition extending more than 5 mm. from the edge of the strip were typed by the capsular swelling technique, using the full range of 46 sera supplied by the State Serum Institute, Copenhagen. Cultures showing no zone of inhibition were classified as *Streptococcus viridans*. Cultures showing zones of inhibition intermediate in width were uncommon, and whenever tested for bile solubility proved to be *Str. viridans*.

#### Staphylococci

Staphylococci were classified mainly by means of the slide coagulase test. Strains with typical morphology on Gram-staining were classified as *Staphylococcus pyogenes* if the coagulase test was positive and *Staph. albus* if it was negative. Only *Staph. pyogenes* was recorded as a positive finding in the analyses.

#### Haemolytic Streptococci

Representative colonies from both blood plates showing zones of complete or partial haemolysis were subcultured on to B.A. plates. If the subculture was pure, haemolysis complete, and morphology typical in a Gram-stained film, those cultures were classified as beta-haemolytic streptococci. This last step was important in excluding haemolytic haemophili, which were often present. During the first year of the survey most of the beta-haemolytic streptococci were tested for Lancefield group A by the precipitin test, using the enzyme produced by Streptomyces albus to prepare the extracts (Maxted, 1948). Six months after the survey began the bacitracin method of identifying group A streptococci (Maxted, 1954) was adopted by placing bacitracin squares on the subculture plates. The use of the two methods together during the second six months provided a valuable check on technical methods. From the end of the first year the bacitracin-sensitive strains were sent to the Streptococcal Reference Laboratory at Colindale, where Dr. R. E. O. Williams kindly arranged for them to be typed.

### Haemophili

In cultures from the fortnightly swabbings attention was confined to the capsulated strains of *H. influenzae* because these are typable and therefore of epidemiological interest. During the period of frequent swabbing non-capsulated haemophili were also recorded.

During the first year of the survey small mucoid translucent colonies on B.A. or B.V., often suspected of being haemophili by showing satellitism with colonies of staphylococci and other bacteria, were subcultured on to boiled blood agar. If the growth showed the typical morphology of *H. influenzae* on a Gram-stained film it was tested for slide agglutination with the antisera to Pittman types *a* to *f*. About half the cultures suspected of being capsulated from their colonial appearance were not typable: many of these agglutinated slowly in one or more of the six antisera or occasionally in saline.

In the second year F.V. was used as a primary plate to help isolate and identify the Pittman strains. It was found that preparation of the peptic blood broth by incubation in the 50° C. water-bath for as long as 48 hours gave the best results both in increasing the size of the colonies of haemophili and in reducing the growth of streptococci and pneumococci. So prepared, F.V. is a relatively selective medium for haemophili, but it allows the growth of coliform bacilli, which produce large semi-translucent iridescent colonies, and of crystal-violet-resistant neisseriae and micrococci, which usually produce opaque colonies. The capsulated haemophili form translucent, strongly iridescent, and coalescent colonies. The non-capsulated strains form translucent colonies which usually lack both iridescence and coalescence.

The growth requirements of the haemophili were not determined routinely, but 27 colonially typical strains isolated on F.V., two of them capsulated, were investigated by the plate method of Everall (1953) in which two blottingpaper strips, one containing X factor and the other V factor, are laid on nutrient agar at right angles to the

inoculum: 24, including the two capsulated strains, required both X and V factors (*H. influenzae*), and three required X factor only. Four haemolytic haemophili isolated from blood agar required V factor only.

# Coliform Bacilli

Under this heading were included Gram-negative bacilli producing large greyish-white colonies, and also proteuslike strains. No attempt was made to investigate them further in the routine survey.

#### **Enrichment** and Frequent Swabbing

In December, 1953, one family was swabbed daily, except on Sundays, for four weeks. In each of the months January, February, and March, 1954, three families were swabbed three times a week for four weeks. Three of these nine families were chosen from each of the overcrowded, crowded, and uncrowded categories (Brimblecombe *et al.*, 1958). Finally, in June, 1954, one of the families swabbed in March was swabbed thrice weekly for another four weeks.

During the period of frequent swabbing an enrichment technique was used in addition to the standard methods just described. After being used for plating, each swab was thrust into a solid enrichment medium (CVN<sub>3</sub>) described by Holmes and Lermit (1955) and adapted from an enrichment broth described by Pike (1944). The medium consisted of 5% horse blood in nutrient agar (blood agar base, 'oxoid") containing 1:500,000 crystal violet and 1:16,000 sodium azide. It was distributed in 5-ml. amounts in testtubes 6 by § in. (15 by 1.6 cm.) with aluminium caps. When a swab was thrust into the medium its cotton-wool plug replaced the aluminium cap. After incubation at 37° C. for 18 hours each swab was taken out and used to inoculate F.V. plates for haemophili and a medium consisting of 5% horse blood in nutrient agar containing 1:500,000 crystal violet and 2 µg. of streptomycin per ml. (B.V.S.), which is a selective medium for pneumococci and streptococci.

Before inoculation of the B.V.S. plates an optochin strip was placed on the medium across the middle of each plate. A quarter of a plate was used for each swab, which was streaked out at right angles to the optochin strip. In this way most growths of pneumococci were identified without subculture.

#### Results

The results of the fortnightly swabbings have been analysed in the report by Brimblecombe *et al.* (1958) mainly for comparison with the clinical data in relation to factors which may affect the incidence and spread of acute respiratory infections. They were also used to follow seasonal fluctuations in the isolation rates of pneumococci, *Staph. pyogenes*, and haemolytic streptococci over a period of two years. There was a tendency for pneumococci to be isolated more often in the winter months and for *Staph. pyogenes* to be isolated more often in the summer months, while haemolytic streptococci showed no consistent seasonal trend in isolation rate. The findings reported here concern mainly the incidence of different respiratory pathogens related to age and site in the upper respiratory tract.

The results of the frequent swabbings have also been analysed to show the effect of nasal discharge on the isolation rates of some respiratory pathogens and the effect of the enrichment method on isolation rates in relation to age and nasal discharge. On the epidemiological side the combination of the enrichment technique with frequent swabbing enabled the spread of specific bacterial types within the families to be followed with greater accuracy than was possible with the fortnightly swabbings.

The findings from 520 pernasal and throat swabs (swabpairs) taken from the nine families swabbed frequently during January-March, 1954, were used to assess the effect of the enrichment technique on the isolation rates of five respiratory pathogens. The percentage of positive swabpairs by direct plating and the percentage positive after adding the results of enrichment were as follows: pneumococci, 51 and 70; non-capsulated haemophili, 50 and 70; group A streptococci, 13 and 23; coliform bacilli, 10 and 22; and capsulated haemophili, 3 and 5. There were considerable differences between families in the extra yield of organisms from enrichment.

#### Pneumococci

Fig. 1 compares the isolation rates of pneumococci from the nose and throat swabs taken fortnightly with those taken from the nine families frequently swabbed during January-March, 1954. From the nose swabs high isolation

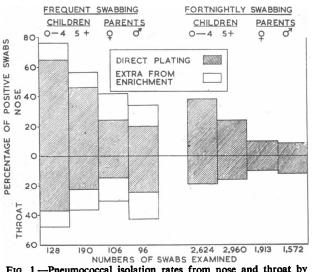


FIG. 1.—Pneumococcal isolation rates from nose and throat by age groups.

rates of pneumococci in the young children and a steady decline with age were found in both groups. Differences in the isolation rates from throat swabs at different ages were slight. The proportion of extra isolations obtained by enrichment increased with age, but not enough to eliminate the downward trend in nasal isolation rates with age. The higher rates for the frequently swabbed families compared with the fortnightly swabbings are attributable largely to the higher pneumococcal incidence associated with nasal discharge found in the winter months, but family differences play a part. The considerable differences between families in pneumococcal incidence are illustrated by Table I, in which the effect of seasonal variations was avoided by including only the swabbing results of the first 12 months for the 45 families which were swabbed for at least a year.

 TABLE I.—Differences of Pneumococcal Incidence in the 45

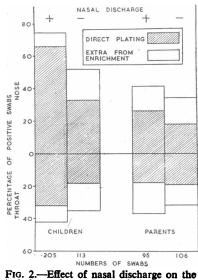
 Families Swabbed for at Least a Year

	Percentage Range of Positive Swab-pairs						
	1-10	11-20	21-30	31-40	41-50	Over 50	
No. of families	0	8	19	12	6	0	

Fig. 2 compares the effect of nasal discharge in the children and parents on the pneumococcal isolation rates from nose and throat swabs. If the results of direct plating are considered, nasal discharge in the children is associated with a higher isolation rate from both nose and throat swabs, while nasal discharge in the parents is associated with a higher isolation rate from nose swabs only. If the results of enrichment are included, only the children show a higher isolation rate in the presence of nasal discharge. This difference from the results of direct plating is due to the enhancing effect of enrichment being greater in the parents than in the children and in the absence of nasal discharge than in its presence. Fig. 3 shows for the survey as a whole the age distribution of the subjects with the seven commonest pneumococcal types as a percentage of the pneumococci isolated from

each of these age groups. The four commonest types -6, 23, 19, and 14 -show a disproportionately high incidence in the pre-school (0-4years) group.

The results of the frequent swabbing show that many of the individuals were persistent carriers. Sixteen of the 27 children and 6 of the 18 parents carried pneumococci continuously during the four weeks for which they were swabbed. In five of the children and two of



isolation of pneumococci in the frequently swabbed families.

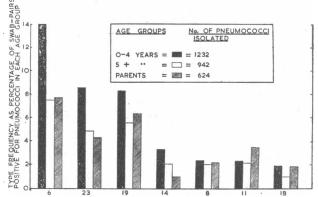


FIG. 3.—Age distribution of subjects with the commoner pneumococcal types.

the parents there was a change in the type of pneumococcus carried: in one instance a new type was added to the old. The types of pneumococcus carried persistently usually appeared sporadically in the nose or throat of other members of the family. One family swabbed during March was swabbed for a second four-weeks period during June. The three children were carrying the same types of pneumococci throughout both periods.

With the fortnightly swabbings a particular pneumococcal type was often isolated from consecutive swabs of an individual for several months. Prolonged carriage was most frequent with types 6, 19, 23, and 34.

Table II analyses the results of the appearance of new pneumococcal types in the frequently swabbed families. Thirteen new types were introduced into the nine families.

TABLE II.—Introduction, Persistence, and Spread of New Pneumococcal Types in Frequently Swabbed Families

Age Groups:	04	5+	Mother	Father	Total
No. in each age group	11	16	9	9	45
Introducers	1	5	2	5	13
Persistence in introducers	1	5	2	0	8
Spread from ,,	0	3	2	0	5

Eight of them persisted at least until the next swabbing two or three days later, and five of these eight spread to a total of eight other members (within three days in four instances rates according to

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and after four to sixteen days in the remaining four). The transferred pneumococci persisted in four of the recipients. Table II shows that the schoolchildren and fathers were the main introducers, although persistence in the fathers and spread from them did not occur. If the introducers and recipients are considered together, persistence of the new types occurred 9 out of 10 times in the children and only 3 out of 11 times in the adults.

### Haemophili

Non-capsulated haemophili were not recorded for the fortnightly swabbings, but their incidence in the frequently swabbed families is shown in Fig. 4. The nasal carrier

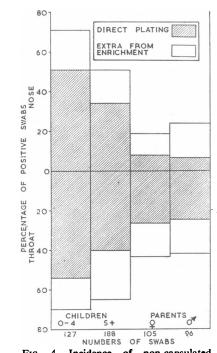
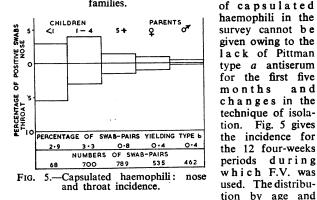


FIG. 4.—Incidence of non-capsulated haemophili in the frequently swabbed families.



between nose and throat is similar to that of the noncapsulated haemophili, although the incidence is only about a tenth as great. The distribution of the six Pittman types shown in Table III is not a representative sample, since about half of the type b strains were isolated during the last three months of the survey. However, the age incidence of Pittman b strains (Fig. 5) shows that four-fifths of them were isolated from the pre-school children (0-4 years). Fothergill and Wright (1933) found that over four-fifths of cases of meningitis due to H. influenzae type b occurred in children up to 3 years old.

Two children in the frequently swabbed families carried capsulated strains (types b and e) of H. influenzae throughout the four-weeks period of swabbing. One child be-

TABLE	III.—	<b>Capsulated</b>	Haemophili:	Incidence o	f Pittman Types

Type	 	a	ь	с	<i>d</i>	e	<u>у</u>
No.	 	17	35	5	1	13	

longed to the family which was swabbed again three months later : she was found still to be carrying a type b strain during the second four-weeks period.

#### Haemolytic Streptococci

Fig. 6 shows the incidence of haemolytic streptococci in the nose and throat swabs taken fortnightly. Owing to incomplete Lancefield grouping in the first nine months of

the survey the results for this period have been omitted in the figure. They do not differ materially in other respects from those in the remainder of the survey.

The incidence of haemolytic streptococci was about twice as great in the schoolchildren and pre-school children of 1 to 4 years as in the parents, and the excess was almost entirely due to group A strepto-

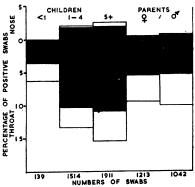


FIG. 6.-Haemolytic streptococci: nose and throat incidence by age groups. Black areas=Group A streptococci. White areas=Other haemolytic streptococci.

cocci. This group also accounted for nearly all the haemolytic streptococci isolated from nose swabs. Both in children and in adults isolations from throat swabs were much commoner than from nose swabs.

Typing of the group A streptococci enabled data to be collected on the carriage and spread of specific types. Among the 45 members of the frequently swabbed families there were 9 continuous carriers, 8 of them children. One child showed a change of type carried. Sporadic appearance of the carried type in the nose or throat of other members of the family was common. Three new types of group A streptococci appeared in the nine families. One replaced another type in the throat of a boy aged 3 years who developed sore throat and fever two days after the new type appeared, followed by nasal discharge three days later and earache with discharge nine days later. Another appeared in the throat of a girl aged 8 years who developed cough and nasal discharge one day later. The third new type appeared in the nose of another girl of 8 years who developed nasal discharge two days later. Although the appearance of all three types was followed by upper respiratory symptoms, this does not imply that all the symptoms were necessarily due to streptococci. None of the three types spread to other members of the family during the remainder of the four-week periods (one, one, and three weeks).

Table IV analyses for the fortnightly swabbings the introduction and spread of 74 new types of group A streptococci into the families. On the average a child introduced and received more than twice as many new types as did a parent.

 TABLE IV.—Introduction and Spread of Types of Group A Streptococci in Families Swabbed Fortnightly

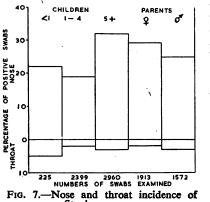
Category of Streptococcal Carrier		No. of Children	No. of Parents	Adjusted* Ratio	
Introducers Spreaders			58 19	16 4	2·4 3·2
Recipients			41	10	2.7

\* As each family consists of three children and two parents, each adjusted ratio equals <u>number of children</u> divided by <u>number of parents</u>.

The connexion between the nose and throat distribution of the streptococci in the person introducing the new type and the spread of the organism to other members of the family was investigated. All 74 introducers had positive throat swabs, while 13 of them also had positive nose swabs. Six of these 13 and 17 of the 61 with negative nose swabs were spreaders. Thus, while 17 out of 23 (74%) of the spreaders had negative nose swabs, there was a higher incidence of transfers from combined nose and throat carriers than from throat carriers.

# Staphylococci

Fig. 7 shows the incidence of Staph. pyogenes in the nose and throat swabs taken fortnightly. The main features are the predominantly nasal distribution and the higher inci-



dence of staphylococcal nose carriers in school than in pre-school children.

The isolation rates of Staph. pyogenes from nose swabs taken from the frequently swabbed families were not significantly affected by nasal discharge.

Penicillin sensi-

Staph. pyogenes.

tivity tests were not done routinely, but, of 30 strains

isolated from members of the survey during 1954, 12 were resistant to a concentration of penicillin of one unit per ml. (method of May and Morley, 1952).

# **Discussion on Methods Optochin Sensitivity**

During the first year of the survey most of the Grampositive cocci showing flat or smooth colonies with green zones were tested for bile solubility, using sodium desoxycholate as described by Mackie and McCartney (1953), as well as for sensitivity to optochin. All organisms showing a zone of inhibition more than 5 mm. wide were found to be bile-soluble, and all but a few of these were typable pneumococci. The few exceptions had tiny colonies and were considered to be "rough" pneumococci. Most other colonies of Gram-positive cocci with green zones showed no zone of inhibition by the optochin strip. Both these and the few organisms showing zones of inhibition between 0 and 5 mm, were invariably bile-insoluble. However, some subcultures of pneumococci included a few colonies more resistant to optochin than the rest, although identical in appearance. Such colonies gave rise to uniformly resistant growths on subculture (zones of inhibition less than 5 mm. wide) and were shown by capsular swelling to belong to the same type as the parent strain.

The use of optochin for identifying pneumococci has been described by Bowers and Jeffries (1955), who used a disk method and a concentration of optochin of 1 in 4.000. Our results agree closely with theirs, and, like them, we consider that optochin sensitivity is a satisfactory and simple way of identifying pneumococci.

# Use of Selective and Enrichment Media

In nose and throat bacteriology the mixed flora is the main difficulty in isolating individual species. Large colonies, such as those of staphylococci and Neisseria catarrhalis, tend to obscure small colonies, such as those of pneumococci, haemolytic streptococci, and haemophili.

CVN<sub>2</sub> medium contains crystal violet, which inhibits staphylococci and diphtheroids, and sodium azide, which

inhibits neisseriae. The medium was used by Holmes and Lermit (1955) for the selective growth of beta-haemolytic streptococci, but its value for us was that it also enriched pneumococci, haemophili, and coliform bacilli. The concentration of azide was, at 1:16,000, less than that ultimately adopted by Holmes and Lermit because this is the maximum concentration which allows growth of haemophili. Unfortunately this concentration permits the growth of some strains of Neisseria, but the difficulty was overcome for the isolation of pneumococci and haemolytic

for subculture from CVN<sub>8</sub>. The use of streptomycin in a selective medium for pneumococci and streptococci was suggested to us by Dr. C. W. Morley, who had found from routine sensitivity tests (May and Morley, 1952) that pneumococci consistently grew in the presence of 5  $\mu$ g. of streptomycin per ml. We found that some strains of pneumococci and haemolytic streptococci were partially inhibited by 5 µg./ml., but none by This concentration was sufficient to inhibit  $2 \mu g./ml.$ almost all strains of crystal-violet-resistant neisseriae and most strains of Haemophilus, including many haemolytic strains which are difficult to distinguish from haemolytic streptococci. Most coliform bacilli failed to grow on B.V.S. or produced a scanty growth of small colonies, while the spreading of most strains of Proteus was prevented.

streptococci by using a selective medium containing B.V.S.

### **Discussion on Epidemiology**

The seasonal variations in the incidence of the main respiratory pathogens showed interesting contrasts. Our findings that pneumococcal isolation rates were higher in the winter may be contrasted with an investigation of the nasopharyngeal flora of adults working in the London School of Hygiene (Straker et al., 1939). In that investigation there were no consistent seasonable fluctuations in the isolation rates of pneumococci, although there was a distinct fall during July or August in three of the seven years of the study.

For haemolytic streptococci Straker et al. (1939) and Blackburn et al. (1930) in this country and Pike and Fashena (1946) in the U.S.A. have, like us, found no consistent seasonal trend in isolation rates; but during one or more years of each investigation, including our own, a sharp rise in the autumn has been a feature. On the other hand, Coburn and Pauli (1941), in their studies in New York City, reported a rise in isolation rates of haemolytic streptococci from throat swabs during the first quarter of the year, and a peak in the second quarter.

The isolation rates of the main respiratory pathogens according to age group showed considerable differences. For pneumococci and haemophili, children under 5 years showed the highest incidence and there was a steady reduction with age. Straker et al. (1939) were unable to establish this trend with age because they investigated separate communities of adults and children living under different environmental conditions.

Schoolchildren showed the highest incidence of staphylococci and group A streptococci. Cunliffe (1949), in an investigation of staphylococcal incidence in the nose, found an age distribution very similar to ours. Holmes and Williams (1954) investigated the incidence of group A streptococci in the throat swabs of children in three Middlesex boroughs. They found that children of 1 to 4 years not attending day nurseries showed a streptococcal incidence about a quarter of that of the schoolchildren, while children attending day nurseries showed a higher incidence than the schoolchildren. In our survey the children from 1 to 4 vears old did not attend day nurseries but showed an incidence of group A streptococci almost as high as the schoolchildren. This finding might be due to close contacts outside the home between children under 5 years in the Paddington district compared with the Middlesex boroughs sampled by Homes and Williams, although we have no precise data on this point.

The differences in nose and throat distribution between the respiratory pathogens indicate the practical importance of choice of swab in clinical bacteriology. While a nose swab is adequate for the detection of staphylococci and a throat swab for haemolytic streptococci, both swabs should be taken for the detection of pneumococci and haemophili.

Analysis of the spread of specific types of pneumococci and group A streptococci within the survey families shows differences between the children and the adults. In the frequently swabbed families about as many new pneumococcal types appeared in the noses or throats of the parents as of the children, but the pneumococci persisted in 9 out of 10 children and only 3 out of 11 adults.

The higher pneumococcal carrier rates of children compared with adults appears from this evidence to be due not to greater exposure of children to pneumococci but to persistence of the pneumococci more often in children than in adults. The disproportionately high incidence of pneumococcal types 6, 19, 23, and 14 in the pre-school children, the age group showing the highest pneumococcal isolation rate, suggests that persistence in the upper respiratory tract of young children is a special property of these types.

A similar analysis of new types of group A streptococci in the frequently swabbed families could not be carried out, as only three new types appeared. Table IV shows that new streptococcal types were detected by the fortnightly swabbings more than twice as commonly in the children as in the adults. As new bacterial types are unlikely to be detected by infrequent swabbing unless they persist, this result is comparable with the greater frequency of persisting pneumococci in the children of the frequently swabbed families. It does not necessarily imply that the children were exposed to new streptococcal types more often than the adults.

It has been shown that nasal carriers are more likely to disseminate streptococci than throat carriers (Hamburger, Green, and Hamburger, 1945). Our figures also showed this, although 74% of the spreaders had negative nose swabs, indicating that throat carriers were numerically more important than nasal carriers in disseminating streptococci. It is noteworthy that only a third of the specific types of group A streptococci introduced into a family spread to another member, although isolation of the specific type from a member of the family continued for an average period of 10 weeks.

Figs. 2 and 5 show how nasal discharge increases the nasal isolation rates of pneumococci and haemophili. These increases might be due to (1) an increase in the number of these organisms; (2) forward extension of the area of mucous membrane in the nares colonized by them, their normal habitat being the nasopharynx; or (3) an increased incidence by acquisition from outside sources. Factor 1 would affect the isolation rate less the more efficient the method of isolation, and would account for the greater enhancing effect of enrichment in the absence of nasal discharge. Similarly, the greater enhancing effect of enrichment in the parents compared with the children suggests that pneumococci and haemophili are scantier or less accessible in the noses of adults compared with children. The contrast between the effect of nasal discharge on the normal isolation rates of the children and the adults obtained with the aid of enrichment is likely to be associated with factors 2 and 3.

#### Summary

Simple techniques for the isolation of the main respiratory pathogens are described and the value of optochin for the identification of pneumococci is confirmed. The use of an enrichment method consisting of incubation of the swab in crystal-violet/sodium-azide/blood-agar and subsequent streaking on selective media resulted in a 40% increase in the yield of pneumococci and noncapsulated haemophili and a doubling of the yield of group A streptococci and coliform bacilli.

The distribution of the principal respiratory pathogens in the nose and throat is given for pre-school and schoolchildren and for mothers and fathers. Pneumococci and staphylococci predominated in the nose and haemolytic streptococci in the throat, while haemophili were evenly distributed between nose and throat. The nasal incidence of both pneumococci and haemophili showed a steady decline with age, while staphylococci and haemolytic streptococci had their highest incidence in children of school age.

Analysis of the introduction of new pneumococcal types into the frequently swabbed families indicated that the higher carrier rates of the children compared with the parents were due not to a larger number of acquisitions of new types but to a greater tendency of the pneumococci to persist in the children's noses and throats.

The fortnightly swabbings showed that an individual often carried the same type of pneumococcus, group A streptococcus, or capsulated haemophilus for months. Spread of pneumococci to other members of the families was usual with some types and unusual with others. Spread of types of group A streptococci was detected in a third of the introducers. Not only did families show considerable differences in isolation rates of respiratory pathogens, but family members often differed among themselves in the specific type of organism which they carried at any one time.

The use of the enrichment method of culture for the frequent swabbing indicated that nasal discharge was associated with a rise in nasal incidence of pneumococci and haemophili in the children but not in the parents.

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The following were the winners of prizes in the Southwest Metropolitan Regional Hospital Board's 1957 competition for clinical research reports. The Board is holding a similar competition this year:

Consultants and S.H.M.O.s.-Dr. A. A. Cunningham and Dr. Towers, 200 guineas each. Senior registrars, registrars, and J.H.M.O.s.-Mr. J. C. Gazet and Dr. A. J. Taylor, 100 guineas each; Mr. P. W. Seargeant, Dr. J. R. H. Pinkerton, and Dr. P. Farnan, 75 guineas each. Senior house officers and house officers .- No entries.