

THE HAEMOPHILIC BACTERIA OF THE UPPER RESPIRATORY TRACT, THE APPEARANCE OF VIRULENT FORMS IN RELATION TO UPPER RESPIRATORY INFECTIONS

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(With Chart including Figures 1-6)

IN previous studies of the bacterial flora of the upper respiratory tract and its variations during attacks of acute coryza (Hoyle, 1932), it was found that there was in many cases a striking relationship between the appearance of certain organisms with definite pathogenic properties towards the lower animals, in the upper respiratory tract, and the occurrence of acute infections. This relationship was especially marked in the case of the influenza bacillus, and it was suggested that this organism played a prominent part in the aetiology of upper respiratory infections. The significance of the influenza bacillus in acute coryza has been noted by various workers (Noble, Fisher and Brainard, 1928; Burky and Smillie, 1929; Webster and Clow, 1932; Kneeland and Dawes, 1932), but on the other hand various observers have found the incidence of influenza bacilli in the respiratory tract to be the same in normal health and in acute coryza, and Fleming (1929) and Fleming and Maclean (1930) have devised a technique by means of which they claim to have isolated influenza bacilli in 100 per cent. of normal throats. One possible explanation of these conflicting results may be found in the absence of any very exact criterion of what constitutes a typical influenza bacillus.

The author had previously observed that the influenza bacillus possessed a definite though low degree of pathogenicity towards mice, and that it could frequently be isolated by a mouse inoculation method when other methods failed, and it was thought that a more complete study of the haemophilic bacteria of the upper respiratory tract especially in relation to their virulence for animals might yield results of some value.

Serial observations of the flora of the upper respiratory tract were made in a group of seventeen individuals, examinations being made at 14-day intervals during normal health, and more frequently during infections. Special attention was given to all haemophilic bacteria isolated, especially in relation to their virulence for mice. The seventeen individuals were selected from a group of forty previously studied, in such a way as to include representatives of all the types of upper respiratory flora previously encountered (Hoyle, 1932).

The technique of the examinations was as follows. Swabs were taken from the nose and throat, the nasal swab being plated on ordinary chocolate agar,

and the throat swab inoculated on to ordinary chocolate agar, to chocolate agar containing a suitable dilution of penicillin (Fleming, 1929), and to a tube of 5 per cent. unheated rabbit's blood broth which was incubated overnight, and then $\frac{1}{2}$ c.c. doses injected intraperitoneally into each of two mice, cultures being made from the heart blood of any mice dying within three days. All haemophilic bacteria isolated were tested for requirement of *X* and *V* factors, and their virulence for mice was determined by inoculating $\frac{1}{2}$ c.c. doses of 18 hours heated blood broth culture intraperitoneally into each of two mice, cultures being made from the heart blood of any mice which died.

PROPERTIES OF HAEMOPHILIC BACTERIA ISOLATED

By the use of Fleming's penicillin medium it was found possible to isolate organisms requiring both *X* and *V* factors in 88 per cent. of all throat examinations made. On grounds of morphology and cultural characteristics these organisms could be divided into two fairly distinct groups, described as typical influenza bacilli and atypical strains.

Typical influenza bacilli

These showed a predominantly cocco-bacillary morphology, gave smooth colonies with a consistency like oil paint, and suspended readily in water and saline.

Atypical strains

Morphologically these were usually short thin bacilli, though some strains showed filamentous forms. The colonies were very rough, fragile, and difficult or impossible to suspend in water or saline.

The typical and atypical groups were not absolutely distinct, a few strains being isolated with properties intermediate between the two.

Carbohydrate fermentation tests were done on a series of sixty-four typical and seventy-six atypical strains isolated consecutively. These proved to be of little value for purposes of classification, because in the majority of cases the fermentative powers of the strains were slight, in some cases they were irregular, and many of the strains possessed the property of producing acid in media free from carbohydrate. The typical strains usually fermented glucose and never saccharose, while many of the atypical strains fermented saccharose. The actions on maltose and lactose were very irregular.

Indol production was tested on a series of twenty-eight typical and twenty-seven atypical strains. Eighteen of the typical strains produced indol. The atypical strains were all indol-negative.

An interesting comparison was made between the results of the penicillin method of isolation and the mouse inoculation method. Atypical strains, even when present in large numbers in the penicillin culture, were never isolated by mouse inoculation, but whenever typical strains appeared in the penicillin culture, even in small numbers, they were almost invariably isolated also by mouse inoculation. It would therefore seem that the typical strains were pathogenic and the atypical strains were not. When, however, the virulence of

the strains was determined by inoculating mice with pure cultures, it was found that only about 30 per cent. of the typical strains were capable of producing a fatal septicaemia in the mouse, the others usually failing to kill or sometimes producing death without septicaemia, death apparently being due to the action of toxins. The atypical strains were all non-pathogenic. These pathogenic typical strains showed certain differences from the non-pathogenic typical strains, they were morphologically 100 per cent. cocco-bacilli, and their colonies were more mucoid in consistency and in some cases almost watery.

In this connection the work of Pittman (1931) is of interest. Pittman noted that certain strains of influenza bacilli isolated from various infections were different from strains isolated from the normal respiratory tract, in that on subculture *S* and *R* variants emerged. The colony of the *S* variant was more mucoid in consistency than that of the *R*, and the *S* variant was characterised by the ability to produce a septicaemia in animals, which the *R* variant did not do even in doses which were consistently lethal. The organisms from the normal respiratory tract apparently contained no *S* variants.

It may be suggested that the difference between the pathogenic typical strains and the non-pathogenic typical strains described above is an artificial one due to only two mice being used for each virulence test, and that the differences would disappear if a larger number of animals were used. This is, however, not the case. A considerable amount of experimental evidence in the past has shown that influenza bacilli are in general not pathogenic to laboratory animals; even when death is produced by the inoculation of massive doses there is no evidence that the bacilli have multiplied in the body, death apparently being due to their toxins. From time to time, however, certain strains have been noted which will produce a true infection in animals, intraperitoneal injection resulting in death from septicaemia. Pittman stresses the point that the essential difference between the *S* and *R* variants is that the *S* variant regularly produces a septicaemia in animals, the differences in toxicity being slight. In the author's own work it has frequently happened that a pathogenic typical strain has been inoculated into more than two mice, as for instance when at a given examination strains were isolated from the nasal culture, both throat cultures, and by mouse inoculation, when eight mice would thus be inoculated. In these cases a septicaemia would consistently result in all eight mice. Again, when non-pathogenic typical strains were inoculated into more than two mice, although occasionally some of the mice might die, a septicaemia would never result. The essential difference between the pathogenic and non-pathogenic typical strains is that the former produce a true septicaemia while the latter either do not kill at all, or kill only by toxic action, no multiplication occurring in the animal body.

There would not seem to be any rigid line of distinction between the typical and atypical strains, but it would appear that while organisms requiring both *X* and *V* factors can be isolated from a very considerable proportion of normal throats, these organisms show considerable differences in

their virulence towards mice, and these differences can be broadly related to differences in morphology and cultural characteristics, as indicated below:

(1) *Strains virulent in pure culture.* Morphologically 100 per cent. cocco-bacilli, colonies mucoid or watery, suspending readily in water or saline.

(2) *Strains virulent in mixed culture but avirulent in pure culture (i.e. can be isolated by the mouse inoculation method).* Morphologically predominantly cocco-bacillary, colonies smooth sometimes with a finely granular surface, consistency like oil paint, suspending readily in water or saline.

(3) *Completely avirulent strains.* Bacillary or filamentous morphology, colonies rough, fragile, and difficult to suspend in water or saline.

RELATIONSHIP BETWEEN VIRULENCE OF HAEMOPHILIC BACTERIA ISOLATED AND OCCURRENCE OF UPPER RESPIRATORY INFECTIONS

Serial observations were made as described above on a group of seventeen individuals for a period of eight months, namely from September, 1932 to April, 1933 inclusive. A chart was drawn up for each individual indicating the variations in virulence of haemophilic bacteria isolated, and any infections which occurred were represented on the chart by shaded areas. Representative charts are shown in Chart, Fig. 1-6. During September the haemophilic bacteria isolated were almost consistently of the avirulent atypical type. Typical strains began to appear in October and November, and almost all such strains were fully virulent. The appearance of these fully virulent strains coincided with an outbreak of severe acute coryza, and the relationship between the appearance of the strain and the onset of coryza was exceedingly striking: fully virulent strains were never isolated during normal health, but appeared coincident with or in some cases immediately preceding the onset of coryza. In the whole group of individuals, fully virulent influenza bacilli appeared on nineteen occasions during the period September-January, all the appearances being associated with attacks of coryza. During late November and December sporadic attacks of acute coryza occurred, many of which were not associated with the appearance of fully virulent influenza bacilli, and in the period September-January fourteen acute infections occurred in which influenza bacilli appeared to play no part, and of these ten were associated with the appearance of pneumococci in the individual's flora for the first time or with a considerable increase in the incidence of pneumococci in some cases in which they were already present.

In February and March eight out of the seventeen individuals became carriers of typical influenza bacilli, the carrier period varying from 2 to 8 weeks, the organisms sometimes being fully virulent and at other times virulent only in mixed culture. In six out of the eight cases the carrier state was associated with attacks of acute coryza, sore throats, or chronic catarrh, but in two cases the carrier state was not associated with any respiratory discomfort. Several of the individuals studied became pneumococcal carriers during this period. The carrier period came to an end at the end of March, and in April virulent

Dates of examinations indicated along abscissae

Virulence of haemophilic bacteria isolated indicated along ordinates:
 Pure—Virulent in pure culture; Mixed—Virulent in mixed culture.

Presence of pneumococci in significant numbers in infections indicated: Pn+++.

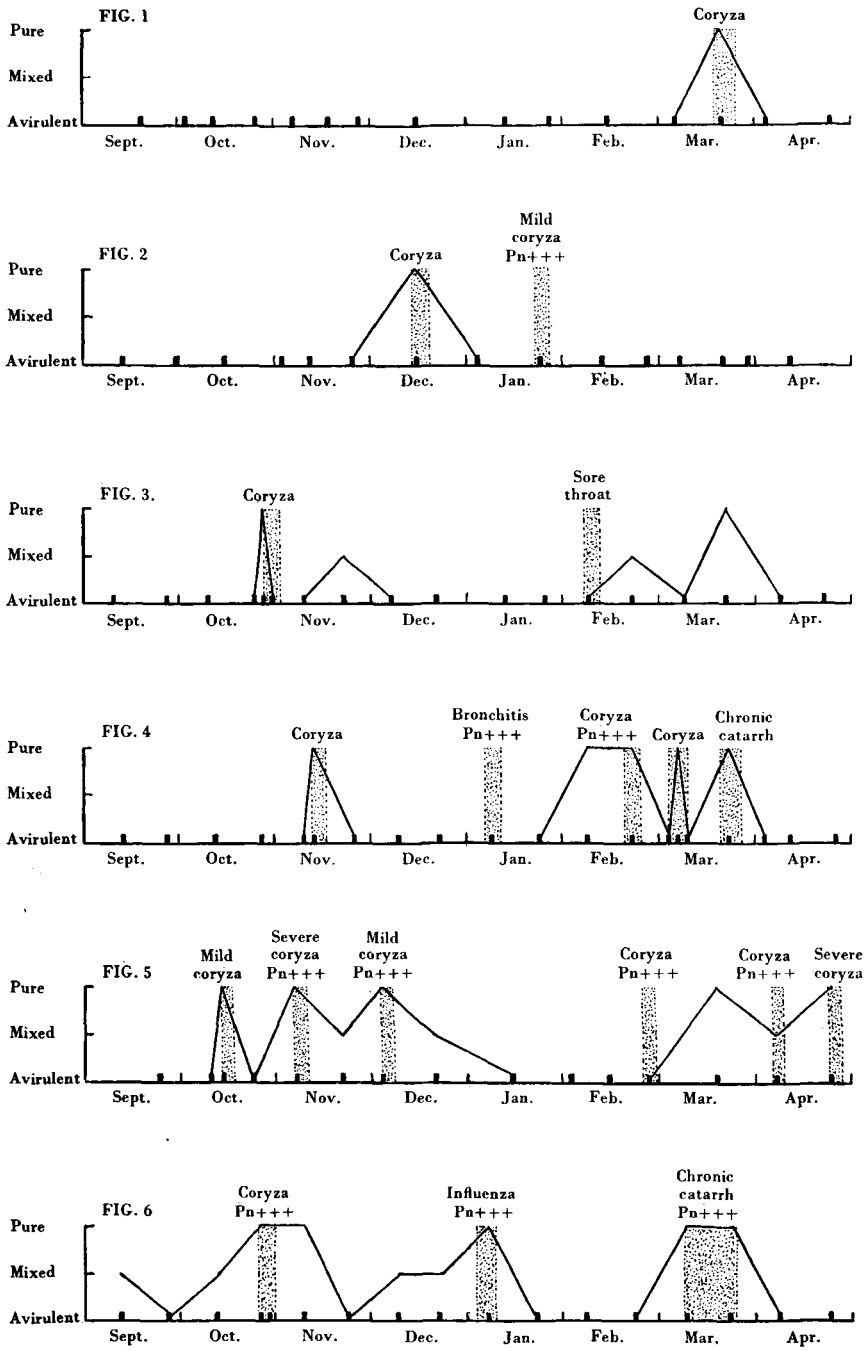


Chart showing the results of investigation in six individuals

influenza bacilli were only twice isolated, both appearances occurring in association with attacks of acute coryza.

The results obtained throughout the whole period of observation strongly supported the view previously put forward by the author, that the majority of attacks of acute coryza were due to infection by influenza bacilli or pneumococci. The severe colds which occurred in the autumn were so definitely associated with the appearance of fully virulent influenza bacilli in the flora that it was difficult to believe that one was not dealing with cause and effect, and apart from the carrier period of February and March these organisms were never isolated in the absence of symptoms. During the whole period of observation fully virulent influenza bacilli appeared on thirty-two occasions. Twenty-four times in association with attacks of acute coryza, influenza, or acute sore throat, four times during periods of chronic catarrh, and four times in the absence of symptoms, these latter four occasions all occurring during the carrier period in February and March. During the period sixteen attacks of acute coryza occurred in which fully virulent influenza bacilli were not isolated; in twelve of these attacks pneumococci were isolated in considerable numbers.

DISCUSSION

By the use of penicillin medium it has been found possible to isolate haemophilic bacteria requiring both *X* and *V* factors from 88 per cent. of all normal throats, thus confirming the observations of Fleming and Maclean (1930). The majority of strains isolated from the normal throat showed considerable differences in morphology and cultural characteristics from the typical Pfeiffer's bacillus. Fleming and Maclean also noted that the majority of strains isolated were of a very aberrant morphology. It would appear that haemophilic bacteria requiring *X* and *V* factors form a group of organisms similar to the streptococci, in that although the group contains organisms of widely differing properties it is not possible to draw rigid dividing lines between the different types. The most definite and most significant difference observed between the various types was that of pathogenicity. Strains showing the typical morphology of Pfeiffer's bacillus could almost invariably be isolated by the mouse inoculation technique, while strains of aberrant morphology were never isolated by that method. It had been previously observed by Jacobson (1901), Kamen (1901), Wolf (1920), and others, that although typical influenza bacilli were not pathogenic to the lower animals except in very large doses, if the bacilli were inoculated along with other organisms especially streptococci, a fatal septicaemia could be regularly produced. This observation has been confirmed by the author, since although typical strains could be isolated with ease by the mouse inoculation method, consisting of the inoculation of a mixed throat culture into mice, when such strains were subsequently tested for virulence by the inoculation of pure culture, 70 per cent. were not virulent. Thirty per cent. of the strains, however, were capable of producing a fatal septicaemia in mice even in pure culture. These fully virulent strains have an

absolutely typical morphology consisting of pure cocco-bacilli, and the colonies are more mucoid than those of strains of lower virulence.

Reference has been made above to the work of Pittman (1931), and it would seem probable that the fully virulent strains correspond to the *S* variants described by Pittman, although the author was unable to demonstrate the existence of a capsule in fully virulent strains, which Pittman had found to be a characteristic of *S* variants. In other respects, however, the strains appear to correspond.

Dochez, Mills, and Kneeland (1932) have recorded some observations of great interest. They found that influenza bacilli were normally present in the throat flora of chimpanzees, but that the strains correspond to the *R* variants described by Pittman. When, however, spontaneous outbreaks of colds occurred in the chimpanzees, the *R* forms became replaced by *S* variants. They also found that if the filtered nasopharyngeal washings of a case of human coryza were inoculated into healthy chimpanzees, the *R* variants in the monkey's flora became replaced by *S* variants. In one animal these *S* variants rapidly disappeared, and in this animal no symptoms resulted, but in another they persisted for several days and the monkey developed an acute cold. It would therefore seem that attacks of acute coryza in the chimpanzee are auto-genous in origin, resulting from a change of *R* variants normally present in the flora into *S* variants. Dochez and his collaborators, however, put forward the hypothesis that the filtered nasopharyngeal washings contained a filterable virus possessing the property of converting *R* variants of the influenza bacillus into *S* variants. It seems, however, quite unnecessary to suppose the existence of a filterable virus to explain the findings, the mere introduction of the washings might be expected to produce a temporary lowering of the resistance of the monkey's respiratory mucosa, possibly rendering the conditions more favourable to the development of *S* variants. Or again it is possible that the washings might contain the type specific substance of the influenza bacillus which would facilitate a change from *R* to *S* forms. It is in the opinion of the author not improbable that the whole of the reported successful transmissions of the common cold by filtered washings may be due to autogenous infection by influenza bacilli or pneumococci consequent upon a lowering of the resistance of the nasopharyngeal mucosa produced by the introduction of a toxic liquid. In this connection it is of interest to note that the same workers (1933) inoculated a number of human volunteers with filtered nasopharyngeal washings from a case of influenza and with supposed tissue cultures derived therefrom. The inoculated individuals developed, not influenza, but acute coryza. The authors concluded that it was not possible to distinguish the virus of influenza from that of the common cold, but again a more probable explanation would be that the colds were autogenous in origin.

The author (1932) had previously observed the existence of a definite relationship between the appearance of typical influenza bacilli in the upper respiratory tract and the occurrence of attacks of acute coryza, and this

relationship has been confirmed and extended by a study of the virulence of the strains isolated. The clear-cut association of attacks of acute coryza with the first appearances of fully virulent influenza bacilli in the autumn, followed by the development in many individuals of a carrier state tending to be accompanied by chronic catarrh or sore throat in the winter months, and the subsequent disappearance of virulent organisms in the spring, is a sequence of events which would suggest that these virulent influenza bacilli were of primary aetiological significance in upper respiratory infections. It is of significance to note that Blake and Cecil (1920) inoculated influenza bacilli of raised virulence into the noses and throats of monkeys and produced a febrile coryza.

The significance of the pneumococcus in the aetiology of acute coryza has again been observed, the first appearances of this organism in the late autumn being associated with the occurrence of attacks of acute coryza.

CONCLUSIONS

Haemophilic bacteria requiring both *V* and *X* factors can be almost constantly isolated from the normal throat, but the majority of strains show considerable differences from the typical Pfeiffer's bacillus, and are avirulent for mice.

Among strains of typical influenza bacilli variations in virulence occur, and the appearance of certain strains in the upper respiratory tract, capable of producing a fatal septicaemia in mice when inoculated in pure culture, was associated in so definite a manner with the onset of acute coryza, that it would seem probable that many attacks of acute coryza are due to these organisms.

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