

THE RELATIONSHIP OF VIRULENT TO AVIRULENT DIPHTHERIA BACILLI.

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THE question of the relationship of the virulent diphtheria bacillus to avirulent forms of similar morphology and fermentative properties has always attracted the interest of bacteriologists. Practically all careful observers agree that avirulent bacilli can never become virulent either *in vivo* or *in vitro*. It has been also the experience of most observers that no virulent strain can be made avirulent by laboratory methods. Crowell (1926) and Cowan (1927) have however reported that they had succeeded in isolating avirulent variants from a pure culture of a virulent strain. From the nature of Crowell's experiment, which only records one such change, it seems possible that he may have chanced on a true biological (*i.e.* uncontrolled) variation, but Cowan suggests that it might be possible to isolate with some degree of regularity "rough" avirulent colonies from virulent cultures. As Cowan points out the procedure is both lengthy and difficult. My own experiments in this direction have been entirely negative, although I have on many occasions tried to isolate avirulent variants from virulent cultures by colony selection and other methods. I have not even been successful with the cultures with which Miss Cowan worked and which she very kindly put at my disposal. Individual colonies of the Park 8 strain have been also tested on more than 700 occasions and have always proved fully virulent when pure. Passage both through a large variety of artificial media and through animals failed to engender avirulent variants.

It does not seem unreasonable then to suppose that such variations as those reported by Crowell and Cowan are more in the nature of infrequent "genetic" variations than of a "phasic" variation, such as the usual "rough" and "smooth" variations of bacteria, which are in a measure subject to experimental control.

In the course of some work done on the serological grouping of the diphtheria bacilli, the problem of relationship of virulent to avirulent forms was approached from a somewhat different angle.

In 1923 Eagleton and Baxter made a comprehensive examination by the method of agglutination and absorption of the strains of virulent diphtheria bacilli then prevailing. They found that 332 out of 348 consecutive strains from very diverse sources fell into 10 fairly definitely constituted serological groups, while 16 strains out of the 348 constituted 16 further groups. Using the 26 sera which covered all Eagleton and Baxter's virulent strains, Miss Baxter and I attempted to classify avirulent strains collected at the same

time as the virulent strains. The technical methods used for agglutination experiments were identical with those described by Eagleton and Baxter. The titre of the rabbit sera used was usually about 1/400. The methods used in testing for virulence were described in a previous paper (Okell and Parish, 1926). Over 300 strains were originally examined by direct agglutination, but as it soon appeared that the fallacies of this method rendered it useless, 100 of the strains were examined more completely by agglutination and absorption methods. Considerable difficulty arose from the fact that avirulent strains appear to make more unstable suspensions than virulent ones. It was found that by agglutination and absorption methods only 2 out of 100 avirulent strains fell into any virulent group. An attempt was then made to make sera from the avirulent strains with a view to classifying them into sub-groups. Difficulty was met with in producing appreciable agglutinins in the rabbit as well as in obtaining stable suspensions. In the case of only 10 strains out of over 50 used was it possible to produce an agglutinating serum in the rabbit with which final experiments could be carried out. No avirulent strains, except the one used as antigen, agglutinated with any of the 10 sera, nor were any of the sera absorbed by any but the homologous antigen.

It appeared then that the avirulent group was antigenically heterogeneous and had little relationship with the virulent group. With these facts in mind, a small group of cultures was examined which had been sequestered from the rest because the virulent and avirulent strains had been found in some sort of relationship. To these we gave the name of "associated" cultures. Three types of such association had been met with:

1. Virulent and avirulent coexisting at the same time in the throat or nose of the same patient.
2. Virulent followed at a later date by avirulent in the same throat or nose.
3. Virulent and avirulent strains coexisting in different patients in the same epidemic, *i.e.* epidemic association.

N.B. In order to eliminate the possibility that cultures were impure, numerous platings were done with each strain and many colonies tested for virulence and fermentation reactions. The agglutination and absorption experiments were repeated on several occasions. The serology and virulence of the strains were consistent even when tested after an interval of several months.

I. VIRULENT AND AVIRULENT COEXISTING AT THE SAME TIME IN THE THROAT OR NOSE OF THE SAME PATIENT.

In the first group there were *three* pairs of strains. In one case the virulent type was not represented in Eagleton and Baxter's groups, the avirulent strain was also never typed, and owing to difficulties in preparing agglutinating serum it was not proved if the virulent and avirulent had any serological relationship. In the second case the virulent strain was of Eagleton

and Baxter's Type III, but the avirulent strain could not be typed and belonged to a group not represented by any virulent or avirulent group.

The third example was however more interesting. The virulent bacillus from the case was an unusual one and could not be typed by any of Eagleton and Baxter's sera. The avirulent was identical with it, sera obtained from both cultures behaving alike in direct agglutination and absorption experiments.

2. VIRULENT FOLLOWED AT A LATER DATE BY AVIRULENT IN THE SAME THROAT OR NOSE.

In this group there were also three pairs of strains. In the first case a Type IV virulent was followed by an untypeable avirulent strain, the latter showing no serological relationship with the former. In the second case a Type III virulent was followed by a Type III avirulent. We had however met with one strain corresponding to virulent Type III in 100 miscellaneous avirulent strains examined. In the last case a virulent strain unrepresented in Eagleton and Baxter's series was followed by an avirulent strain which was identical with it by agglutination and absorption.

3. VIRULENT AND AVIRULENT STRAINS COEXISTING IN DIFFERENT PATIENTS IN THE SAME EPIDEMIC, I.E. EPIDEMIC ASSOCIATION.

Out of 13 virulent strains, 4 fell into Group VII of Eagleton and Baxter; out of 15 avirulent strains, 7 fell into this group, though none of the 100 miscellaneous avirulent strains had fallen into this group. In the same epidemic 2 further virulent strains fell serologically into the same group—one not represented in Eagleton and Baxter's series—and 5 avirulent strains also fell into this group.

DISCUSSION.

From these observations it would appear that some "associated" virulent and avirulent strains show serological identity. When it is considered that only 2 out of 100 of the miscellaneous group of avirulent strains fell into any of Eagleton and Baxter's virulent groups, the occurrence together of virulent and avirulent strains of identical serology appears to be outside the realm of any reasonable chance. The occurrence of several examples of such an event in the material we examined would demand chances of a very high order.

If serological identity can be relied on as evidence of a common derivation, the deduction is evident that virulent strains can from time to time throw avirulent variants, though of course it gives no support to the view that all avirulent strains have once been virulent.

If we accept the rather high figures of 2 per cent. for the virulent and 2 per cent. for the avirulent carrier-rate of the normal population, the chance of virulent and avirulent forms being isolated at the same time and from the same patient would be 1 in 2500. This assumes that it is technically as easy to isolate the two forms as one. The two forms were actually isolated together three times from some 1500 carriers. The likelihood of isolating both

varieties even when they coexisted was probably very small as we usually only picked two colonies off a plate for virulence testing. S. F. Dudley (personal communication) shares with me the impression that carriers of avirulent bacilli are more common among those who have recently been virulent carriers or convalescents from diphtheria than among the general population.

Reference should be made to the remarkable observations of Griffith (1928) on changes in pneumococcal types which may have a bearing on the foregoing observations. Griffith showed that by certain methods a transformation of pneumococcal types could be affected. For example, if a rough form of Type I is inoculated subcutaneously into mice together with a heated smooth culture of Type II, a virulent smooth form of Type II may be developed. "The apparent transformation is not an abrupt change of one type into another, but a process of evolution through an intermediate stage, the rough form, from which the type characters have been obliterated." The great tendency for avirulent strains of the diphtheria bacillus to form unstable suspensions in 0.9 per cent. saline and also their lack of "type character" have already been remarked upon. The acquisition of definite type characters in certain of the avirulent strains of the "associated" series may then possibly be secondary and similar in mechanism to the change in type which Griffith describes—a rough avirulent intermediate stage occurring between the smooth virulent stage and the smooth avirulent stage which is serologically identical with it.

Whatever explanation is accepted for these observations, there seems no reason to modify the prevailing view that the avirulent diphtheria bacillus is not even potentially a cause of disease. On this view some of the most useful measures of controlling diphtheria depend.

CONCLUSIONS.

By serological methods observations have been made suggesting that virulent diphtheria bacilli occasionally throw avirulent variants with an identical constitution from the point of view of agglutination. No evidence has been discovered that suggests that an avirulent bacillus can engender virulent variants.

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