A CRITICAL EXAMINATION OF THE ORGANISM DESCRIBED AS *BACILLUS WAKEFIELD*

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In a paper which was published recently in this *Journal*, Dr F. M. Berger (1945) described certain bacilli which had been isolated from cases of gastro-enteritis. These organisms, three in number, were observed to be non-motile, Gram-negative rods. From their biochemical and serological behaviour they were considered to be members of the Flexner group and to constitute a hitherto undetected type. Dr Berger suggested that this organism should be known as *Bacillus wakefield*.

The biochemical reactions of these strains were shown to be similar to those which are commonly seen in the Flexner group, with the exception that there was no fermentation of mannitol. This fact would not, however, exclude an organism from the group, since we know that many strains of the Newcastle dysentery bacillus (*Shigella flexneri* VI) are unable to attack this sugar.

A more important finding was the fermentation of saccharose. Dr Berger found that on first isolation the Wakefield strains were able to render saccharose medium slightly acid in 8 days. After further subculture this period was reduced to 3 days. While it is true that old laboratory cultures of Flexner bacilli may sometimes ferment saccharose, we have never yet encountered a freshly isolated strain which has this power, even when incubation has been continued for 21 days. This finding, then, was sufficient to throw some doubt on the conclusion reached by Dr Berger.

Our doubt as to the nature of the Wakefield strains was not lessened by an examination of the serological results presented in the paper. Dr Berger based his claim that these organisms should be regarded as Flexner bacilli on the suggestion by Boyd (1940) that 'membership of the Flexner group should be extended to, and limited to, those races which, in addition to possessing a distinct specific antigen, are also endowed with the common group antigen'. We now know that the group antigen of the Flexner bacilli is of two varieties, exemplified by the organisms Flexner X and Flexner Y. These so-called types possess no specific antigen but are composed of group antigen only. According to Wheeler (1944) Flexner X contains the non-specific antigenic factors 1, 7, 8 and 9; while Flexner Y is composed of factors 1, 3 and 4. In Table 1 in his paper Dr Berger gave the results of testing Flexner sera against the Wakefield strains. He showed that these organisms were agglutinated to full homologous titre by both X and Y sera. It was sufficiently remarkable that freshly isolated strains, which might be presumed to consist mainly of specific antigen, were agglutinated to such high titres by pure group sera. It was even more remarkable that they were affected equally by the sera of both Flexner X and Flexner Y, organisms which in their antigenic content differ from one another so markedly.

In his second table Dr Berger presented the results obtained with antiserum prepared in the rabbit by inoculation of one of the Wakefield strains. This serum had an homologous titre of 1/5000, and at the same time was found to agglutinate several Flexner types. The highest titre obtained was that against Flexner Y, which was agglutinated in a dilution of 1/320. On these results Dr Berger concluded that the Wakefield strains possessed the group antigen common to the Flexner bacilli and a type-specific antigen of its own. This conclusion appeared to us unjustified. In the first place a Flexner serum with an homologous titre of 1/5000 would normally be expected to agglutinate one of the non-specific races Flexner X or Y to a much higher titre than 1/320; and secondly, a titre of 1/320 against Flexner Y is no higher than may be found in the serum of normal healthy rabbits (Boyd, 1940).

We obtained subcultures of the Wakefield strains (nos. 50, 58511 and 53028) from the Curator, National Collection of Type Cultures. A small quantity of antiserum prepared in the rabbit against one of the strains was kindly supplied to us by Dr Berger.

In general we found that the morphological and biochemical characters of the strains conformed to the description given by Dr Berger. There was no fermentation of lactose or mannitol. Saccharose was fermented by all three strains within 7 days.

Since the biochemical reactions of the Wakefield strains were in some respects similar to those of the Newcastle bacillus, our first serological tests were carried out with serum of this type. We were immediately confronted with the unexpected result that certain batches of Newcastle serum agglutinated the Wakefield strains to full titre, while other batches were completely negative. Absorption tests proved that the Wakefield strains were unable

to remove the homologous agglutinin from any of these sera.

Tests were then carried out with other Flexner sera, and similar results were obtained. Thus, one batch of Flexner V serum strongly agglutinated the Wakefield strains, whereas another batch failed to do so. Further tests were carried out with sera of organisms unrelated to the Flexner group, and it was found that the same conditions were often present. A recently prepared paratyphoid B serum agglutinated the Wakefield strains to 1/500, whereas a batch prepared some months previously gave negative results.

There was only one possible explanation of these findings: that we were dealing with strains of paracolon bacilli containing α-antigen (Stamp & Stone, 1944). That this was the true explanation was quickly proved by testing all sera against the strain paracolon F (Francis & Buckland, 1945), which is known to contain this antigen. It was found that all sera which agglutinated the Wakefield strains agglutinated paracolon F to the same titre. Those sera which did not agglutinate the Wakefield strains were negative with paracolon F. Absorption of any of the positive sera with the Wakefield strains removed all agglutinins for paracolon F.

Tests with the Wakefield serum supplied to us by Dr Berger provided further proof. This serum in our hands had an homologous titre of 1/3200, and agglutinated paracolon F to the same titre. Absorption of the serum with paracolon F removed all agglutinins for the Wakefield strains.

In order to try the effect of immunization with the Wakefield strains on the agglutinins for the Flexner bacilli, two rabbits, nos. 50 and 53, were inoculated respectively with the Wakefield strains 58511 and 53028. The sera of the rabbits were tested before and after immunization, and the results are shown in Table 1. It will be seen that rabbit no. 50 acquired an homologous titre of 1/28,000 with no appreciable effect on the Flexner agglutinins. In the case of rabbit no. 53 it will be observed that this animal possessed some α-agglutinin in its serum in low titre before immunization. This titre was raised to 1/10,000 after immunization without any increase in agglutinins for the Flexner bacilli. These tests were sufficient to prove that there is no antigenic relationship between the Wakefield strains and organisms of the Flexner group.

Table 1. *Effect of inoculating rabbits with the Wakefield bacillus*

Test suspension	Rabbit no. 50 (strain 58511) titre of serum		Rabbit no. 53 (strain 53028) titre of serum	
	Before inoculation	After inoculation	Before inoculation	After inoculation
Shigella flexneri V (I)	0	0	0	0
,, ,, W (II)	0	0	0	0
,, ,, X	0	0	0	0
,, ,, Y (Hiss-Russell)	35	35	85	70
,, ,, Z (III)	0	0	0	0
,, ,, 103 A (IV) (specific)	0	17	0	0
,, ,, 103 B (non-specific)	20	35	35	30
,, ,, P119 A (V) (specific)	0	0	0	0
,, ,, P119 B (non-specific)	20	17	25	17
,, ,, Newcastle (VI)	0	0	0	0
Bacillus wakefield, strain 50	0	28,000	175	10,000
,, ,, strain 58511	0	28,000	200	10,000
,, ,, strain 53028	0	28,000	175	11,000
Bacillus paracolon F	0	20,000	100	10,000

0 = no agglutination in 1 in 25 dilution. Incubation for 4½ hr. at 52° C.

SUMMARY

The organisms described as *Bacillus wakefield* and considered to be members of the Flexner group are not dysentery bacilli. They are paracolon bacilli containing α-antigen.

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