THE TYPING OF STRAINS OF *BACT. PARATYPHOSUM* B BY THE BIOCHEMICAL METHOD OF KRISTENSEN AND BÒJLEN

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Strains of *Bact. paratyphosum* B were originally classified into different types by Kristensen & Bòjlen (1929) according to the rate of acid production from rhamnose and inosite and this method has been suggested by Warren & Iredale (1934) and Vogelsang (1934) for epidemiological studies, such as in the correlation of cases, the tracing of sources of infection and the spread of outbreaks.

In the present study the method has been applied in the typing of 290 strains of *Bact. paratyphosum* B isolated from 121 patients suffering from paratyphoid fever during an epidemic of the disease in the north of Scotland in the early part of the year 1941, and of a further seventy-four strains derived from forty-four cases of the disease occurring in small groups or isolated cases in Edinburgh and its neighbourhood during the latter part of the same year.

About 230 cases of paratyphoid fever occurred during the epidemic in the north of Scotland, which was centred mainly in Ross-shire and Inverness-shire, though a small number of cases occurred in the neighbouring counties of Sutherlandshire and Nairnshire. The source of the epidemic was traced to a bakery in the district which distributed its products over a wide area in the counties involved. Three or four employees of this bakery were found to be ambulant cases of the disease, and another employee, who had suffered from a febrile illness a few months before the outbreak of the epidemic, was found to be excreting *Bact. paratyphosum* B in the faeces. In the great majority of cases the diagnosis was confirmed by the isolation of *Bact. paratyphosum* B, from the faeces or urine. As the typing of the strains was not begun until after the epidemic had subsided a number of cultures were not available for this work, but the strains investigated were fairly representative.

All the strains were identified by means of sugar-fermentation reactions and complete agglutination tests, and were retested after typing by slide agglutination.

The tests were carried out by the methods described by Kristensen & Bòjlen, i.e. according to the rate of acid production in three fermentation tests: (*a*) fermentation of rhamnose, (*b*) fermentation of inosite, and (*c*) the so-called Bitter test (a modified rhamnose fermentation using methyl red as indicator to ascertain whether the *p*H has been lowered to a certain point) (Bitter, Weigmann & Habs, 1926). The rhamnose and inosite media consist of 1 % peptone, 1 % meat extract, 0·5 % sodium chloride, and 0·5 % of the fermentable substance, standardized to a *p*H of 7·5–7·6, bromo-thymol blue (12 ml. of a solution containing 1 g. of 500 ml. *N*/200 NaOH) being added as an indicator. The rhamnose tests are read after 12 hr., and the inosite after 18–24 hr., a yellow colour being 'positive'. The medium used for the Bitter Test contains 0·5 g. of di-sodium hydrogen phosphate, 1·0 g. ammonium sulphate, 2 g. sodium citrate, 5 g. sodium chloride, 0·05 g. peptone, 1000 ml. of distilled water with 0·5 % of rhamnose added, the *p*H being about 7. After 15 hr. incubation 2 or 3 drops of methyl red solution are added to the culture, a 'positive' reaction being indicated by an orange-red to purple colour.

The strains are then classified in the following manner:

	Rhamnose	Bitter	Inosite
R_1I_1	+	+	+
R_1I_2	+	+	−
R_2I_1	+	−	+
R_2I_2	+	−	−
R_3I_1	−	−	+
R_3I_2	−	−	−

It is important that the hydrogen-ion concentration of the media should be carefully adjusted and that adequate controls should be used. Strains may sometimes be encountered that, even on retesting several times, persist in giving a borderline reaction with rhamnose after 12 hr. incubation, i.e. R_{2-3}. From two patients, such strains were consistently isolated.

Of the strains tested, 283 from 114 patients fell into the category R_3I_1, which seems to indicate that one type was responsible for the whole epidemic. In several cases the same type was obtained on as many as seven or eight different occasions from the one patient. There were, however, seven exceptions—strains received from patients in widely separated areas—each type being isolated at different times:

(*a*) R.B. giving R_3I_1, R_3I_1, R_3I_1, R_2I_2, R_3I_1.
(*b*) C.F. giving R_2I_2, R_3I_1.
(*c*) A.M. giving R_3I_1, R_3I_1, R_3I_1, R_2I_1, R_3I_1, R_3I_1, R_3I_1.
(*d*) R.M. giving R_2I_1, only one positive culture obtained.
(*e*) E.R. giving R_3I_1, R_3I_1, R_3I_1, R_2I_1.
(*f*) S.S. giving R_2I_2, R_3I_1, only two positive cultures obtained.
(*g*) C.M. giving R_2I_1, R_3I_1, R_3I_1.

It will be seen from this list that, except in the case of one patient, R.M., from whom only one culture was received, all the patients, from whom strains were typed, gave at some time or other, the common type R_3I_1. This variation of type may be due, as suggested by Kristensen (1938), to (1) changes in the fermentative properties of the infecting strain, (2) infection with two strains, or

(3) the possible confusion of specimens. The last two seem unlikely as the specimens were received from several sources and at varying times. Several of these strains were retested at varying intervals, a dozen colonies being chosen from each subculture, in order to see whether any variation might occur in vitro, but the fermentative properties of the strains remained constant over a number of subcultures.

It, therefore, seems important in determining *the type of Bact. paratyphosum* B by these fermentation reactions, for any patient, that several strains, if possible, should be tested, before the typing is accepted.

In the 74 strains of *Bact. paratyphosum* B derived from forty-four patients residing in the south-east of Scotland, there were two main types, R_2I_1 (seventeen cases) and R_3I_1 (twenty-two cases), and three cases showed variation. Two strains of R_2I_2 were isolated from a husband and wife, living in a small village in Berwickshire. No other strains of R_2I_2 were obtained from the south-east of Scotland during this period, and it was not possible to trace the source of infection.

The source of infection in most of the Edinburgh cases could not be determined, but five, due to *Bact. paratyphosum* B of the type R_3I_1, were found to have been infected by consuming food from a bakery. One of these cases was removed to a general hospital, and shortly after, two further cases occurred in the same ward. Type R_3I_1 was also isolated from these patients. Also in another small outbreak in Innerleithen, which occurred about a month before, the same type, R_3I_1, was isolated from specimens from four patients.

Three patients, as stated above, however, showed variations in type in cultures isolated at different times:

(1) J.G.—R_3I_1 and R_2I_1.
(2) B.—R_2I_1 and R_3I_1.
(3) W.—R_2I_1 and R_3I_1.

Consequently, when two cases of *Bact. paratyphosum* B infection occurred in one household, practically simultaneously, and a third member was shown to be a carrier, it was thought that it might be of value to test strains from these three cases at frequent intervals to determine whether any fluctuation in type occurred. Attempts at isolation of *Bact. paratyphosum* B were made daily by direct plating on MacConkey's medium, and by brilliant green and tetrathionate enrichment methods, and 107 cultures of *Bact. paratyphosum* B were obtained over a period of 35 days. These all proved to be of the type, R_2I_1.

From these investigations, it would appear that on the whole, the types of *Bact. paratyphosum* B, as shown by these fermentation tests, are constant, as already suggested by various authors (Kristensen & Bòjlen, 1929; Vogelsang, 1934; Warren & Iredale, 1934; and Frazer, Glover & Glass, 1937), and are therefore of epidemiological significance. Variants, however, may arise in a small percentage of cases (Kristensen, 1938), and from some of these cases the original type may again be isolated at a later date. It is therefore desirable, if possible, to test several strains from each patient to determine if there is variation, and if so, to establish the most constant type.

SUMMARY

Results are given of the typing (by biochemical fermentation tests) of 290 strains of *Bact. paratyphosum* B isolated during an epidemic in the north of Scotland. Of these strains 283 were of type R_3I_1. Seventy-four strains isolated in or around Edinburgh later in the same year, and 107 strains from three patients were also typed. Some variation (2·4 % in the north and 4 % in Edinburgh) was observed, but, in general, typing by the fermentation of rhamnose and inosite is of epidemiological significance.

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