# STUDIES ON THE TYPE DIVISION OF THE TYPHOID AND PARATYPHOID B BACILLI BY FERMENTATION

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## I. Introduction

In 1926 Henriksen and I gave an account of the fermentative features of the typhoid bacillus, with special reference to the differences demonstrated by xylose and *l*-arabinose fermentation reported in 1901 by C. O. Jensen. In 1929 Bojlén and I devised a system of type division for paratyphoid B bacillus by fermentation, based on the behaviour of the bacillus in the presence of *l*-rhamnose and of *i*-inositol. The typhoid bacillus, type I (in a meat-extract-peptone solution) ferments xylose in 24 hr., whilst type II ferments either later (by mutation) or not at all.

Types $R_1$ and $R_2$ of paratyphoid B both form acid in rhamnose in the course of a few hours. $R_1$ produces a positive reaction in "Bitter rhamnose" in contradistinction to $R_2$. $R_3$ shows no distinct formation of acid until after about 24 hr., $R_4$ does not ferment until after about two or more days (by mutation) or, sometimes, does not ferment at all. We did not mention $R_4$ in the work referred to above as a special type, but merely described it as a particularly slowly fermenting variety ("Haslev type") of $R_3$. We later realized that it is sufficiently constant to be designated as a separate fermenting type. We differentiate as regards behaviour with inositol between $I_1$, which forms acid in less than 24 hr., and $I_2$, which either does not ferment inositol at all or not until after several days. Combination of the rhamnose and inositol types gives the eight types, $R_1I_1$, $R_2I_1$, $R_3I_1$, $R_4I_1$, $R_1I_2$, $R_2I_2$, $R_3I_2$, $R_4I_2$. We have, however, never seen the last-mentioned type and have only met $R_1I_2$ in a *d*-tartrate fermenting paratyphoid bacillus.

In the work mentioned above we made a special point of investigating the constancy of the fermenting types we had established, in particular their constancy *in vivo*, i.e. the question of the immutability of the type as regards the single individual and also as regards transmission from one person to another. We found that the paratyphoid type, as well as the typhoid, remained very constant both *in vivo* and when cultivated on ordinary media. Type classification is therefore of epidemiological interest, quite apart from the fact that one type can be transformed to another by cultivation in special conditions; as, for instance, the transformation of typhoid bacilli type II to type I by the mutational fermentation which results from cultivation for a long

period in xylose. On the other hand, paratyphoid strains, as well as typhoid, will remain unimpaired for many years in ordinary agar and broth media, no change of a fermentational kind occurring.

We shall not review here all the literature on the variations of typhoid and paratyphoid bacilli, but will refer only to the more recent work dealing especially with the classification of the typhoid bacillus by means of xylose, and of the paratyphoid B bacillus by means of rhamnose and inositol.

The following authors have dealt with the classification of the typhoid bacillus by means of the xylose test: Joffe & Linnikova (1926), Schiff (1928), Ivanić & Dimitrijević-Speth (1929, 1930), Dechigi & Musettini (1930), Reitano (1930), Schmidt (1930), Eckstein (1931), Silberstein (1931), Hirvisalo (1932), Rimpau (1932), Shousha & Cossery (1932), Dechigi (1933), Giovanardi & Mondolfo (1933), Hirszfeld *et al.* (1933), Lawrynowicz & Bohdanowicz (1933), Amzel (1934), Constantino (1934), Mennonna (1934), Shimajo (Soda (1934)), and other Japanese authors, Strietter (1934), Strietter & Chachaewa (1934), Vater (1934), Habs (1935), Klinge (1935), Röderer (1935), Dimitrijević-Speth (1936), Gorrieri (1936), Montagnini (1937), Tesdal (1938). In the aggregate these authors examined over 8000 strains (from a somewhat smaller number of patients, as some authors reckon only one strain from each patient, others repeated tests from the same person); general agreement prevails that the type remains very constant, both *in vitro* (when cultivated in ordinary conditions) and *in vivo*. Strietter (1934), however, found both types in the same person in one case, and Rimpau (1932) on one occasion found both types in the same epidemic. Rare observations such as these can, of course, be explained quite as well as being due to mixed infection as to transformation of one type to the other.

All the authors who have dealt with sufficient material found that type I was more frequent than type II. Strains that fermented arabinose quickly (type III) were only seen in single cases.

It is generally agreed that the behaviour of the xylose-positive and the xylose-negative typhoid bacilli is the same as regards other cultural properties, serological features, and the clinical course of the infections they produce in human beings. Some of Hirvisalo's type II strains, however, agglutinated more slowly than his other strains, and Reitano found type II to be more virulent (or toxic) for rabbits than type I.

Shimajo combined classification by xylose fermentation with four different types of growth on solid media, from which a total of eight types appeared; and these he stated were constant. Soda (1934) added four types to these. Klinge (1935) found five types, taking into consideration arabinose, dulcitol, sucrose (one strain fermented sucrose!), and xylose; he included in the positive type all the strains that fermented in the course of 10 days, a proceeding which is not to be recommended, as general experience shows that it is the late fermentation of xylose, arabinose, and dulcitol, that is of a mutational character, and can therefore appear at very varying periods when the same strain is subjected to repeated tests. The reason that Klinge found the type constant, nevertheless, may be that he had only had a few cases of such late fermentation in his material.

It would, however, be desirable if the classification into xylose-positive and xylose-negative types could be supplemented by an epidemiologically constant classification according to other properties. Fermentation tests with *d*-tartrate and citrate, in particular, are possibly applicable (Kauffmann & Burón, 1935; Tesdal, 1937, 1938), but greater experience is necessary before a definite opinion can be given.

Up to a few years ago interest was so centred on the question of differentiation of the paratyphoid B bacillus from the related *Salmonella* types, especially *S. typhi murium*, that but slight interest was taken in the cultural classification of the serologically defined paratyphoid B type.

The first detailed test of Kristensen & Bojlén's rhamnose-inositol system was made by

Vogelsang (1932*b*, 1934), who found that the types were very constant. Warren & Iredale (1934), Röderer (1935), and Frazer *et al.* (1937) came to the same conclusion. These authors examined altogether 541 strains. Tesdal (1938), however, found three examples in 432 strains from 145 patients of possible transformation of one type to another. In one case (the appearance of $R_1 I_1$ and $R_2 I_1$, in the same epidemic) he allowed, however, the possibility of double infection; in the second case a strain was found which split into inositol-positive and inositol-negative variants; the third case is the most extraordinary, as it consisted of the appearance of $R_1 I_1$ and $R_4 I_1$, respectively, in two persons who had been infected from the same source.

The most important of the other cultural differences among the paratyphoid B bacilli is the distinction between the *d*-tartrate-negative and *d*-tartrate-positive strains. In spite of complete serological agreement the *d*-tartrate-positive strains must, from a practical point of view, be kept clearly apart from the true paratyphoid B bacillus and be placed among the gastroenteritis bacilli (see Kristensen & Kauffmann, 1937).

A certain epidemiological importance must be attached to the demonstration of ammon-negative (Tesdal) and anaerogenic strains; both these, however, are rather rare, and deficient formation of gas is, according to the experiences of several authors, not one of their most constant properties. Paratyphoid B strains with slight ability or without ability to ferment dulcitol or xylose are even rarer. Stern's glycerol fermentation reaction is hardly a suitable criterion for the type classification of paratyphoid B bacillus, as the boundary between Stern-positive and Stern-negative strains is not clearly defined (Vogelsang, 1932*a*; Kristensen & Bojlén, 1936).

Examination of paratyphoid B strains should entail not only adherence to the fermentative classification of the type but also tests for factor I and factor V (the latter is found, as is well known, in by far the majority of strains), as well as for factor IV, which is common to all paratyphoid B strains (Kauffmann, 1934; Zahn, 1935; Christensen, 1937). We propose, however, to restrict ourselves to the Institute's experience of fermentational classification by means of the *RI* system, as this is the only method that has been systematically used for a number of years. The typhoid and paratyphoid material comprises only strains from patients not included in Kristensen & Henriksen's (1926) and Kristensen & Bojlén's (1929) works. The word "patient" is used to mean a person from whom typhoid or paratyphoid bacilli have been cultivated, whether or not clinical symptoms have been present. A "strain" means a culture from one single sample received.

## II. Typhoid strains

The material on which the present work is based comprises typhoid strains, the type of which had been determined, isolated before 15 March 1938 from patients whose first type-classified culture originated from a sample submitted to the Institute after 30 November 1925. In a number of the cases the disease began before 1 December 1925, and these were mostly chronic carriers for whom either typhoid bacilli had not been demonstrated previously or the previous finding dated from before the commencement of Kristensen & Henriksen's work.

In none of the cases was more than one type found in the same patient; and the classification of the strains was always unmistakable, with one exception (a strain from a patient in the Faroe Islands) which was classified as type II, although a partial yellowish colouring of the xylose tube was observed at 37° C. as early as the first day (inconstant, however, in repeated tests);

the tube was not completely yellow until the third day at the earliest. The same phenomenon was observed on plating-out and inoculation from single colonies. There was no formation of acid when the test was carried out at 30 and 22° C.

Table I gives a summary of the total number of cultures of which the types were determined.

Table I.* *Number of patients for whom the total number of strains is shown in the top line.*

| Type | Arabinose | Xylose | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0 | + | 284 | 115 | 61 | 34 | 18 | 8 | 5 | 11 | 7 | 7 | 1 | 3 | 2 |
| II | 0 | 0 | 96 | 40 | 17 | 8 | 4 | 4 | 1 | | | 1 | 4 | 1 | |
| III | + | + | 1 | | | | | | | | | | | | |
| | | | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 23 | 24 | 27 | 29 | 33 |
| I | 0 | + | 2 | 2 | 4 | 1 | 3 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 |
| II | 0 | 0 | 2 | | | | | | | 1 | | 1 | | | |
| III | + | + | | | | | | | | | | | | | |

| Type | Patients | Strains |
|---|---|---|
| I | 575 | 1668 |
| II | 180 | 449 |
| III | 1 | 1 |
| Total | 756 | 2118 |

* In Tables I–V a blank means 0.

Of these 2118 cultures 1954 came from faeces (or other intestinal content) or from urine, 144 from blood, eleven from cultures submitted (probably all cultures from blood); three were from bile, two from pus, one from sputum, two from glands, and one from spleen. The findings from the faeces and the urine will be dealt with under one heading "FU" as regards the typhoid and paratyphoid material, for the bacillus found in the urine is certainly often due to contamination from the faeces.

Type I was found in the FU and blood of sixty-eight patients, in the FU and cultures submitted of five patients, in the FU, blood, and culture submitted of one patient, in the FU and bile of three patients, in the blood and culture submitted of one patient, and in the spleen and mesenteric gland of one patient.

Type II was found in the FU and blood of five patients, in the FU and cultures submitted of two patients, in the FU and pus of one patient, and in the blood and retroperitoneal gland of one patient.

Table IIA gives a summary of the duration of the excretion of the bacillus, reckoned from the commencement of the disease. The only persons included here are, therefore, those who were reported as being ill and for whom information was available as to the time when the disease began. Chronic carriers, for whom no such information has been forthcoming, have therefore not been included, even though over a year had elapsed between the first and last finding of the bacillus. Strains that were not subjected to type determination and were found later than the last determination are included.

Table IIA indicates that patients with type II become chronic carriers just as frequently as those with type I, and thus does not confirm Ivanić & Dimitrijević-Speth's assumption that type II creates fewer carriers of bacilli (in proportion to the number of cases) than type I. In view of the possibility that the distribution of the two types may vary during the course of years,

all the cases in which the disease began before 1 December 1925 or after 31 December 1936 have been withdrawn from Table IIA and the result given in Table IIB. No essential difference between the tendencies of the two types towards prolonged excretion of bacilli were found even when this allowance was made.

Table II. *Duration of excretion of typhoid bacilli, in months, reckoned from the commencement of the disease.*

Cases for which the date of commencement of disease is not available are not included, even though one year has elapsed between the finding of the first and last bacillus. Findings without type determination (for patients whose type is otherwise verified) are included.

| | A. Total no. of cases | | | | | | B. Cases in which the disease began within the period 1. xii. 25 to 31. xii. 36 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type | 0–1 | 1–2 | 2–3 | 3–6 | 6–12 | ≧12 | 0–1 | 1–2 | 2–3 | 3–6 | 6–12 | ≧12 |
| I | 284 | 91 | 31 | 19 | 10 | 56 | 264 | 86 | 30 | 19 | 10 | 24 |
| II | 76 | 49 | 8 | 6 | 1 | 12 | 75 | 49 | 8 | 6 | 1 | 5 |
| III | 1 | | | | | | 1 | | | | | |

By way of elucidating the question of the constancy of the type when the infection passes from one person to another, an account will be given of the state of affairs in the Faroe Islands, where only type II was found in the material previously examined. In the present material seventy-three strains from twenty-nine patients proved to belong to type II; only one strain, from a patient in whom the typhoid bacillus was only found once, belonged to type I. The source of infection is unknown in this case, but there is a possibility of infection from foreign tourists. An epidemiological investigation of typhoid in the Faroe Islands has been made by Bojlén (1936).

Only partial information is available as to infection in the rest of Denmark, but no example has been observed that indicates the transformation of one type to another by transmission from individual to individual.

## III. Paratyphoid B strains

### (*a*) *Distribution of types*

The material comprises strains of *d*-tartrate-negative paratyphoid B bacilli, the types of which were completely determined (i.e. with rhamnose and inositol), and isolated from patients the type for whom was first determined from a sample submitted after 31 December 1928, the last determination being made from a sample submitted 6 October 1937.

The account of the duration of the excretion of the bacillus includes strains of which the types have not been determined, or only incompletely so. With these strains the period for material ceased at the end of February 1938.

Notice has been taken of the anaerogenic strains as well as of the *RI* system; where no mention is made of the formation of gas the strains concerned were aerogenic. Transitional forms among the *RI* types had also to be included.

Contrary to the findings for the typhoid bacillus types, more than one type was found here in the same patient in several cases. We will first, therefore, give a summary (Table III) of the cases in which only one type was found in each patient, and later discuss the cases with different types.

Table III. *Number of persons from whom the number of type-classified paratyphoid B strains shown in the top line have been isolated, all of which have been of the type shown in the first column*

| Type | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1I_1$ | 5 | 1 | 4 | 1 | | | 1 | | | | | | | | |
| $R_{1-2}I_1$ | 2 | 1 | | | | | | | | | | | | | |
| $R_2I_1$ | 399 | 153 | 88 | 51 | 36 | 23 | 22 | 12 | 9 | 8 | 2 | 4 | 4 | 4 | |
| $R_2I_1$ anaer. | 3 | 4 | 1 | | | | | | | | | | | | |
| $R_{2-3}I_1$ | 1 | 1 | | | 1 | | | | | | | | | | |
| $R_3I_1$ | 342 | 252 | 139 | 71 | 47 | 25 | 16 | 13 | 12 | 12 | 6 | 10 | 6 | 9 | 5 |
| $R_4I_1$ | 2 | 2 | 1 | 1 | | 1 | | | | | | | | | |
| $R_{1-2}I_2$ | 1 | | | | | | | | | | | | | | |
| $R_2I_2$ | 15 | 3 | 4 | 3 | 1 | | | 1 | | | | | 1 | 1 | |

| Type | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 29 | 32 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $R_1I_1$ | | | | | | | | | | | | | | | |
| $R_{1-2}I_1$ | | | | | | | | | | | | | | | |
| $R_2I_1$ | 3 | 4 | 1 | 4 | 1 | 2 | | | 1 | 3 | | 1 | | 1 | |
| $R_2I_1$ anaer. | | | | | | | | | | | | | | | |
| $R_{2-3}I_1$ | | | | | | | | | | | | | | | |
| $R_3I_1$ | 7 | 3 | 4 | 4 | 2 | | 2 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 |
| $R_4I_1$ | | | | | | | | | | | | | | | |
| $R_{1-2}I_2$ | | | | | | | | | | | | | | | |
| $R_2I_2$ | | | 1 | | | | | | | | | | | | |

Of the 6071 cultures recorded in Table III, 6030 come from FU, thirty-three from blood, and eight from bile. Type $R_2I_1$ was found in the FU and blood of thirteen patients, and in the FU and bile of two patients. Type $R_3I_1$ was found in the FU and blood of twelve patients, in the FU and bile of three patients and in the FU, blood, and bile of one patient. Type $R_2I_2$ was found in the FU and bile of 1 patient.

Table IV shows the duration of the excretion of the bacillus (undetermined types included), A comprising all the cases for which information was available as to the time when the disease began, and B only the cases in which the disease began within the period 1 January 1929 to 31 December 1936.

It is not possible to decide, on the basis of the material reported here, whether there is any real difference as regards the duration of the excretion of the bacillus between the typhoid and paratyphoid B bacillus and the various types of paratyphoid B.

It has been calculated from Table IIB and Table IVB that carriers (i.e. individuals who have excreted bacilli at least one year after the disease began) form the following percentage of all the patients in whom bacilli were found:

Typhoid I, 5·5; typhoid II, 3·5; paratyphoid B $R_2I_1$, 2·8; paratyphoid B $R_3I_1$, 4·0. Without going into statistical calculations it may just be stated that these differences cannot be considered to be significant.

More than one type of paratyphoid B bacillus was found in fifty-three patients. I have divided these cases into two groups:

I (26 cases). (*a*) Inconsiderable variations (alternation between one of the

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Table IV. *Duration of excretion of paratyphoid B bacilli, in months, reckoned from the commencement of the disease*

Cases for which the date of commencement of disease is not available are not included, even though one year has elapsed between the finding of the first and last bacillus. Findings without type determination (for patients whose type is otherwise verified) are included.

| | A. Total no. of cases | | | | | | B. Cases in which the disease began within the period 1. i. 29 to 31. xii. 36 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type | 0–1 | 1–2 | 2–3 | 3–6 | 6–12 | ≧12 | 0–1 | 1–2 | 2–3 | 3–6 | 6–12 | ≧12 |
| $R_1I_1$ | 6 | 2 | 2 | | | | 6 | 2 | 2 | | | |
| $R_{1-2}I_1$ | 3 | | | | | | 3 | | | | | |
| $R_2I_1$ | 360 | 239 | 34 | 34 | 10 | 20 | 306 | 193 | 25 | 25 | 7 | 16 |
| $R_2I_1$ anaer. | 4 | 3 | 1 | 1 | | | 4 | 3 | 1 | 1 | | |
| $R_{2-3}I_1$ | 2 | 1 | | | | | 2 | 1 | | | | |
| $R_3I_1$ | 426 | 301 | 58 | 35 | 8 | 38 | 421 | 291 | 56 | 35 | 7 | 34 |
| $R_4I_1$ | 1 | 4 | | 1 | | | 1 | 4 | | 1 | | |
| $R_{1-2}I_2$ | 1 | | | | | | 1 | | | | | |
| $R_2I_2$ | 17 | 3 | 2 | | 1 | 3 | 16 | 3 | 2 | | 1 | 2 |

normal types and a closely related intermediate type; alternation between aerogenic and anaerogenic forms of the same *RI* type).

(*b*) Occurrence of two normal types and a transitional form from one to another; in these cases we have probably to deal with strains occupying in reality on intermediate position between the two types.

(*c*) Some cases (indicated with a question mark) in which it might be assumed that the particular finding was due to confusion of the samples or a clerical error.

II (27 cases). Other variations.

The cases will be reported briefly, in the alphabetical order of the patients' names, the findings for each patient being shown chronologically. Where there is no other indication the finding originated in the FU.

I. ♂ W.A.: $R_2I_1$ anaer.; blood $R_1I_1$?; $R_2I_1\times2$. ♀ H.B.: $R_{1-2}I_1\times2$; $R_2I_1\times6$. ♀ C.C.: $R_2I_1$; $R_{1-2}I_1\times2$; $R_1I_1\times4$. ♀ K.D.: $R_{1-2}I_1$; $R_2I_1\times8$. ♀ K.F.: $R_2I_1\times3$; $R_{2-3}I_1$. ♀ A.G.: $R_2I_1$; $R_2I_1$ anaer.$\times2$. ♀ C.H.: $R_2I_1\times16$; $R_{1-2}I_1$. ♂ A.H.: $R_3I_1\times10$; $R_3I_2$ (inositol $(+)^3$, after which the degree of acidity decreased. On further reinoculation in a fresh inositol tube $+^{\frac{1}{2}}$); $R_3I_1\times2$. ♀ A.J.: $R_{1-2}I_1$; $R_2I_1$. ♀ I.J.: $R_2I_{1-2}$ (inositol $+^2$); $R_2I_2$; $R_2I_{1-2}$ (inositol $+^2$); $R_2I_1$. ♀ K.J.: $R_{1-2}I_1$; $R_2I_1$. ♀ K.K.: $R_1I_1\times2$; $R_{1-2}I_1\times2$. ♀ M.L.: $R_2I_1\times2$; $R_{2-3}I_1$; $R_2I_1$; $R_3I_1$; $R_2I_1\times3$; $R_{2-3}I_1$; $R_3I_1$; $R_{2-3}I_1$. ♀ M.M.: $R_2I_{1-2}$ (inositol $+^2$)$\times2$; $R_2I_2$; $R_3I_2\times2$; $R_{2-3}I_2$; $R_2I_2\times2$; $R_2I_{1-2}\times5$; $R_3I_{1-2}$; $R_{2-3}I_2\times2$. ♀ E.N.: $R_2I_1$; $R_{1-2}I_1$. ♂ V.N.: $R_{2-3}I_1$; $R_2I_1$. ♀ A.O.: $R_1I_1$; $R_{1-2}I_1$; $R_1I_1\times2$; $R_{1-2}I_1$. ♀ O.O.: $R_2I_1\times2$; $R_{1-2}I_1$; $R_2I_1\times4$. ♀ E.P.: $R_2I_1$?; $R_3I_1\times3$. ♂ K.P.: $R_3I_1\times9$; $R_{1-2}I_1$?; $R_3I_1$. ♀ E.R.: blood $R_2I_1$; $R_2I_1$; $R_{1-2}I_1$; $R_2I_1$; $R_{1-2}I_1$. ♀ I.R.: $R_2I_1$; $R_{1-2}I_1$; $R_1I_1\times7$; $R_{1-2}I_1$; $R_2I_1$; $R_1I_1\times9$; $R_{1-2}I_1$; $R_1I_1\times10$. ♀ P.S.: $R_3I_1\times6$ (the first two strains only slight formation of gas); $R_3I_{1-2}\times2$. ♀ B.S.: $R_2I_1\times20$; $R_{1-2}I_1$; $R_2I_1\times7$. ♀ O.T.: All strains $R_2I_1$. The first six were anaer. in mannitol; no. 6, however, gave slight formation of gas in sorbitol ($\frac{1}{20}$ volume of a Durham's tube). No. 7 gave inconsiderable formation of gas in mannitol and some other "sugars"; $\frac{2}{5}$ volume of a Durham's tube of gas after 9 days in sorbitol. Nos. 8 and 9 are only designated aerobic in mannitol, no tests having been made for formation of gas in the other "sugars". ♀ K.T.: $R_2I_1$ anaer.$\times20$; $R_2I_1$?; $R_2I_1$ anaer.$\times2$.

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There can hardly be any doubt that the variations in the majority of these twenty-six patients were genuine, as transitional forms between the types are concerned here. The variations for the other twenty-seven patients, on the contrary, occurred "without motive".

II. ♀ A.A.: $R_3I_1\times3$; $R_2I_1\times2$. ♀ H.A.: $R_2I_2\times2$ (inositol 0²); $R_2I_1$. ♀ V.B.: $R_3I_1\times4$; $R_2I_1$ anaer.; $R_3I_1$. ♀ A.G.: $R_2I_1\times4$; $R_3I_1$; $R_2I_1$; $R_3I_1$. ♀ V.H.: $R_2I_1\times8$; $R_3I_1$; $R_2I_1\times6$; $R_3I_1\times2$. ♀ C.J.: $R_2I_2\times7$; $R_2I_1\times2$; $R_2I_2\times13$ (the last from bile). ♀ J.J.: $R_3I_1\times8$; $R_2I_1$. ♀ A.J.: $R_3I_1\times4$; $R_2I_1$. ♀ G.J.: $R_3I_1\times4$; $R_2I_1$. ♂ S.J.: $R_3I_1$; $R_2I_1$. ♂ B.K.: $R_2I_1\times5$; $R_3I_1$. ♂ J.K.: $R_3I_1$ anaer.; this strain formed gas in a subsequent test; $R_2I_1$ anaer.$\times2$. ♀ L.K: $R_2I_1\times7$; $R_3I_1$. ♀ G.L.: $R_2I_1$; $R_3I_1$; $R_2I_1$. ♀ A.L.: $R_2I_1$; $R_1I_1$; $R_2I_1\times5$. ♀ A.N.: $R_2I_1\times2$; $R_2I_2$. ♂ I.N.: $R_3I_1\times15$; $R_2I_1$; $R_3I_1$; $R_2I_1$. The two $R_2$ strains fermented rhamnose comparatively slowly. ♀ G.O.: $R_2I_2\times2$; $R_2I_1$; $R_2I_2\times2$. ♀ C.P.: $R_2I_1\times2$; $R_3I_1$, rough; $R_2I_1\times2$; $R_3I_1$, slimy. The four $R_2$ strains were cultivated from faeces, the two $R_3$ from urine. ♀ E.P.: $R_3I_1$; $R_2I_1\times23$. ♀ G.P.: $R_2I_1\times2$; $R_3I_1$; $R_2I_1\times2$. ♀ H.R.: $R_2I_1\times2$; $R_3I_1$; $R_2I_1\times3$. ♂ A.S.: $R_2I_1\times4$; $R_4I_1$. ♂ S.S.: $R_2I_1\times3$; $R_3I_1$. ♀ M.S.: $R_3I_1\times2$; $R_{1-2}I_1$; $R_3I_1\times2$. ♂ S.T.: $R_2I_1\times2$; $R_1I_1$; $R_2I_1$. ♂ W.W.: $R_3I_1$; $R_2I_1$.

When these variations are considered it should be borne in mind that they need not all be due to changes in the properties of the strain but may originate in other causes: (1) infection of a patient with two different strains; (2) contamination of a sample with material from another patient (opportunity for this has probably been greater in paratyphoid than typhoid); (3) confusion of samples, either at the hospital or at the laboratory; (4) faulty recording of the results. It is highly probable that some of the variations for the twenty-seven patients are due to intermediate strains that were not recognized as such. It is an open question whether strains do not also occur which, although they cannot be described as intermediate, have a particular tendency to vary.

From a purely practical point of view, though, the variations registered indicate the uncertainty that must be reckoned with in the determination of a patient's paratyphoid B type by fermentative methods used in the present work.

It looks as though the rhamnose fermentation of the strain in case ♀ C.P., simultaneously with a morphological and serological change, was weakened by localization in the urinary system. In most of the other cases, however, in which a different type was found in the urine from that in the faeces (not specified in the summary above) the strains from the urine showed the greatest fermentation. No general relation between variation and the localization in the organism can thus be established on the basis of the present material.

Table V gives a synopsis of (1) the patients, and (2) the strains, that appear in Table III, (3) the number of strains from the fifty-three patients for whom the types varied, and (4) the total number of strains.

Of the 6510 strains 4563 come from repeated examinations of 1177 patients. 232 of the 386 repetitions for the fifty-three patients whose types varied were of the same type as the first strain; 154 were different from the first strain. A small calculation, the details of which will be omitted, shows that if no constancy at all were found, but the types were distributed uniformly without relation to the first examination, though in the same numerical proportion

as that in which they were found in the entire material, 2117 of the repetitions would be identical with the first examination, and 2446 would be different. Apparent or actual change of type has thus occurred with about $\frac{1}{16}$ the frequency that might have been expected if no constancy at all had been found. This fraction is unpleasantly large even though a large number of incompletely changed types are included.

Table V

| Type | Cases with constant findings No. of patients | No. of strains | Cases with varying types No. of strains | Total no. of strains |
|---|---|---|---|---|
| $R_1I_1$ | 12 | 30 | 40 | 70 |
| $R_{1-2}I_1$ | 3 | 4 | 22 | 26 |
| $R_2I_1$ | 836 | 2460 | 185 | 2645 |
| $R_2I_1$ anaer. | 8 | 14 | 34 | 48 |
| $R_{2-3}I_1$ | 3 | 8 | 5 | 13 |
| $R_3I_1$ | 994 | 3432 | 101 | 3533 |
| $R_3I_1$ anaer. | | | 1 | 1 |
| $R_4I_1$ | 7 | 19 | 1 | 20 |
| $R_2I_{1-2}$ | | | 9 | 9 |
| $R_3I_{1-2}$ | | | 1 | 1 |
| $R_{1-2}I_2$ | 1 | 1 | | 1 |
| $R_2I_2$ | 30 | 103 | 34 | 137 |
| $R_{2-3}I_2$ | | | 3 | 3 |
| $R_3I_2$ | | | 3 | 3 |
| Totals | 1894 | 6071 | 439 | 6510 |

There is therefore reason to examine the question whether the type classification should be simplified and made more strict. But the intermediate forms should not be ignored; on the contrary, the ideal would be to replace type classification by its original basis, i.e. registration of the number of hours that elapse, in every case, before the rhamnose, or inositol, tube becomes yellow, a method of procedure that will, however, only be adhered to in special cases. On the other hand, it is strongly recommended that the strains that do not ferment inositol in 24 hr. should be observed for a longer period (from 3 to 4 days). The differentiation which might most advantageously be omitted is that between types $R_1$ and $R_2$, as it appears from Table V that more than half the bacilli of type $R_1I_1$ are found among the patients with the varying type; in all these cases it was the rhamnose, and not the inositol, type that varied; furthermore, a considerable number of the transitional form $R_{1-2}I_1$ are found, in proportion to the frequency of $R_1I_1$. As appears from the work of Kristensen *et al.* (1937), the value of the Bitter rhamnose test has also been too greatly emphasized in other fields of salmonella diagnosis. The other Bitter reactions, as well as this one, should be reserved for diagnostic use in the special fields in which a special examination has shown that they are sufficiently constant. If it is so desired, of course the Bitter rhamnose test can be applied to paratyphoid B bacilli, but it must be clearly understood that the constancy of the reaction is poor. If it is desired to omit this, a designation common to both $R_1$ and $R_2$ must be introduced; $R_{1 \text{ or } 2}$, or $R_{<3}$ could be written.

As in the case of typhoid, we have not undertaken a systematic investigation of the constancy of the types when paratyphoid is transmitted from

person to person. The general impression, however, is that the differences between strains from different patients, within the same epidemic, are no greater than the differences between a corresponding number of cultures from the same person. The information obtained from the Faroe Islands may be quoted in illustration of this assertion. Type determination was used in the examination of paratyphoid B bacilli from 109 patients. 107 of these showed type $R_2I_1$, in altogether 221 tests. The other two are mentioned in the above list of twenty-six patients with inconsiderable variations, etc.: (1) ♀ K.D. for whom $R_{1-2}$ was found once, otherwise $R_2I_1$, and (2) ♀ P.S. for whom $R_3I_1$ was principally found. The latter patient, a chronic carrier, is not known to have had any connexion with the other cases.

### (*b*) *Clinical data*

With reference to the above-mentioned experiences of a special clinical course for the infections produced by the *d*-tartrate-positive paratyphoid B bacilli I thought it would be of interest to find out whether there might be any difference among the cases produced by the various fermenting types of the *d*-tartrate-negative paratyphoid B bacilli. With this object in view the records of Blegdams Hospital were examined systematically for patients that had been discharged before the end of 1936. One of these patients was admitted in 1928, the others all after 1 January 1929. The rarer types ($R_1I_1$, $R_4I_1$, $R_2I_2$) from Blegdams Hospital are supplemented by cases from the Copenhagen County Hospital at Frederiksberg. The following cases from Blegdams Hospital have, however, been omitted: five patients whose case histories could not be found (two had type $R_2I_1$, three type $R_3I_1$) and the patients (chronic or temporary carriers) who—in spite of the fact that the bacillus was found—had no disease when they were in Blegdams Hospital that could be described as paratyphoid (six of these had type $R_2I_1$, four type $R_3I_1$). Table VI comprises the material less these cases. Four patients with anaerogenic strains are included in $R_2I_1$, one patient with varying bacilli (♂ J.K., mentioned above) in $R_3I_1$, and one patient with $R_{1-2}I_1$ in $R_1I_1$.

For the sake of comparison the corresponding figures are given for ninety-one *typhi murium* patients for whom information concerning the duration of fever was available, and for 140 *typhi murium* patients for whom seven had sufficient data for classification of diarrhoea. None of the *typhi murium* patients had roseola, setting aside one patient who excreted also paratyphoid B bacilli. A distinct impression of the difference between paratyphoid B and the *typhi murium* infection is thus obtained.

It might perhaps be thought that the cases of paratyphoid B with diarrhoea "3" could be identified clinically with the *typhi murium* cases. But this is not so, for eleven of the twenty-two paratyphoid B patients with diarrhoea "3" had roseola, the fever in none of the twenty-two cases lasting fewer than 10 days.

Table VI. *Clinical course of paratyphoid B cases with various fermentative types*

| | $R_2I_1$ | $R_3I_1$ | $R_1I_1$ | $R_4I_1$ | $R_2I_2$ | *Typhi murium* |
|---|---|---|---|---|---|---|
| Number of patients | 161 | 162 | 5 | 1 | 7 | |
| Treated with: | | | | | | |
| *Staphylococcus* vaccine | 96 | 92 | 2 | 0 | 6 | |
| Yatren-casein | 0 | 8 | 0 | 0 | 0 | |
| *Staphylococcus* vaccine and Yatren-casein | 0 | 1 | 0 | 0 | 0 | |
| *Staphylococcus* vaccine and coloxin | 0 | 0 | 0 | 0 | 1 | |
| Duration of fever where there was only one period of fever. Fatal cases not included: | | | | | | |
| 1–5 days | 1 | 0 | 0 | 0 | 0 | 43 |
| 6–10 ,, | 5 | 4 | 0 | 0 | 0 | 36 |
| 11–15 ,, | 21 | 12 | 2 | 0 | 0 | 4 |
| 16–20 ,, | 19 | 29 | 1 | 0 | 1 | 4 |
| 21–25 ,, | 31 | 35 | 0 | 0 | 1 | 3 |
| 26–30 ,, | 21 | 24 | 1 | 0 | 1 | 1 |
| 31–40 ,, | 24 | 20 | 0 | 1 | 1 | 0 |
| 41–50 ,, | 7 | 8 | 0 | 0 | 2 | 0 |
| 51–60 ,, | 3 | 2 | 0 | 0 | 1 | 0 |
| >60 ,, | 3 | 3 | 0 | 0 | 0 | 0 |
| ? | 5 | 4 | 0 | 0 | 0 | |
| Two periods of fever | 17 | 13 | 0 | 0 | 0 | |
| Four ,, ,, | 1 | 0 | 0 | 0 | 0 | |
| Continued fever stage | 55 | 51 | 1 | 1 | 3 | |
| Roseola | 90 | 91 | 1 | 1 | 4 | |
| Enlargement of spleen | 25 | 16 | 0 | 0 | 1 | |
| Diarrhoea* 0 | 51 | 55 | 1 | 0 | 3 | 1 |
| ,, 1 | 51 | 35 | 2 | 1 | 1 | 2 |
| ,, 2 | 49 | 61 | 0 | 0 | 3 | 61 |
| ,, 3 | 9 | 11 | 2 | 0 | 0 | 76 |
| Intestinal bleeding (without slime) | 8 | 11 | 0 | 0 | 0 | |
| Slimy stools (without blood) | 15 | 12 | 0 | 0 | 0 | |
| Blood and slime in stools | 7 | 11 | 0 | 1 | 0 | |
| Symptoms of appendicitis | 4 | 9 | 0 | 0 | 0 | |
| Symptoms of disease of the liver and biliary system | 1 | 8† | 0 | 0 | 0 | |
| Patients who became chronic carriers | 3 | 9† | 0 | 0 | 0 | |
| Catarrhal infections in upper air passages | 11 | 21 | 1 | 0 | 0 | |
| Pneumonia | 13 | 11 | 0 | 0 | 0 | |
| Epistaxis | 22 | 17 | 0 | 1 | 1 | |
| Phlebitis | 6 | 8 | 0 | 0 | 2 | |
| Prostration, typhoid state, etc. | 20 | 12 | 1 | 0 | 2 | |
| Fatal cases | 3 | 7 | 1 | 0 | 0 | |

* 0 = No record of diarrhoea (apart from diarrhoea after purgative).
1 = Single fluid or semi-fluid stools now and then, or a few fluid evacuations on one single day.
2 = Several thin evacuations daily during most of the duration of the disease.
3 = Severe diarrhoea, as in typical acute gastroenteritis.

† Six of these were adult women.

Several other complications (meningitis, nephritis, pyuria, etc.) were observed, as well as those shown in Table VI, but each of them in only one case, or in quite few cases, for which reason their occurrence is of no significance to the comparison of the progress of infections produced by different fermentation types.

On the other hand, in addition to the data given in Table VI, extracts were taken from the case records giving information on the following points:

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length of period in hospital, duration of disease before admission, duration of the continued fever, if present, relation of temperature to pulse, haemoglobin per cent and the proportion of white blood corpuscles (where such examination was made). No essential difference among the types was found under any of these headings.

Table VI shows that there is clinically very close agreement on most points, between the two types. It is, however, surprising that both patients with symptoms of diseases of the liver and of the biliary system and chronic carriers are more strongly represented among the $R_3I_1$ cases than among the $R_2I_1$ cases, but it must be borne in mind that there are considerably more adult women with $R_3I_1$ than with $R_2I_1$. Strangely enough, none of the patients with disease of the liver or biliary system is identical with any of those who became chronic carriers of bacilli.

The figures for the three rarer types are of course too small for any general conclusion to be drawn from them; but nothing indicates that their clinical behaviour is essentially other than that of types $R_2I_1$ and $R_3I_1$.

## SUMMARY

No case of transformation (*in vivo*) of the "xylose-positive" typhoid bacillus to the "xylose-negative", or vice versa, has been observed in any of the material dealt with at the State Serum Institute, Copenhagen.

On the other hand, the types of paratyphoid B bacilli that can be established on the basis of their relation to rhamnose and inositol did not prove to be absolutely constant; actual or apparent transformation of one type to the other having been observed in several cases.

In this connexion the suggestion is made that Bitter's rhamnose reaction be omitted; the distinction between types $R_1$ and $R_2$ would thus disappear.

Patients with symptoms of diseases of the liver and biliary system and also chronic carriers were represented in greater numbers for type $R_3I_1$ than for type $R_2I_1$, but it is doubtful whether this observation can be taken as a general one. Apart from this, the behaviour of these two types was very uniform as to the clinical course of the infections they produced. The cases of infection with the rarer types, also, did not seem to vary clinically from those with the more frequent types. On the other hand, the difference between the clinical course of paratyphoid B and the infection with *Salmonella typhi murium* was very marked.

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and the testing of the paratyphoid B bacilli for inositol fermentation were carried out by Dr F. Kauffmann and other assistants in the Diagnostic Department. Dr Erlingur G. Tulinius has rendered no inconsiderable assistance in going through the case histories, the records of the Institute, and the card index of the typhoid and paratyphoid patients, the keeping of which has been for some years in the hands of Dr C. Faarup.

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