

NOTES ON ORGANISMS SEROLOGICALLY RELATED
TO *S. ENTERITIDIS* GÄRTNER. II.

OBSERVATIONS ON *S. PARATYPHUS* N 2 (SALMONELLA, TYPE
MOSCOW): AN ORGANISM COMPLICATING RELAPSING FEVER.

BY E. P. HICKS, M.D., D.T.M. AND H.

(From the Bacteriological Department, National Institute
for Medical Research, Hampstead, N.W. 3.)

INTRODUCTORY.

IN 1922 Iwaschenzoff described from Russia an epidemic of relapsing fever attended by high mortality. From many of the sufferers organisms of the Salmonella group were isolated. These organisms, termed *S. paratyphus* N¹, were apparently responsible for the severity of the cases. In 1921-2 the mortality rate in uncomplicated cases of relapsing fever was 5.5 per cent.; whereas it was 50 per cent. in cases showing secondary infection by Paratyphus N bacilli.

The bacillary disease, referred to by the Russian authors as N-Paratyphusbazillose, seems to have appeared first among Tartar fugitives from the famine districts in the Volga area, and occurred only in those suffering or recovering from relapsing fever. The cases were clinically divisible into two groups: those of typhoid type and those of septic type. In the typhoid type, the course of relapsing fever is altered by a sudden rise of temperature, often accompanied by rigors, and the patient's condition becomes worse. There are pains in the extremities, in the lumbar and sacral regions and elsewhere; spleen and liver are enlarged; there is deep jaundice increasing to a saffron colour; delirium and prostration are usual, and some meningeal symptoms seem to be constant; the urine as a rule shows no striking changes.

In the septic type, high temperature continues without remission until the patient dies in the course of 2 or 3 days with symptoms of profound intoxication.

In non-fatal cases recovery is slow, and obstinate joint pains remain. Further complications are arthritis, abscesses, otitis media and pulmonary affections (Hesse, 1924).

The causative organisms have been isolated by culture from the blood, urine, stools and pus, and from the organs at *post-mortem* examination; they have been described by Kulescha and Titowa (1923), Sütterlin (1923), Weigmann (1925), and Iwaschenzoff (1926).

It was soon discovered that the Paratyphus N bacilli concerned in these cases, while all showing the general biochemical features of the Salmonella

¹ See Editor's note, p. 445.

group, are separable into two serological types—*S. paratyphus* N 1 and N 2. The Paratyphus N 1 strains, which were by far the more frequent, have been identified with the majority of the Erzindjan strains of Neukirch (Weigmann, 1925, who termed the organism *B. paratyphus* C 2) and with Hirschfeld's bacillus (White). The Paratyphus N 2 strains, which form the subject of this note, were found by their discoverers to have affinities with Gärtner's bacillus. Weigmann (1925), who termed the organism *S. paratyphus* C 1, believed it to coincide with a strain, received by him under the title "Erzindjan," which differed sharply from the remainder of Neukirch's Erzindjan cultures. He states that the serological cross-reactions of the Paratyphus N 2 (or C 1) bacilli and *S. enteritidis* Gärtner, noted by Russian workers, depends solely on similarity of the O stable and finely agglutinating antigens, the H labile, coarsely clumping antigens of the two forms being sharply distinct.

RE-EXAMINATION OF STRAINS OF *S. PARATYPHUS* N 2.

Among Salmonella cultures sent in 1926 by Prof. Ludwig Bitter of Kiel to Mr Bruce White were several strains of *S. paratyphus* N 2 drawn from the series already investigated by Weigmann. It seemed of interest to continue the study of these organisms. Since, however, the time available for such study was limited, attention was restricted to five of these strains: four strains of Russian origin (N 1, N 5, S 127 and S 1409) and the Turkish strain Erzindjan referred to in the introductory note.

The reader is warned against confusing the strains N 1 and Erzindjan with the type Paratyphus N 1 or true Erzindjan bacillus.

MORPHOLOGY AND FERMENTATIVE ACTIVITY OF THE STRAINS.

All five organisms are short, motile gram-negative bacilli of the usual Salmonella pattern. Strains N 1, N 5, S 127, S 1409 produce acid and gas in 24 hours in media containing glucose, mannite and rhamnose; in dulcitate media they produce acid and gas in 3 to 4 days; lactose, saccharose and salicin are unfermented. The strain Erzindjan shows the same range of fermentative activity, but attacks dulcitate rapidly and fails to produce gas in any of the carbohydrate media tested.

SEROLOGICAL COMPARISON.

A serum was prepared against strain N 5. As shown in Table I, this serum agglutinated fresh cultures of the four Russian strains to the same approximate titre; the strain Erzindjan rather less vigorously. In all cases, in association with the small compact granules of somatic (O) agglutination, the large loose flocculi of flagellar (H) agglutination could be differentiated. The somatic reactions of the several strains, studied by use of alcohol treated bacilli, showed no appreciable differences. In absorption tests, the results of which are also indicated in Table I, it was found that while strains N 1, S 127, and S 1409 all exhausted the serum more or less completely, the strain Erzindjan,

though removing somatic agglutinins, left well-marked flagellar agglutinins for N 5 and the remaining Russian strains. These tests, while confirming belief in the identity of the Russian strains, separated them from strain Erzindjan.

Table I.

| Culture agglutinated | | Titre of strain N 5 antiserum | | | | |
|----------------------|---|-------------------------------|-----------------------|--------------|---------------|------------------|
| Strain | Fresh or alcohol treated (alc. tr.) bacilli | Before treatment | After absorption with | | | |
| | | | Strain N 1 | Strain S 127 | Strain S 1409 | Strain Erzindjan |
| N 5 | Fresh | 4000 F (g) | 200 F | <200 | <200 | 2000 F |
| | Alc. tr. | 1000 g | <200 | <200 | <200 | <200 |
| N 1 | Fresh | 4000 F (g) | 200 F | <200 | <200 | 2000 F |
| | Alc. tr. | 1000 g | <200 | <200 | <200 | <200 |
| S 127 | Fresh | 4000 F (g) | 200 F | <200 | 200 F | 4000 F |
| | Alc. tr. | 2000 g | <200 | <200 | <200 | <200 |
| S 1409 | Fresh | 4000 F (g) | 200 F | <200 | 200 F | 4000 F |
| | Alc. tr. | 1000 g | <200 | <200 | <200 | <200 |
| Erzindjan | Fresh | 2000 F & g | 200 F | <200 | 200 F | <200 |
| | Alc. tr. | 1000 g | <200 | <200 | <200 | <200 |

F = floccular (flagellar) clumping.
 g = granular (somatic) clumping.
 F & g = mixed floccular and granular clumping almost up to the titre limit.
 F (g) = dominantly floccular clumping with granular clumping in lower serum dilutions.

Without entering into details, it may be said that the last-named organism was fully identified serologically with typical strains of *S. enteritidis* Gärtner. The strain N 5 was now compared with Gärtner's bacillus (Tables II and III).

Table II.

| Culture agglutinated | | Titre of <i>S. enteritidis</i> (Wallasey) serum | | Titre of <i>S. paratyphus</i> N 2 (strain N 5) serum | |
|--------------------------------------|---|---|----------------------------------|--|---|
| Strain | Fresh or alcohol treated (alc. tr.) bacilli | Before treatment | After absorption with strain N 5 | Before treatment | After absorption with <i>S. enteritidis</i> |
| | | | | | |
| <i>S. paratyphus</i> N 2, strain N 5 | Alc. tr. | 2,000 g | <200 | 1000 g | <200 |
| | Fresh | 4,000 F (g) | <200 | 4000 F (g) | 1000 F |
| | Alc. tr. | 2,000 g | <200 | 2000 g | <200 |

Symbols as in Table I.

Table III.

| Culture agglutinated | | Titre of <i>S. enteritidis</i> serum | | |
|--------------------------------------|---|--------------------------------------|--|---|
| Strain | Fresh or alcohol treated (alc. tr.) bacilli | Before treatment | After absorption with | |
| | | | <i>S. enteritidis</i> (boiled) Derby (fresh) | <i>S. enteritidis</i> (boiled) N 5 (fresh) and strain |
| <i>S. enteritidis</i> | Fresh | 40,000 F (g) | 20,000 F | 20,000 F |
| | Alc. tr. | 2,000 g | <200 | <200* |
| <i>S. paratyphus</i> N 2, strain N 5 | Fresh | 10,000 F (g) | 10,000 F | <200* |
| | | | | |
| <i>Salmonella</i> , type Derby | Fresh | 4,000 F | <200 | ? 400 F* |

Symbols as in Table I.

* In the series so marked there occurred, up to dilution 400, a slight indefinite precipitation, apparently due to substances absorbed by the serum during its preliminary treatment.

Contrary to the statement of Weigmann, it was found that in the cross agglutination reactions of these organisms, the H labile or flagellar components are definitely involved. Where actively motile cultures of both organisms were employed, a coarsely floccular type of clumping was superimposed upon the granular clumping noted by Weigmann in similar cross agglutination tests. Agreement was, however, only partial and in reciprocal absorption tests the two organisms were sharply defined by the disparity of their flagellar components. The somatic constituents of the two organisms on the other hand, and in harmony with the statement of Weigmann, reacted as if identical. In similar tests made in comparison of strain N 5 and the Dublin type (see immediately preceding communication) results took the same form, viz. identity of somatic, overlapping of flagellar antigens. Agglutinative affinity with the Derby type was limited to the flagellar elements.

An attempt was made to orientate strain N 5, as representative of *S. paratyphus* N 2, in the system of antigenic formulae presented by Mr Bruce White in the immediately preceding communication. The work had to be abandoned before completion: certain points were, however, determined.

From observations already discussed, it follows that strain N 5 possesses in common with *S. enteritidis* and the Dublin type the somatic factor III and also a distinctive flagellar factor which may be indicated by the symbol P_4 .

Since strain N 5 exhausts *S. enteritidis* serum of agglutinins for the Derby type (Table III) and Derby type serum of those for *S. enteritidis*, it (N 5) presents the flagellar factor R .

The further relations of N 5 to *S. enteritidis* and the Dublin type have been only partially elucidated. Since *S. enteritidis* serum is not exhausted of flagellar agglutinins for strain N 5 by treatment with the Derby or Dublin type, N 5 must possess some portion of the factor group $P_1 + P_2$ —and certainly a portion of P_2 . Whether N 5 possesses a part of the factor P_3 of the Dublin type was not determined. The information collected may be summed up in the formula

| | | | | | |
|--------------------|-----|-----|-----|-----|-------------------|
| Somatic antigens | ... | ... | ... | ... | III |
| Flagellar antigens | ... | ... | ... | ... | R |
| | | | | | $(P_1 + P_2) - *$ |
| | | | | | P_4 |

* - sign indicates that the factor group $(P_1 + P_2)$ is incompletely represented.

NOMENCLATURE.

As the terms *S. paratyphus* N 1 and *S. paratyphus* C 2 have no present usage in British nomenclature and since there is no good reason for replacing the well-established title Hirschfeld type or *S. paratyphosus* C of Hirschfeld by either, it is desirable to adopt a new name for *S. paratyphus* N 2 (or C 1). With Mr Bruce White I suggest the name *Salmonella*, type Moscow.

TECHNIQUE.

All organisms used for agglutination or absorption were grown on nutrient agar for about 20 hours at 37° C. The absorption doses were, for boiled

organisms, 50 plates, and for unheated organisms, 100 plates per cubic centimetre of undiluted serum. Absorption was carried out for 1 hour in a water bath at 50° C., and 3 hours at room temperature. Agglutination was studied by the macroscopic method. The tubes were placed in a water bath at 50° C. for 2 hours. The results were read at the end of this period and again after the tubes had stood on the bench overnight. The final reading only is given in the tables.

For study of the somatic reactions the flagellar antigens were destroyed by placing the organisms, suspended in absolute alcohol, in a water bath at 50° C. for 1 hour. The alcohol was then decanted, and the organisms were suspended in normal saline solution.

CONCLUSIONS.

Detailed study shows that the so-called Paratyphus N 2 strains of Russian authors are much more closely related serologically to *S. enteritidis* Gärtner than has hitherto been suggested. The agglutinative cross-reaction between the two organisms and their antisera involves not only the somatic but also the flagellar antigens. The organism is, however, readily differentiated from *S. enteritidis* and other known *enteritidis*-like forms by means of the absorption test and is a distinct *Salmonella* type. It is proposed to replace the original title of the strains by the name of *Salmonella*, type Moscow.

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