A STUDY OF THE SO-CALLED *BACTERIUM TYPHI FLAVUM*

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During the last eight years, a number of Continental workers, particularly in Germany, have described an organism to which the name "*Bacterium typhi flavum*" has been given. A motile, gram-negative rod, characterised by the production of yellow colonies on agar, it was originally isolated from the ejecta of typhoid contacts, but has since been obtained from water, soil and numerous other sources. The particular interest of the organism lies in the claim made by certain workers that they have observed the transformation of certain strains of "*Bacterium typhi flavum*" into true *Bact. typhosum* in the course of more or less frequent subcultures—a claim which has, in their view, explained the appearance of enteric infections in closed institutions, the pigment-producing organism being regarded as a reversible variant of *Bact. typhosum*, non-pathogenic in its pigmented form or capable only of producing a mild disease. The recommendation that persons excreting the organism should be treated as typhoid carriers has endowed the problem with some importance from the public health standpoint, and the present study consists of an investigation of the characters of strains of *Bact. typhi flavum* obtained from various sources, their relation to *Bact. typhosum*, and an attempt to confirm the occurrence of a mutation into that organism.

Dresel and Stickl (1928) had reported the production by growth in dilutions of tulip juice of variants of *Bact. typhosum* which produced yellowish brown pigment on agar, fermented sucrose and were of lowered agglutinability by anti-typhoid serum. In 1930, they described an epidemic of enteric infection in an institution in Greifswald. Commencing with 4 frank cases of typhoid fever, the source of which was not apparent, 50 inmates fell ill with atypical symptoms during the next 6 months. *Bact. typhosum* was not isolated from these individuals, but from a few of them and from a number of healthy persons in the institution *Bact. typhi flavum* was obtained. At this stage, 3 typical new cases of typhoid were reported. By the time a year had elapsed from the original outbreak, *Bact. typhi flavum* had been isolated from 26 persons, out of a total of 996 in the institution; of these 20 had had no symptoms, 3 had had atypical symptoms and 3 typical typhoid. The organism was obtained 26 times from the urine, once from stool and twice from blood. The serum of 120 individuals agglutinated a strain of *Bact. typhi flavum* either alone (28) or in addition to *Bact. typhosum* (92). It would appear that only about half of those persons

from whom the organism was isolated or whose serum agglutinated the organism had any symptoms. In 6 neighbouring hamlets, *Bact. typhi flavum* was isolated from typhoid cases after *Bact. typhosum* had disappeared, from atypical cases and "suspects" and from healthy contacts. Pointing out that 118 villages in which typhoid fever was reported in 4 years had but one case each, the authors formulate the theory referred to above, that *Bact. typhi flavum* may be a typhosum variant of lowered virulence, playing an epidemiological role in that it may be responsible for the survival of the infective agent from one outbreak to another.

In support of this theory, v. Gara and Stickl (1930) published the results of researches on 40 strains of *Bact. typhi flavum* obtained from human sources—enteric suspects, convalescents and healthy persons—mostly from urine, and 6 from water. Having classified *Bact. typhosum* into groups I (typical), III (failing to ferment maltose but fermenting sucrose) and IV (fermenting neither maltose nor sucrose), and *Bact. typhi flavum* into groups II (typical), V (failing to produce pigment) and VI (failing to ferment maltose), the authors tabulate the results of almost daily subculture on agar plates. Six of the human strains and 1 water strain ("L"), starting as typical *Bact. typhi flavum*, are stated, after 3, 136, 40, 144, 206, 65 and 127 subcultures respectively to have, with or without intermediate change into one of the atypical groups, produced variants which had lost the characters of *Bact. typhi flavum* and acquired those of *Bact. typhosum*, satisfying biochemical and serological tests as to identity with that organism.

Neisser (1930) came to the conclusion that *Bact. typhi flavum* and *Bact. typhosum* were fundamentally different, and was unable to confirm the findings of the previous workers with regard to change of species on repeated plating.

Sonnenschein (1930) studied 4 strains which he had obtained from the Greifswald workers, including the water strain "L"; he recorded many differences from *Bact. typhosum* culturally, and was able to demonstrate neither serological relation of the original strains with *Bact. typhosum* nor transformation of type. Lotze (1931), who was working on bacterial variation in the colityphoid group under the influence of growth in various body fluids and in media of varying acidity and alkalinity, claimed, amongst other surprising changes, to have transformed *Bact. typhosum* into *Bact. typhi flavum*, and the latter organism into *Bact. typhosum* and *Bact. paratyphosum* B.

The matter was discussed at the German Microbiological Society's meeting at Heidelberg (1931), where the Greifswald workers stoutly asserted their previous claims. Stickl (1931) stated that 203 strains had been isolated from 179 persons in 4 years, that 12 reports of change into *Bact. typhosum* were now on record, and that an agglutinating titre of 1/100 in a patient's serum for *Bact. typhi flavum* is of diagnostic significance, in his opinion, as regards enteric infection. He cited an epidemic of clinically typical typhoid fever, where *Bact. typhi flavum* was the only organism isolated, mostly from the urine. Dresel (1931) reviewed the epidemiological findings, laying stress upon the

isolated occurrence of single cases of typhoid in districts apparently remote from chance of contact infection, and suggesting that carriers of *Bact. typhi flavum* were responsible. Kathe (1931), who had studied 3 of the Greifswald strains and 28 isolated by himself from urine (25), stools (1) and water (2), had carried out subcultures which produced colourless variants but no colonies of *Bact. typhosum.* Sonnenschein (1931) remarked upon the more frequent finding of the organism in healthy persons and upon its occurrence in nature; failing to credit it with relation to *Bact. typhosum* or power to change into that organism, he drank a large dose of two of the Greifswald cultures without ill-effect. Kauffmann (1931) also commented upon the widespread occurrence of *Bact. typhi flavum* and suggested that Stickl's alleged production of these organisms from *Bact. typhosum* by growth in tulip juice could be explained by the frequency of the yellow organisms in plants. He could not confirm the transmutation findings and believed that an original mixture of *Bact. typhi flavum* and *Bact. typhosum* must be presumed, most of the strains concerned being from typhoid or obscure cases. Lentz (1931) similarly recorded negative findings, while Gotschlich (1931) pointed out that single-cell culture would be of assistance in assessing the claims.

Obrtel (1931) records the isolation of *Bact. typhi flavum* from the blood of a case of typhoid fever, from the stool of a non-enteric case and from water. He noted no change during 9 months' subculture and considers that there is little evidence in favour of its being a variant of *Bact. typhosum.* He regards it as a saprophyte able to reach the blood stream by the breakdown of the intestinal barrier in ulcerative disease.

Grossmann (1932) carried out a study of eight strains—4 from Greifswald, 1 from mouse faeces and 3 from human stools (2 healthy persons, 1 a paratyphoid case). The strain from the paratyphoid case and 2 of the Greifswald strains are stated to have given rise to true *Bact. typhosum* as variants after 55, 63 and 29 subcultures respectively on agar at daily intervals at 37° C. The same worker, in another paper (1933*a*), reports a similar transformation of another of his strains after a large number of subcultures into an organism which, he considers, satisfies the cultural and serological criteria of *Bact. typhosum.* He took all precautions to preclude contamination or error, repeatedly plating his original cultures to ensure purity. In a further paper (1933*b*), Grossmann records details of the same and of later experiments, including a series in which single-cell cultivation (Wámoscher, 1932) was done prior to subculture—some variants were produced in these latter experiments but none had the characters of *Bact. typhosum.*

Strószner (1932), who had access to a large hospital for enteric cases, failed to isolate *Bact. typhi flavum* from blood, stools and urine, but isolated a strain from milk, which he studied and subcultured 50 times without noteworthy result.

v. Gara (1933) gives the results of attempts to induce variants by growth of *Bact. typhi flavum* in broth of increasing acidity and alkalinity, and by growth

in egg, the appearance of *Bact. typhosum* being in each series once recorded. Negative results are reported with reference to subculture in bile-containing media and media to which sterile sea-sand was added.

Seydel (1933, 1934) isolated 180 strains in 3 years and made a close study of 80 of these—including 41 from blood, 6 from stools, 13 from urine, 6 from water, 1 from butter and 1 from nasal secretion—without noting any evidence in support of the claims of the Greifswald workers.

Hirsch (1933) similarly failed to confirm the reports of transmutation, whereas Sobernheim (1934*a*), working with Dresel's 4 original strains, made daily subcultures on agar and in broth containing *Bact. typhosum* killed by heat and tested for sterility, and claimed to have observed the appearance of organisms with the cultural and serological characters of *Bact. typhosum*, after 42, 65, 71 and 96 transfers. Further experiments with the same strains are said in 3 cases to have given rise to gas-producing variants identical with *Bact. paratyphosum* B, but a series commencing with a single-cell culture by Burri's method achieved no noteworthy results. In another paper (1934*b*) Sobernheim attempts to explain by such bacterial variation various epidemiological problems, without producing, however, much evidence to support his theory.

Castro (1934*a*, *b*) published two papers giving the results of study of the Greifswald strains and 76 strains isolated by himself. Variants were examined, mostly obtained by plating on glycerine agar after treatment of the organisms with bile, but none of these variants had the characters of *Bact. typhosum*. He is of opinion that *Bact. typhi flavum* is a common saprophyte.

Fortner (1934) likewise carried out 220 successive subcultures of 10 strains including 4 from Greifswald on agar at intervals of 1 or 2 days at 37° C., without noting any variants, other than the usual colourless ones, differing strikingly from the original strain. He insists that single-cell cultivation must precede all such experiments, and suggests that the results of Dresel and Stickl are accounted for by technical error.

Hirsch (1934), in a further contribution to the subject, arrives at the same conclusion, laying stress upon the appearance of certain aggregated masses in the colonies, discussed below, which may, in his view, favour the persistence in subculture of contaminating organisms.

Claims to have noted the appearance of *Bact. typhosum* during subculture of *Bact. typhi flavum* have thus been made by the following workers: Dresel, Stickl and v. Gara; Grossmann; Lotze; Sobernheim. Failures to confirm these findings have been much more numerous.

SOURCES OF THE STRAINS USED

From Prof. Bürgers (Königsberg):

3200.	Obtained 2 years previously from Prof. Dresel, Greifswald.
H.M.	From grass.
3932.	From urine.
6899.	,,

163 *g*. From urine

163 *b*. A colourless variant of 163 *g*.

Luft 1. From air.

517. From urine.

Tute 5. From grass.

(Strains 3200 and 6899 are referred to by Hirsch, 1933.)

From Prof. Grossmann (Göttingen):

625 III. From stool of a healthy person. This original strain has been subcultured every 4 weeks in agar stab.

625 IV. Is the same strain subcultured on agar every 1–2 days at 37° C. It was said to be agglutinated by a *Bact. typhosum* antiserum (titre 1/100,000) at 1/800.

625 D. Another variant of 625 III appearing after subculture on agar. Mucoid, producing gas in dextrose, no papillae on rhamnose agar.

These strains are referred to in a paper by Grossmann (1933*a*).

6247. From the stool of a case of paratyphoid fever, isolated simultaneously with *Bact. paratyphosum.*

6247 XI/85. A single-cell culture of 6247, the culture being carried out by Wâmoscher. It is said to be agglutinated by *Bact. typhosum* antiserum at 1/200–1/800 titre. Subcultured about every 2 days on agar at 37° C.

666. From the stool of a healthy person. Subcultured every 4 weeks in agar stab.

These strains are referred to by Grossmann (1933*b*).

149. From the blood of a typhoid suspect.

From Prof. Dresel, Greifswald:

000. From urine. Said to be agglutinated by *Bact. typhosum* antiserum at 1/100 ±.

001. This strain is said to be a variant produced from 000 after 9 subcultures on agar. It is said to be agglutinated by *Bact. typhosum* antiserum to high titre and to fulfil all the criteria of identity with *Bact. typhosum.*

L. Obtained from the slime on a filter at a waterworks.

L 239. Stated to have been obtained from L after 170 subcultures and to be *Bact. typhosum.*

v. Gara and Stickl refer to these strains in a paper (1930).

From Miss Seydel, Warsaw:

13840. From blood. Stated to be agglutinated by antityphoid serum 1/200.
3193. From stool. ,, ,, 1/400.
11752. From urine. ,, ,, 1/100.
6087. From water. ,, ,, 1/200.
J.M. From air. ,, ,, 1/100.

Of these strains record of transformation into *Bact. typhosum* is made in reference to Wasserstamm L, 000, 625 III, 6247 and 666.

I have to thank Prof. Bürgers (Königsberg), Prof. Grossmann (Göttingen), Prof. Dresel (Greifswald) and Miss Seydel (Warsaw) for their kindness in sending me the various strains.

A systematic study of these strains was made. A separate account of strains 001 and L 239 is given later.

MORPHOLOGY

All the strains consisted of rods, varying considerably in size, but mostly small, being 1–3μ in length by 0·5–0·7μ in thickness. They were examined in films made from 24-hour growth on agar plates at 22 and 37° C., and on other media, such as Löffler's serum and blood agar, but the appearances of each strain did not differ materially on the various media. The commonest appearance was of a short stout rod with rounded ends and bulging sides, or of a rather longer coliform rod, straight or slightly curved and with parallel sides. They were arranged in groups, pairs end-to-end, or singly, frequently assuming the polygonal arrangement found in films from smooth colonies of the coli-typhoid group (Wilson, 1930). Sometimes a palisade arrangement was observed.

Some strains (J.M. for example) consisted predominantly of rods so short as to be almost coccal, whereas on the other hand 8 of the strains showed filaments in addition to the shorter rods. These filaments were mostly unjointed and up to 15–20μ in length (*e.g.* strain 6087), straight, curved or S-shaped. Strain 625 III consisted of such filaments in addition to palisades of long slender rods, whereas 625 D showed short, stout and coliform rods only.

Staining. All stained well with the usual aniline dyes and all were Gram-negative and non-acid-fast. Staining was uniform, except that some of the short forms showed bipolar staining (*e.g.* strain 3193).

Motility. Hanging-drop preparations were made from 24-hour broth cultures at 22 and 37° C. At 22° C. all strains were motile, the movement being very brisk and of a darting and wriggling character. At 37° C., however, the organisms were non-motile, except in 2 or 3 cases in which an occasional poorly motile organism would be seen. Peritrichate flagella were demonstrated by a modified Fontana's method.

A peculiar feature of cell arrangement, which has been remarked upon by some of the German workers, particularly by Kathe (1931) and by Hirsch (1934), was noted. In a hanging drop made from the condensation water of an agar slope at 22° C. from most of the strains, it was easy to demonstrate elongated masses segmented into roughly angular shapes. These are variously described as sausage forms, caterpillar formations ("räupchenartige Bildungen"), "symplasmata" and "Bakterien-verbänden". They are granular in structure and, when they are viewed by dark-ground illumination or lightly stained, as by Giemsa's method, it becomes apparent that they consist of masses of organisms aggregated together. Kathe demonstrated them best in ascites broth after 24 hours, or on Löffler's serum or blood agar after a week, and Hirsch illustrates them as they occur in the edge of a colony on a glycerine-agar plate.

Cultural reactions

Heart broth. All the strains grew well in 18 hours at 22 and 37° C.; in Hartley's tryptic-digest broth growth was still better. Uniform turbidity was produced, mostly with a fine powdery white or yellowish white deposit which easily swirled up and dispersed on shaking. Further incubation produced increased turbidity and deposit with a fine surface scum or ring.

Nutrient agar plate. There was ready growth of all strains, but it is to be noted that growth occurred more rapidly and profusely at 37° than at 22° C., except for 3 strains which grew equally well at either temperature. After 24 hours' growth at 37° C., the diameter

of the colonies was from 0·5 to 3·0 mm., usually 1–2 mm., whereas at 22° C. the usual colony was punctiform or pinhead in size. Individual colonies were typically round, low convex, amorphous, smooth, glistening, opaque and with entire edge; the consistency was butyrous and emulsifiability easy. All the strains except 163*b*, which was white, were pigmented, the colour being ochre or rusty yellow; the pigment was confined to the colonies and did not diffuse into the medium. While there was no differentiation after 24 hours, after further incubation a usual appearance noted was that of a central flattening or plateau, sometimes with a granular surface, and a smooth bevelled periphery. A boss sometimes appeared in the centre of the colony, which, in the case of strain 3193 at 22° C., assumed a markedly worm-cast structure. Strain 625 III had from the start a rough surface and crenated edge, strains 11752 and 6087 assuming a rough surface after 48 hours, the latter strain producing eventually a rosette with radial striations and scalloped edge. A number of the strains gave a distinctly mucoid type of growth.

On examining with a lens, by transmitted light, colonies on an agar plate, in about half the strains, particularly 149, 3932, 6899, 163*g*, T 5, 517, L and 000, certain bodies or spots were noted. Investigation with the low power of the microscope showed that these consisted of granular clumps situated centrally, one in each colony, usually with lines of similar granular aggregations tapering radially therefrom in a manner suggestive of the conventional "bursting bomb". Alone, or in the centre of such a mass, were frequently found very distinct, clear-cut and striking biconvex bodies—the "Wetzsteinformen" of Hirsch, who discusses them at length (1934). These appearances were best seen after 48–72 hours' incubation at 22° C. The granular structures would seem to be simply aggregations of organisms analogous to the masses observed in the hanging drop from the condensation water of the slope. Hirsch suggests that they may include contaminants which may thus fail to be freed until after a succession of platings, when they ultimately are separated and give the impression of appearance of variants. The whetstone bodies are, in my own view, down-growths of the colony into the medium, the evidence for this being that this biconvex form is familiar as the most frequent form of a colony in the depths of a solid medium, and that, if one lightly removes, under the dissecting microscope, the surface growth of a colony or of a confluent growth throughout which "whetstones" are scattered, by passing a loop along it, the "whetstones" remain.

Agar slope. There was abundant confluent, smooth, glistening, yellow, opaque growth with entire or slightly undulate edge.

Five per cent. glycerine-agar slope. At 22° C. growth was found to be particularly mucoid in character. The German workers lay stress on this—so mucoid, frequently, is the growth that, when an area is inoculated on a glycerine-agar plate, which subsequently is placed on edge, the growth will run down to the lower edge of the plate. This excessive production of mucus they know as the "Fliessphänomen".

Agar pour-plate. Deep colonies were biconvex, sometimes with lateral knobs or projections.

Agar or glucose-agar shake. A yellow surface growth was produced with a few small colonies a short distance below the surface.

Horse-blood agar plate. Growth did not appear to be favoured by blood, the colonial appearance at 37° C. being the same as on an agar plate. No haemolysis was produced.

MacConkey's agar. Growth was very poor and hardly perceptible in 24 hours. On further incubation, colourless colonies with a rather rougher surface and more crenated edge than on nutrient agar were produced.

Löffler's serum slopes. All the strains gave in 24 hours at 37° C. a good confluent, glistening, yellow growth with smooth or slightly contoured surface and undulate edge. No liquefaction of the medium was observed.

Potato. The organisms grew readily, a confluent, yellow, glistening, mucoid growth being produced.

Fifteen per cent. Gelatin Stabs. After 24–48 hours at 22° C., a filiform growth occurred along the line of inoculation. After 6–10 days liquefaction commenced and progressed during the next 10–15 days until the medium was entirely liquefied. Liquefaction was infundibuliform, a yellow surface pellicle forming and later sinking to the bottom.

Resistance and viability

The bacilli were non-sporing.

An experiment to determine the resistance to heat was carried out with 3 of the strains. The growth from a 24-hour agar slope was washed off in sterile Ringer made with glass-distilled water, and a tube containing 6 c.c. of a suspension of 500,000 viable organisms per c.c. was placed in a water-bath at 56° C. Counts made at intervals showed that the suspension was sterilised within 10 min. in each case.

The strains remained alive for several months in agar stabs at room temperature.

1/1000 phenol incorporated in agar plates inhibited the growth of all the strains.

Castro (1934*b*) tested 102 strains and found that they died in 1–2 days in 40 per cent. bile at 37° C., as opposed to *Bact. typhosum* which survived for lengthy periods. Schaede made a similar observation (1933).

Metabolism

The organisms were aerobic and facultatively anaerobic. In the anaerobic jar on agar slopes poor growth was apparent after 3 days, but pigmentation was absent.

Growth appears to take place over a wide range of temperature with optimum nearer to 37 than 22° C. Although this observation agrees with the finding of v. Gara (1935), it is at variance with the records of most workers quoted above, who assert that the optimum temperature for growth is 22° C. (Kauffmann, 1931). Ströszner's strain grew best at 37° C.

Pigment. All strains, except the variant 163 *b*, produced on solid media, such as agar, Löffler's serum or potato, at 37 and 22° C. a pigment of ochre or rusty yellow colour. It was not produced under anaerobic conditions. It became more pronounced after a few days' incubation, but did not diffuse into the medium. Growth washed from plates, centrifuged, dried in a desiccator and ground, was tested with various solvents. The pigment was found to be insoluble in water, whereas a brownish yellow pigment dissolved out in alcohol and a bright yellow one in ether, in each case leaving the dried powder still pigmented even after some days' treatment with these solvents. Chloroform had practically no effect on the pigment.

Fermentation of carbohydrates

All the strains were inoculated into peptone water containing 1 per cent. of the carbohydrate and incubated at 37° C.

In 24 hours acid, without gas, was produced by all *Bact. typhi flavum* strains in glucose, mannite, sucrose, salicin (except 163*b*), rhamnose, arabinose and xylose. On further incubation for 24–48 hours 12 of the strains produced acid in maltose, but lactose, inosite and dulcite were not fermented even after prolonged incubation.

While these findings are mainly in agreement with those of the Continental workers, mention must be made of the numerous reports of the production of gas in dextrose broth or dextrose agar shake tubes (Neisser, 1930; Sonnenschein, 1930; Dresel, v. Gara and Stickl, 1930; Kathe, 1931; Grossmann, 1933*a*; Fortner, 1934). Though these observers, working with strains some of which were used in the present work, recorded production of gas variously at 22° C. or at 37° C., Kauffmann (1931), who regards the activity of the organism at its maximum at 22° C., states that gas was produced at that temperature only. In a recent study by Castro (1934*a*) of 76 strains from vegetable and animal sources, 23 are reported to produce gas in glucose, 53 acid only. None of the 80 strains studied by Seydel (1934) produced gas at either 37 or 22° C. v. Gara, who did not observe gas formation in glucose-agar shakes, suggests that Sonnenschein's findings in that medium are due to artefacts (1931).

In view of these discrepant observations, all the strains were inoculated again into sets of sugars and into glucose-agar shake tubes, and were kept under observation at 22° C. for 3 weeks. No gas was produced by any of the strains in either medium. In order to determine whether these organisms might be able to produce gas from a simpler substance which might be present as a breakdown product of glucose, the strains were grown in peptone water to which was added, in one series, sodium lactate, in another, sodium pyruvate—in neither series was gas produced.

It is universally agreed that sucrose is constantly fermented and that lactose is not attacked by *Bact. typhi flavum*, although Castro (1934*a*) reports slight formation of acid in the latter sugar by 6 of his 76 strains.

Litmus milk in each case remained neutral for 24 hours or showed a faint and transient acidity, but after 3–7 days the medium became alkaline in all except 3 cases. With a few strains a soft clot was produced after about 10 days. Slight differences in the reactions in litmus milk recorded in the literature are probably accounted for by variation in the nature of the medium. Some workers, such as Sobernheim (1934*a*), stress the spongy coagulation said by him to be constantly produced in 5–10 days.

Indol was not produced by any strain. The methyl-red reaction was positive and the Voges-Proskauer reaction negative. All strains reduced nitrates to nitrites.

Presence of H_2S was tested for by introduction of a piece of lead acetate paper between plug and glass of an agar-slope tube—at 37° C. this was negative, but at 22° C. there was just perceptible blackening of the edge of the strip after 2 days.

Catalase is produced.

Pathogenicity

Five of the strains (625 III, 13840, 149, 517, L) were tested on batches of 5 mice. Saline suspensions of 24-hour growth washed from agar slopes were injected intraperitoneally. The strains did not kill in a dose of 1,000,000 organisms, but when an enormous dose (1/5 slope) was injected, death usually took place in 2–3 days, the organism being recovered from spleen and heart blood.

Serology

Antisera were prepared in rabbits by the intravenous inoculation at weekly intervals of 500 million organisms washed from 24-hour agar slopes grown at 22° C. and killed by heating in the water-bath at 56° C. for ½ hour. Macroscopic agglutination tests were carried out, dilutions of sera from 1/25 to 1/25600 being set up in duplicate with H (flagellar) and O (somatic) suspensions of all the strains. The H suspensions were made by growing in broth at 22° C. for 18 hours, adding formol to 0·25 per cent. and heating for ½ hour at 56° C. The O suspensions were made by growing the organisms on agar plates at 37° C. for 24 hours, washing off with absolute alcohol, heating to 60° C. for 1 hour, washing and resuspending in saline. All agglutinations were carried out in the 55° C. water-bath, the H and O readings being taken respectively after 4 hours and overnight.

At the same time the sera were tested against members of the *Salmonella* group—as regards flagellar antigens, formolised suspensions of *Bact. typhosum*, *Bact. paratyphosum* A, *Bact. paratyphosum* B in the type and in the group phase, *Bact. aertrycke* (type) and *Bact. enteritidis*; as regards somatic antigens, *Bact. typhosum*, *Bact. aertrycke*, *Bact. paratyphosum* A, *Bact. paratyphosum* C, *Bact. Newport*. A wide range of *Salmonella* antigenic factors was thus covered

(Report by the Salmonella Sub-Committee of the Nomenclature Committee of the International Society for Microbiology, 1934)—actually flagellar factors *a*, *b*, *c*, *d*, *i*, *g*, *o*, *m*; 1, 2, and somatic factors I, II, IV, V, VI, VII, VIII, IX.

Further, two *Bact. typhosum* antisera, one with high H titre (1/25600), the other with high O titre (1/6400) were set up against all the suspensions, and antisera prepared against the strains 001 and L 239 were likewise included in the series. Finally, a serum prepared against a totally rough strain of *Bact. aertrycke* (R/Z) was tested against all suspensions.

The results, as regards the interrelation of the *Bact. typhi flavum* strains, are set out in Tables I and II. The conclusions to be drawn from these are as follows:

(1) The organisms known as *Bact. typhi flavum* do not form an antigenically homogeneous group.

(2) Unlike certain organisms which have a common H antigen and several varieties of O antigen, both H and O antigens are diverse.

(3) While thus a heterogeneous group, they show antigenic relationship in that the serum prepared against one strain will agglutinate the organisms not only of that strain but, as a rule, those of several other strains. This applies both to H and O suspensions.

(4) There appears to be a more widespread relation between the H than the O antigens of the strains studied.

(5) Although no definite classification of the strains into groups could be made, certain strains with more or less close relationship to one another can be detected—for instance, the antigenic make-up of H.M. and 149 is very similar, and the O antigen distinct from that of the other strains; likewise of 6087, J.M., 666; of 3932, 6899, 625 D and L; and of 11752, 517, 3193, 13840 (H).

The *Bact. typhi flavum* antisera did not agglutinate any of the H or O suspensions of the *Salmonella* group tested, except that 11752 agglutinated *Bact. Newport* "O" to low titre.

One of the *Bact. typhosum* antisera—that with the high "O" titre—flocculated 9 of the 19 *Bact. typhi flavum* "H" suspensions in a dilution which did not in any case exceed 1/100 (11752, 6087, 3200 at 1/100; 163 *g*, 666, 625 D, Luft 1 at 1/50; 517, 149 at 1/25). The flocculation was of a rather granular type but not so fine as a typical somatic agglutination. The alcoholised suspensions of *Bact. typhi flavum* were unaffected by *Bact. typhosum* antiserum.

Strain 11752 "O" was agglutinated to full titre by the rough *Bact. aertrycke* serum—it is to be noted that this strain was colonially somewhat rough. It is of interest to note that the strain of *Bact. Newport* which was agglutinated by antiserum 11752 was found to be in a partially rough state also. None of the other strains were agglutinated by the rough antiserum.

A few comments might be made on the serological findings recorded in the literature. It may be remarked that no distinction between H and O antigens has previously been made.

Table I. "*H*" *suspensions*

"H" suspensions	Sera																	
	11752	517	3193	13840	T 5	6247 I	163 *g*	H.M.	6087	J.M.	666	3932	6899	L	625 D	3200	000	149
11752	1/6400	1/3200	1/3200	1/1600	1/50	1/50	1/400	1/1600	1/50	1/50	1/3200	1/50	1/50	1/50	1/50	1/25	1/50	1/50
517	1/3200	1/3200	1/50	1/800	1/50	1/50	1/100	1/100	1/50	1/100	—	1/800	1/25	1/800	—	—	1/25	1/25
3193	1/3200	1/800	1/3200	1/3200	1/100	1/25	1/400	1/400	1/50	1/100	1/3200	—	1/200	—	—	—	1/50	1/25
13840	1/25	1/100	1/800	1/12800	1/25	1/400	—	1/100	1/50	1/50	1/100	1/200	1/25	—	1/100	—	1/200	—
T 5	—	1/100	1/50	1/25	1/6400	1/6400	1/3200	1/3200	1/6400	1/6400	—	1/1600	1/3200	1/3200	—	—	1/6400	1/800
6247 I	—	1/100	1/25	1/50	1/800	1/12800	1/25	1/400	1/12800	1/800	—	1/200	1/1600	—	1/1600	1/25	1/1600	1/800
163 *g*	—	1/50	1/50	—	1/200	1/100	1/3200	1/200	1/12800	1/12800	1/3200	1/25	1/400	—	1/50	—	1/800	1/200
H.M.	—	1/50	1/100	—	1/400	—	1/800	1/3200	—	—	—	—	1/100	—	—	1/25	—	1/800
6087	—	—	1/100	1/25	—	1/200	1/3200	1/25	1/12800	1/12800	1/6400	1/200	1/100	—	1/200	—	1/1600	—
J.M.	—	—	1/50	1/50	1/400	1/3200	1/1600	1/200	1/6400	1/12800	1/3200	1/400	1/3200	—	1/50	1/25	1/800	1/400
666	—	—	1/50	1/25	1/50	1/100	1/1600	1/25	1/1600	1/3200	1/6400	1/100	1/50	—	1/200	1/25	1/50	—
3932	—	—	—	1/50	1/800	1/1600	—	—	—	—	—	1/6400	1/100	1/3200	1/800	1/400	1/25	1/25
6899	—	—	1/50	1/25	1/25	1/100	—	—	—	—	—	1/800	1/1600	1/6400	1/25	—	1/50	—
L	—	—	1/50	1/50	1/50	1/50	—	1/25	1/25	1/25	—	1/800	1/800	1/12800	1/50	—	1/50	1/25
625 D	—	1/50	1/50	1/100	—	1/6400	—	1/25	1/25	—	1/25	1/800	1/25	—	1/3200	1/50	—	1/25
3200	—	—	1/100	—	—	1/3200	—	—	1/6400	—	—	—	1/25	—	1/400	1/1600	1/6400	—
000	—	—	—	—	—	1/6400	—	—	—	1/25	—	—	1/200	—	—	—	1/6400	—
149	—	1/25	—	—	—	—	—	1/3200	—	—	—	—	—	—	—	1/25	—	1/800
Luft 1	—	—	—	—	—	1/400	—	—	—	1/50	—	—	1/25	—	—	—	—	—

Table II. "*O*" *suspensions*

Suspensions	Sera																	
	11752	517	3193	13840	T 5	6247 I	163 *g*	H.M.	6087	J.M.	666	3932	6899	L	625 D	3200	000	149
11752	1/200	1/100	—	—	1/3200	—	1/800	—	—	—	—	—	—	—	—	1/50	—	—
517	1/25	1/800	—	—	—	—	—	—	—	—	—	1/25	—	1/25	—	—	—	—
3193	1/50	—	1/800	1/25	—	—	1/25	1/25	—	—	1/800	—	—	—	—	—	—	—
13840	—	—	—	1/25	—	1/100	—	—	1/25	1/50	1/25	1/100	—	—	—	—	—	—
T 5	—	—	—	—	1/400	—	—	—	—	—	—	1/25	1/25	—	—	—	—	—
6247 I	—	—	—	—	—	1/50	—	—	—	—	1/25	1/25	—	—	1/200	—	—	—
163 *g*	—	—	—	—	—	1/25	1/200	—	1/50	1/100	—	—	1/25	—	—	—	—	—
H.M.	—	—	—	—	—	—	—	1/200	—	—	—	—	—	—	—	—	—	1/50
6087	—	—	1/50	1/25	1/100	—	1/400	—	1/800	1/3200	1/1600	—	—	—	1/100	1/25	—	—
J.M.	—	—	1/50	—	—	—	1/50	—	1/800	1/1600	1/1600	—	—	—	—	—	—	—
666	—	—	—	—	—	—	1/50	—	1/200	1/200	1/200	—	—	—	—	—	—	—
3932	—	—	—	1/25	—	1/25	—	—	—	—	—	1/200	1/50	1/400	1/100	—	—	—
6899	—	—	—	1/25	1/25	1/25	—	—	—	—	—	1/100	1/800	1/800	1/25	—	1/25	—
L	—	—	—	—	—	1/25	—	—	—	—	—	1/50	1/400	1/400	1/25	—	—	—
625 D	—	—	—	—	—	1/800	—	—	—	—	—	1/50	1/25	—	1/3200	—	—	—
3200	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/50	—	—
000	1/50	—	1/50	1/25	1/800	—	—	—	—	—	—	—	—	—	—	—	1/2000	—
149	—	—	—	—	—	—	—	1/800	—	—	—	—	—	—	—	—	—	1/400
Luft 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Dresel and Stickl (1928) recorded agglutination of 29 out of 41 *Bact. typhi flavum* strains by *Bact. typhosum* antiserum at low titre (1/50, 1/100). v. Gara and Stickl (1930), using an antiserum made from a urine strain, record the wide variation in agglutinability of their strains, unrelated to their groupings on carbohydrate fermentation. They record that a large number of their strains are agglutinated by *Bact. typhosum* antiserum (1/100–1/400). Sonnenschein (1930) differs from v. Gara in that, while that worker records agglutination of strain 3200 by antityphoid serum, Sonnenschein got none with a serum the titre of which was 1/6000. Kathe (1931) found that, of strains 3446, L and 3200 from Greifswald, 1 was agglutinated by an antityphoid serum (titre 1/100,000) and 2 were not—it is not stated which. 11 of his own 28 strains were weakly agglutinated by that serum. Having carried out tests with antisera against 6 strains, he concludes from his results that *Bact. typhi flavum* showed no antigenic unity and he was unable to group the strains serologically. Of his sera, 1 agglutinated *Bact. typhosum* (1/100), 2 at 1/800, the other 4 being negative. Bürgers (1932) likewise found no orderliness in the antigenic structure. The strain studied by Strószner (1932) was agglutinated by a rabbit antityphoid serum (1/25000 titre) but not by another (1/5000), by 2 *Bact. paratyphosum* B antisera, and was apparently related antigenically to 2 of Dresel's strains.

Seydel (1934) records similar antigenic heterogenicity. Of 80 strains, 44 were agglutinated by a *Bact. typhi flavum* antiserum and 65 by antisera against either *Bact. typhosum* or *Bact. paratyphosum* (type not stated), at titres varying from 1/100 to 1/800. A *Bact. typhi flavum* serum agglutinated *Bact. typhosum*, and 1 *Bact. paratyphosum* C strain, but not another. A protocol shows that strain 3193 was flocculated by sera against *Bact. typhosum*, *Bact. paratyphosum* A, B and C, *Bact. enteritidis*, *Bact. Shiga*, *Bact. Flexner* and *Bact. typhi flavum*.

Sobernheim confirms the diversity of antigenic make-up of *Bact. typhi flavum* and finds 8 of his strains related to both *Bact. typhosum* and *Bact. paratyphosum* B, and 1 more to each of these organisms respectively.

From these somewhat chaotic observations, it may be agreed that the organisms known as *Bact. typhi flavum* are antigenically heterogeneous and that a certain number have some slight antigenic relation to *Bact. typhosum*, though this does not appear to involve the characteristic H or O antigen. Some recorded findings are difficult to explain, such as the agglutination of Strószner's strain by both *Bact. typhosum* and *Bact. paratyphosum* B sera, and Seydel's report with regard to strain 3193. Our present knowledge of antigenic structure indicates that, while *Bact. typhosum* and *Bact. enteritidis* are related in their somatic antigens and *Bact. paratyphosum* B and *Bact. paratyphosum* C in the group phase of their flagellar antigens, the relation of strain 3193 to all of these organisms, and to the dysenteries would predicate an unusual make-up of that strain. The most likely explanation that suggests itself is that the strain might be rough (it was slightly so colonially), and we know (White, 1929) that the rough *Salmonella* antigen is widespread. This further presumes at least a partial roughness of the strains from which the various antisera were prepared. Against

any such explanation is the fact that 3193 "O" was not agglutinated by the known rough antiserum *R/Z*.

It was clearly desirable to determine whether rabbits might possess normal agglutinins to *Bact. typhi flavum*—the ubiquity, later demonstrated, of the organism rendered this not improbable. Accordingly serum was taken from 5 normal stock rabbits and tested in dilutions from 1/25 to 1/200 against H and O suspensions of 9 of the apparently most agglutinable strains and 2 other strains isolated in the laboratory (see below). The results are shown in Table III.

Table III. *Normal agglutinins (rabbit)*

	"H" suspensions					"O" suspensions				
	Serum of rabbit					Serum of rabbit				
Suspensions	1	2	3	4	5	1	2	3	4	5
13840	—	—	—	—	1/50	—	—	—	—	1/25
517	1/25	—	—	—	1/50	—	—	—	—	—
163*g*	—	—	—	—	1/25	—	—	—	—	1/25
H.M.	—	—	—	—	—	—	—	—	—	—
6247 I	—	—	—	—	—	—	—	—	—	—
3932	—	—	—	—	—	—	—	—	—	—
6899	—	—	—	—	1/25	—	—	—	—	—
3200	—	—	—	—	—	—	—	—	—	—
Luft 1	—	—	—	—	1/25	—	—	—	—	—
Own 1	1/25	—	—	—	1/50	—	—	—	—	—
Own 2	—	—	—	—	1/25	—	—	—	—	—

It will be seen that the sera of 2 of the rabbits contained agglutinins for a number of the strains of *Bact. typhi flavum*, one serum showing "H" agglutinins at 1/25 dilution, the other serum agglutinating 7 of the 12 "H" suspensions and 2 of the "O" suspensions, the titre, however, in no case exceeding 1/50. In view of the existence of such normal agglutinins, only titres of over 1/100 should probably be regarded as definitely due to artificial immunisation in Tables I and II. Testing of the sera of the rabbits prior to immunisation was precluded by the fact that the necessary knowledge of the antigenic heterogenicity of the species had by that time not been gained.

Variants

During the course of the study, variants appeared from time to time.

(1) Colourless variants were not uncommon. Strain 517, for instance, showed these on agar. Subcultured, they bred true, the fermentation and serological reactions being the same as those of the original strain.

(2) Excessively mucoid colonies.

(3) Colonies with a rough surface and an irregular edge. Strain 149 produced such a variant after a few subcultures.

Prolonged subculture

For a period of 9 months, 2 of the strains (L, 000) and for shorter periods (2–3 months) other strains (163 *g*, 517) were subcultured daily or every second day on agar. Sometimes cultivation in broth was interposed between platings,

but more often a loopful of growth was taken from one plate and spread on a fresh one so as to obtain single colonies. The plates were incubated at 22° C. and any unusual (*e.g.* non-pigmented) colonies investigated by picking off, inoculating into sugars and, if necessary, studying serologically. Though variants of the above nature were encountered, none resembling *Bact. typhosum* was observed. A rapid initial test was to pick the suspected colony into sucrose-peptone-water—in no case was a variant obtained which did not ferment sucrose.

STRAINS 001 AND L 239

These strains were stated to have been derived respectively from 000 and L by v. Gara and Stickl (1930), the former directly after 9 subcultures, the latter after 170 subcultures in the course of which it appeared as a member of group III (colourless, maltose-negative, sucrose-positive), then of group V (colourless, maltose and sucrose positive) before becoming a typical *Bact. typhosum.*

The strains proved to be Gram-negative coliform rods, growing better at 37° C., and motile at that temperature as well as at 22° C. They produced on agar colonies which were smooth, circular, low convex, grey-white, shining, easily emulsifiable and with entire edge. On MacConkey's agar, strain 001 grew as non-lactose-fermenting colonies typical of *Bact. typhosum.* Strain L 239 was peculiar, however, in that it completely failed to grow on MacConkey's medium. The fermentation reactions were found to be those of *Bact. typhosum,* acid being produced in glucose, maltose, mannite and xylose. Gelatin was not liquefied. A very small amount of H_2S was produced.

Antisera were prepared against both strains, and tests were set up with the *Bact. typhi flavum* strains and sera. Towards the somatic antigens the sera behaved exactly as *Bact. typhosum* antisera, agglutinating O suspensions of that organism and of each other as well as the homologous strain. The sera flocculated similar flagellar suspensions but had also some effect on the flagellar suspensions of a few of the *Bact. typhi flavum* strains, particularly J.M., 3193 and 11752, which were stated by Seydel to be agglutinated by antityphoid serum to low titre. The H and O suspensions of strains 001 and L 239 were agglutinated only by the homologous sera, each by the serum of the other strain, by *Bact. typhosum* and very slightly by J.M. antiserum.

Complete cross-absorption tests appeared to show that these strains were antigenically identical with *Bact. typhosum.* It is to be noted, however, that, after absorption of the serum in 1/50 dilution by a thick suspension in equal quantity, tests could only be set up at an initial dilution of 1/200 so that a small amount of some other agglutinin might not be detectable.

As regards pathogenicity, strain 001 in a dose of 1,000,000 visible organisms killed all of 5 mice in 8 days when inoculated intraperitoneally, the organisms being recovered from spleen and heart blood. With strain L 239, 2 of the 5 mice survived in a similar experiment.

Flavobacterium orchitidis (Sherwood, Edwin and Marts, 1933)

In order to determine whether any known strain in the National Collection of Type Cultures corresponded in its characters with *Bact. typhi flavum*, a request was made for any Gram-negative organisms producing a yellow pigment. A number were sent, but only one—*F. orchitidis*—behaved in a manner resembling the organism under investigation. It was a briskly motile Gram-negative rod forming similar colonies on agar at 22° C. but without the aggregated masses. It produced acid only in glucose, maltose, mannite, sucrose and salicin, but H and O suspensions were not agglutinated by any of the *Bact. typhi flavum* antisera.

This organism was described as being isolated from the cerebrospinal fluid of a case of meningitis.

Isolation of new strains

It remained to be determined whether organisms of this type existed as saprophytes in the locality—this important question was rapidly settled.

Owing to rapid overgrowth by other organisms in fluid culture, isolation was attempted directly on agar plates. Plates were left open for ½ hour in the laboratory and in other rooms not devoted to bacteriological work. Further, various substances were shaken over plates. These were incubated at 22° C. for 3 days, any likely colonies picked off, purified by replating and studied. Four strains were easily isolated at the first attempt, (1) from the air of the laboratory, (2) from the animal house, (3) from oats used for feeding, (4) from dried grass from a suburban garden. These organisms appeared morphologically and behaved culturally exactly as the strains from the Continent. They showed the "whetstone-forms" and aggregations, they produced acid in the same carbohydrates and were agglutinated by a varying number of the *Bact. typhi flavum* antisera when set up in a dilution of 1/50.

It was deemed unnecessary to search further for strains of such organisms, particularly since Castro, in a recent paper (1934*a*), records the isolation in 125 attempts of 76 strains from practically all the common vegetables and fruits, from leaves, flowers and trees of many kinds, from practically all samples of hay, from cream, butter, cheese, and from the faeces of dogs. This he did, using mostly glycerine agar on which the colonies are easily recognisable, in the town of Hamburg and the country round about. In this he was but confirming and extending the observations of Seydel, who got strains easily from the air, of Hirsch and other workers.

Discussion

Prior to the work of Stickl, organisms producing yellow pigment had not infrequently been noted, as for instance by Boite (1905) and by Löffler (1906). Köhlisch (1916) and van Hövel (1915) isolated them from stools and urine of typhoid cases and suspects, the latter worker being of opinion that they were accidental and without pathological significance. Eisenberg (1918) described

similar organisms which he regarded as variants of *Bact. coli*, whereas Baumgärtel (1918), who isolated 26 strains during an epidemic of Paratyphoid A infection, thought they were a variant of that organism.

Certain facts emerging from the experimental evidence are beyond dispute:

(1) There exists a group of Gram-negative organisms producing a yellow pigment, with certain fermentative and cultural characters sufficiently distinct and constant to merit a specific name.

(2) Though a species not homogeneous antigenically nor capable of any simple serological grouping, the organisms are related in such a manner that a serum prepared against one will usually agglutinate several other members of the species.

(3) The species exists very commonly as a saprophyte in air, in vegetables and in animal materials.

(4) The species differs from *Bact. typhosum* chiefly in the formation of pigment, the production of acid in sucrose and liquefaction of gelatin. Some strains have a slight antigenic relation to *Bact. typhosum*—so slight that reciprocity is not shown in serological tests.

(5) There is no evidence of pathogenicity except in enormous doses, in which even such organisms as *Chr. prodigiosum* have proved fatal to laboratory animals. Large doses have been swallowed by man without ill-effect.

(6) While they have been isolated from the excreta of typhoid contacts and suspects, they have likewise been frequently found in the excreta of normal individuals. It may be noted that the sera of sick and of healthy individuals have been reported as agglutinating strains of *Bact. typhi flavum* (Dresel and Stickl, 1930; Seydel, 1934).

(7) Some of the strains alleged to have been derived by subculture correspond culturally, biochemically and serologically to *Bact. typhosum.*

Clearly, then, the organism, described as being isolated during the Greifswald epidemic and elsewhere from excreta and blood of typhoid suspects and contacts, is indistinguishable from a ubiquitous saprophyte, so that failure to find it frequently in routine examinations or occasionally as an aerial contaminant in blood culture would be more surprising than otherwise.

The assertion of Dresel and Stickl (1930) that a titre of 1/100 against *Bact. typhi flavum* in the serum of individuals has a diagnostic significance with regard to enteric infection seems incompatible with the observation that *Bact. typhi flavum* possesses none of the characteristic antigens of *Bact. typhosum* or of any other member of the enteric group. Further, it may be noted that the species is itself antigenically heterogeneous and that we have no definite knowledge of the frequency or titre of normal agglutinins acting on this organism. Much of the work of Seydel, although she recognises that there is no antigenic unity, is rendered of little value for a similar reason—agglutination of *Salmonella* suspensions is tested by a single *Bact. typhi flavum* antiserum, and the percentage of sera from enteric cases agglutinating *Bact. typhi flavum* is recorded after testing them on 2 strains of the latter organism.

The alleged transformation of *Bact. typhi flavum* into *Bact. typhosum* has, in the hands of the writer, not been confirmed, and a general survey of the literature suggests that this unlikely variation has not been satisfactorily proved. A number of the strains concerned were isolated from sources suspected of enteric infection or contact, and an original mixed culture may have existed. While the occurrence of *Bact. typhosum* as a fortuitous contaminant is very improbable, in the long series of subcultures performed, mistakes may have occurred. Methods of single-cell culture are open to technical error in view of their difficulty, and Burri's method has been criticised on the grounds of uncertainty that a single cell has been obtained owing to optical defects of the method. Holman and Carson (1935) in a recent paper dealing with a similar problem in bacterial dissociation point out a number of possible sources of error which may arise in such work.

No satisfactory evidence of any relation between the isolation of *Bact. typhi flavum* and epidemiological findings as regards enteric infection has been brought forward. It would appear that the so-called *Bact. typhi flavum* is a saprophyte; that it may reach excreta by the mouth in foodstuffs or as an aerial contaminant, or the blood with or without breakdown of the intestinal barrier as other saprophytes may do (Conradi, 1909); and that its transformation into *Bact. typhosum* is not proven.

There have been described very many organisms closely resembling those dealt with in the present work. The strains studied differ, if at all, only in minor details such as shades of pigment, from such organisms as *Chr. aquatile* (*Bacillus aquatilis*) of the Franklands (1889), *Bac. flavus* (Fuhrmann, 1907) and many others, including a number of the formidable list (68 in all) of species of *Flavobacterium* of Bergey (1934). In any event it would appear desirable to discard the word "typhi", and, as "Bacterium" is reserved for the non-pigmented group of typically intestinal parasites—the coli-typhoid group—this organism, or this group of organisms, would seem to fall more naturally into the genus *Chromobacterium*.

Summary and conclusions

1. The results of a study of 19 strains of the so-called *Bacterium typhi flavum* are given.

2. It is a Gram-negative rod, motile at 22° C., producing yellow colonies on agar and capable of fermenting a range of carbohydrates, but not lactose, with production of acid but no gas. It is not an antigenically homogeneous species.

3. The organism is widespread, especially in air, dust and plants, and thus may be isolated from various animal sources.

4. Prolonged subculture on agar failed to produce confirmation of the claims of certain workers to have observed the appearance of *Bact. typhosum* as variants of *Bact. typhi flavum*.

5. It is suggested that any relation of this organism to enteric infections is unproven and that its appropriate place is in the genus *Chromobacterium*.

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