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Further Studies on the Antigenic Structure of Paratyphoid Bacilli with Special Reference to Three New Species of Salmonella.

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SINCE the establishment of a National Salmonella Centre at Onderstepoort in 1939, the authors, who are working in laboratories widely separated in different parts of the country, have been collaborating in the study of the antigenic structure of different organisms obtained from infections in both man and animals. It is our intention to discuss these results below. For the serological work we have employed the technique described by Henning (1939).

Although sporadic as well as epizootic salmonella infections are very common in domestic animals and birds in South Africa (Henning, 1939; Henning and Haig, 1939), not many outbreaks, other than typhoid fever, have been recorded in man. It is true that Greenfield and Judd (1936), and Henning and Greenfield (1937), have described an outbreak of meat-poisoning due to pork infected with *S. bovis-morbificans* and that Henning (1938), recorded a septicaemic disease in a group of natives who had partaken of calf's meat infected with *S. dublin*, while Buchanan and Bevan (1939), reported an outbraek of food-poisoning affecting the passengers of a train in which *S. typhi murium* contaminated ice was incriminated as the cause; but these are probably only a few of the outbreaks that have occurred in South Africa during the last ten years—there are no doubt a number of others that have not been properly recorded.

One of us (W.F.R.) was concerned with the study of 6 outbreaks of food-poisoning that have not been previously recorded; a common feature of most of these outbreaks is the presence of raw eggs (probably duck's eggs) as an ingredient of the food:—

1. In January, 1928, eight persons became sick after they had partaken of a honeycomb mould, made from gelatin, milk and eggs. From one of the patients who died and also from the mould, an organism, probably of the *enteritidis* group, was isolated.

2. In November, 1932, twenty children in a school hostel were involved in an outbreak of food poisoning. A Salmonella, apparently related to *paratyphi A*, was isolated from several of the patients, but the source of of the infection remained obscure.

3. In April, 1934, eight out of nine people living in the same house became sick. All eight had partaken of a honeycomb sponge made of eggs, milk and table jelly, but the ninth person did not. All the ingredients of the pudding were cooked except the white of the eggs which was used raw. A Salmonella, which probably falls in group B of the Kauffmann-White Schema, was isolated from some of the patients and from the remains of the honeycomb sponge.

4. In October, 1925, fifteen people developed food poisoning after they had partaken of ice-cream in which one of the ingredients was raw eggs. No suspecting organism was isolated.

5. In March, 1936, an organism, probably related to *S. typhi-murium*, was isolated from one of a number of sick soldiers, but an examination of the food partaken proved to be negative.

6. In May, 1936, another outbreak occurred in which an organism apparently related to *S. typhi-murium* was isolated from one of a number of patients suffering from food poisoning. The incriminating food was custard, but a bacteriological examination proved to be negative.

In man the disease usually assumes the form of a continued fever, gastro-enteritis or a septicaemia. It is commonly referred to as "food-poisoning" or "meat-poisoning" and in most cases the source of infection is undoubtedly of animal origin. Dack (1940), however, has pointed out that the word "meat-poisoning" is a misnomer; heat-killed cultures of *S. typhi murium* fed to rhesus monkeys failed to produce symptoms of paratyphoid, whereas living cultures of the same organism caused gastro-enteritis. Heat-killed broth cultures or filtrates of virulent *S. typhi murium* and *S. enteritidis* when fed to adult human volunteers failed to produce symptoms of disease. Moreover, custard infected with *enteritidis* produced typical symptoms of "food-poisoning", but heated suspension of the organisms grown in custard or the filtrates of unheated cultures proved to be harmless to volunteers.

Outbreaks of Salmonella "food-poisoning" in adults, or more appropriately "food-infection" has received so much attention during recent years that a further review of the literature in this paper is regarded as superfluous.

In infants as in young animals Salmonella infection is usually much more serious than in adults. According to Savage (1913), Smith (1921, 1933, 1934), Smith and Scott (1930), Bahrenburg and Ecker (1937), Guthrie and Montgomery (1939) and others the infection commonly assumes a septicaemic form and is usually characterised by symptoms of meningitis, enteritis and sometimes cholecystitis. Twenty-seven of the twenty-eight cases reported by Guthrie and Montgomery (1939), occurred in children under 2 years. Bahrenburg and Ecker (1937), described a case of Salmonella meningitis in a 7-week old child and cited a list of 33 outbreaks of the same condition in children reported by other workers. Investigators like Smith, Smith and Scott, Guthrie and Montgomery, Bahrenburg and Ecker performed reliable serological tests for the determination of the identity of the causal organism, but in a number of other outbreaks similar tests were not carried out so that it was not always certain which bacterium could actually be incriminated.

More recently a very severe outbreak of infantile Salmonella meningitis was described by Schiff and Saphra (1941). The outbreak occurred at a Maternity Hospital in Havana where it was studied by Curbelo. Only new-born babies with an average age of 11 days were affected. There were 21 deaths in the hospital and a number outside. Salmonellas were isolated from the spinal fluid and in the blood and stools in a few cases.

Smith (1933, 1934), and Smith and Scott (1930), believe that *S. dublin* has a special predilection for the meninges and that milk is the common vehicle of infection. As this organism is the cause of calf-paratyphoid which is one of the most serious diseases of calves in South Africa (Henning, 1939), milk contamination may occur at any time on infected premises. Kinlock, Taylor and Smith (1926), report a serious outbreak of *enteritidis* infection involving 497 persons in which milk was incriminated as the cause, and the source of the infection was actually traced to a single cow with an indurated udder which later developed septicaemia. Another milk-borne epidemic, affecting 19 persons, recorded by McAllan and Howie (1931), was also traced to one cow. Milk was again incriminated in an outbreak of *dublin* infection described by Conybeare and Thornton (1939), and the organism was isolated from a single cow. A few years ago the attention of one of us (M. W. H.) was directed to a fatal case of Salmonella meningitis in an 18 months old child that occurred on a farm which was known to be badly infected with calf-paratyphoid; the child became infected soon after its diet was changed over from boilt to raw milk. Although the Salmonella isolated was not available for species determination the evidence strongly points to *dublin* infection.

Bourmer and Doetsch (1928), mention a case of paratyphoid caused by the milk of a cow that had to be slaughtered in emergency and they describe an outbreak involving over 80 people who had partaken of some cheese prepared from the milk of a cow which, although apparently healthy, was discharging *S. enteritidis* with her faeces. By carefully examining the milk of two cows that were discharging *enteritidis* with their faeces Standfuss and Wilken (1933), were unable to obtain the organisms from any sample of milk studied. They concluded, therefore, that the presence of paratyphoid organisms in milk should be attributed to contamination.

The description of a case of Salmonella meningitis in an infant by us is apparently the first record of this condition affecting children in South Africa. Moreover, the organism incriminated has not been previously described.

A case of sporadic Salmonella infection in a native woman suffering from a severe attack of dysentery is also described. The responsible organism too was found to be a new species.

Two Salmonellas obtained directly from a pig are described; both were isolated from a pig obviously affected with paratyphoid. Of these the one, *S. heidelberg,* is well-known, while the other one was found to be a new species that had not been described before.

1. SALMONELLA DURBAN.—This organism was isolated by one of us (J. G. J.) from the stools of a native woman at Durban. The woman was admitted to King Edward VIII Hospital on June 8th, 1939, and discharged a month later. On admission she was suffering from severe abdominal pain with the abdomen diffusely tender. There were several motions daily with mucus, blood and pus in the stools, but there was no vomiting. A provisional diagnosis of amoebic dysentery was made, but no parasites could be found. The patient was treated with emetine in spite of the apparent absence of amoebae and the diarrhoea continued for a long time after the administration of emetine was discontinued. A culture made from the stools yielded a Gram-negative, non-lactose fermenting bacterium which showed all the characteristics of a Salmonella.

When the organism was tested serologically it was found to react with the sera of a number of unrelated strains. As the arrangement of its antigenic components did not comply with that of any Salmonella previously described it was raised to species rank and given the name of *Salmonella durban.* For example, *durban* was agglutinated by " O " sera of types falling under group D of the Kauffmann-White schema containing factor IX and also by " H " sera containing factors *a, en, enx* and *eh.* When single colonies of 24 hours' growth were tested it was found that some agglutinated with sera containing factor *a* while others were flocculated only by sera containing either factors *en, enx* or *eh.* It was apparent, therefore, that the organism existed in the α-β phase variation of Kauffman and Mitsui (1934) or the 1-2 phase variation of the International Association of Microbiology (Proceedings 1940).

Absorption tests showed that *paratyphi-A* removed practically all the α-phase (factor-*a*) from *durban* reducing its titre from 6,400 to 200, without altering the titre for its β-phase (factor *enx*), while *abortus-equi* caused a decrease in the β-phase titre from 6,400 to 400 or less, leaving the α-phase agglutinins undisturbed. The β-phase (*en*-factor) of *glostrup* and *mikawashima,* although also reducing the β-phase titre of *durban,* did not absorb as much of the β-phase as *abortus-equi.* A combination of *abortus-equi* and *paratyphi-A* removed practically all the α and β-phase agglutinins from *durban* serum. *Oslo,* α and β-phases, also exhausted nearly all the " H " agglutinins from *durban* serum.

On the other hand, the β-phase of *durban* reduced the " H " titre of *abortus-equi* serum containing factor *enx,* from 12,800 to 400, and the α-phase of *durban* lowered the " H " titre of paratyphi-*A* serum from 25,600 to 400, but the β-phase of *durban* barely lowered the β-phase of *glostrup* serum, containing factor *en*, from 6,400 to 800. It would appear, therefore, that the β-phase of *durban* is more closely related to *abortus-equi* than to *glostrup*. Moreover, it was found that *enteritidis* var. *dublin* completely exhausted the " O " serum of *durban*, while *durban* removed all "O " agglutinins from *dublin* serum.

According to these results it is clear that the " O " antigens of *durban* and *dublin* are identical and that the " H " antigen of *durban* occurs in the α-β phase variation, some colonies resembling the " H " antigen of *paratyphi-A* while others were similar to that of *abortus-equi*. Hence the antigenic structure of IX, ——*a*, *enx* was assigned to *durban*.

It has often been observed by us that, when a diphasic bacterium is absorbed with a similar phase of an organism occurring in another group or vice versa, a fair residue of the homologous agglutinins remains behind in the absorbed serum. The same phenomenon was noticed in the absorption tests with *durban; paratyphi-A* could not remove all the α-agglutinins from *durban* serum and *ebortus-equi* merely reduced the β-titre of *durban* from 6,400 to 200. The α and β phases of *durban* respectively also left significant residues of unabsorbed agglutinins in absorbed " H " sera of *paratyphi-A* and *abortus-equi*.

2. SALMONELLA KAAPSTAD.—This organism was isolated by one of us (W. F. R.) from a child at Cape Town. Fourteen days before admission to hospital the child was suffering slight intestinal trouble. It was admitted to hospital on September 21st, 1939, and 48 hours later suffered from loss of appetite, neck rigidity and restlessness. It died on the sixth day. Cerebro-spinal fever was suspected and three specimens of spinal fluid were taken. All three specimens were slightly turbid on arrival at the laboratory and showed Gram-negative bacilli in the centrifuged deposits. Cultivation on MacConkey's bile-salt agar yielded numerous colonies of non-lactose fermenting, Gram-negative, motile bacilli that gave biochemical tests which were regarded as characteristic for Salmonellas. Agglutination tests were performed with the organism against the sera of the patient and of its mother and father. The results were all negative.

Preliminary serological tests showed that the organism was a Salmonella which falls under group B of the Kauffmann-White schema and that it was diphasic. Agglutination and absorption tests showed that its " O " and type antigens resembled those of *reading* but that its group antigen was different. The organism should, therefore, be regarded either as a variant of *Salmonella reading* or as a new species. It was named *Salmonella reading* var. *kaapstad* or *S. kaapstad*. The " O " antigen resembled that of *copenhagen*. *Copenhagen* removed all the " O " agglutinins from *kaapstad* serum and *kaapstad*, while completely exhausting the somatic agglutinins *from copenhagen* serum, merely reduced the " O " titre of *typhimurium* serum from 800 to 400. Factor IV, the " O " factor of *copenhagen*, was therefore assigned to *kaapstad*.

The specific phase of *kaapstad* agglutinated with sera containing factors *eh, en, enx.* Organisms containing specific factors *eh* (*eastbourne, kottbus, selandia, reading*), removed all the specific agglutinins from *Kaapstad*, while *kaapstad* exhausted the type agglutinins (factors *eh*) from *eastbourne, selandia* and *reading.* The non-specific phase of *kaapstad* barely reduced the group titre of *reading* (factors 1, 2) from 3,200 to 1,600 and *reading* caused no significant reduction of the group agglutinins of *kaapstad.* On the other hand, the non-specific phase of *kaapstad* exhausted the group agglutinins from *selandia* (factors 1, 7) and *new brunswick* (factors 1, 7) while *selandia* and *new brunswick* removed the non-specific agglutinins from *kaapstad* serum. Moreover, a combination of *eastbourne* (containing factors *eh*) and *new brunswick* group phase (containing factors 1, 7) completely exhausted the " H " agglutinins from *kaapstad*, while reciprocal absorptions between *kaapstad* and *selandia* (*eh*; 1, 7) caused the removal of all the " H " agglutinins from both the sera. The non-specific phase of *kottbus* (factors 1, 5) and *cholera suis* (factors 1, 5) also failed to lower the group titre of *kaapstad* more than from 6,400 to 3,200. It is evident, therefore, that *kaapstad* exhibits an arrangement of antigenic components that have not yet been described hitherto, viz. IV—*eh*: 1, 7.

3. SALMONELLA HEIDELBERG and SALMONELLA PRETORIA.—This was a mixed infection. A farmer near Premier Mine in the Pretoria District bought a number of pigs on a sale. The pigs were fed on garbage from the military camp nearby and a number of them died from symptoms that alarmed the owner. One of the pigs was brought to Onderstepoort for investigation. A post-mortem was performed and a tentative diagnosis of paratyphoid was made. Cultures were made by one of us (M. W. H.) from the liver and spleen in tetrathionate broth and on MacConkey's bile-salt agar. A number of non-lactose fermenting colonies appeared on the MacConkey and preliminary testing gave a characteristic reaction for Salmonellas. But it was noticed that all the colonies did not react with the same sera. Some reacted with sera of group B of the Kauffman-White schema, while others were flocculated by sera of other Salmonella types that were included in different groups. The two organisms were labelled culture 810 and culture 811.

Culture 811 proved to be diphasic, falling under Group B of the Kauffmann-White schema. Agglutination and absorption tests carried out showed that its " O " antigen was composed of factors IV, V and its " H " antigen of factors *r*; 1, 2. It, therefore, resembled *Heidelberg.* Moreover, reciprocal absorption tests were now carried out with *heidelberg* and culture 811. These proved that culture 811 completely exhausted *heidelberg serum* and that *heidelberg* removed all " O ", type and group agglutinins from 811 serum.

Culture 810 was also found to be diphasic, but, as the arrangement of its antigenic components did not resemble that of any other known Salmonella, it was decided to raise this organism also to species rank. In compliance with the accepted procedure culture 810 was named *Salmonella pretoria.*

Agglutination and absorption tests showed that the "O" antigen of *pretoria* resembled that of *aberdeen*, and that its diphasic "H" antigen partly resembled *zanzibar*; while the type antigen of *zanzibar* was agglutinated to full titre by *pretoria* serum the group antigen was flocculated only up to part of the group titre.

The specific phase of *pretoria* removed all the specific agglutinins from *zanzibar* serum, but the group phase barely reduced the non-specific titre of *zanzibar* from 6,400 to 3,200. On the other hand, while the type phase of *zanzibar* exhausted all the type agglutinins from *pretoria* its group phase merely lowered the group titre of *pretoria* from 6,400 to 1,600. Moreover, a purely specific *thompson* absorbed all the specific agglutinins from *pretoria* serum and the non-specific phase of *newport*, viz., var. *puerto-rico*, removed all the group agglutinins from *pretoria* serum, whereas the specific phase of *pretoria* completely exhausted the "H" agglutinins from *thompson* (specific) serum. When *newport* var. *puerto-rico* serum was absorbed with *pretoria* all the group agglutinins were exhausted.

On studying the "O" antigen it was found that *aberdeen* removed all the "O" agglutinins from *pretoria* serum and that *pretoria* exhausted the somatic agglutinins from *aberdeen* serum.

These results showed that the somatic antigen of *pretoria* resembled that of *aberdeen*, its specific "H" antigen that of *thompson* and *zanzibar* and its non-specific antigen that of *puerto-rico*. The following antigenic components have therefore, been assigned to *pretoria*:— XI—k: 1, 2.

SUMMARY.

Three new species of Salmonella are described.

1. *S. durban* was obtained from an adult native woman suffering from dysentery. The antigenic formula allocated to it was IX—*a*, *enx*.

2. *S. reading* var. *kaapstad* was isolated from the cerebro-spinal fluid of a child suffering from meningitis. Its antigenic structure was found to be IV—*eh*: 1, 7.

3. Two unrelated organisms were isolated from one of a group of pigs affected with paratyphoid. The one turned out to be *S. heidelberg*, factors IV, V—r: 1. 2, while the other was found to be a new species with the following antigenic structure:— XI—k: 1, 2. The latter was named *S. pretoria*.

4. The biochemical reaction of these organisms are given in Table 1.

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TABLE 1.

The Biochemical Reactions of Various Organisms Studied.

Culture.	[1]Bitter Weigh-mann & Habs.	[2]Jordan's Tartrate Medium.	[3]Stern's Fuchsin Broth.	Glu-cose.	Lac-tose.	Dul-cite.	Saccha rose.	Man-nite.	Mal-tose.	Arabi-nose.	Rham-nose.	Ino-site.	Xy-lose.	Sor-bite.	Indol.	H_2S.
S. kaapstad No. 1013......	+	+	++++	+	−	+	−	+	+	+	+	+	+	+	−	+
S. reading K. 19.........	−	+++	±	+	−	+	−	+	+	−	−	+	+	+	−	+
S. durban No. 717........	+	+	++++	+	−	+	−	+	+	+	+	−	+	+	−	+
S. pretoria No. 810.......	+	+	++++	+	−	+	−	+	+	+	+	+	+	+	−	+
S. heidelberg No. 811......	+	+++	++++	+	−	+	−	+	+	+	+	−	+	+	−	+
S. heidelberg K. 16.......	+	+	+++	+	−	+	−	+	+	+	+	+	+	+	−	+
S. aberdeen K. 90.........	+	+	++++	+	−	+	−	+	+	−	+	−	+	+	−	+
S. zanzibar K. 82........	+	+	+	+	−	+	−	+	+	−	+	−	+	+	−	+
S. thompson specific.......	±	+	++++	+	−	+	−	+	+	−	+	+	+	+	−	+

(1) *Munch. Med. Wschr.* 73, 940–941, 1926.
(2) *Jl. Inf. Dis.* 42, 238–241, 1928.
(3) *Z. bl. Bakt.* 78, 481–492, 1916.

In columns 2 and 3, +, ++, +++, ++++ = degrees of reactions (all positive).
+ = Positive reaction.
± = Incomplete reaction.
− = Negative reaction.

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