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The Antigenic Structure of Salmonellas obtained from Domestic Animals and Birds in South Africa.

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As far as possible an attempt has been made to present the results in the form of tables. These are given on the following pages: —

INTRODUCTION.

The significant part played by *Salmonella* infection in both man and animal and the frequency with which members of this group of organisms have been associated with outbreaks of food-poisoning in man, have led to a detailed study of their antigenic components during recent years. With the advent of reliable methods of serological analysis it has become possible to recognise several new strains of *Salmonella* and to subdivide a number of older forms into distinct types in cases where groups of these were previously grouped in a haphazard fashion under one name on either clinical, zoological or cultural grounds. Where pioneer workers had to rely largely or solely on the fermentation reactions of the types for a differentiation of the groups, a description of the organism at present can be accepted only if based on reliable serological work, involving the complete antigenic analysis of the bacterium.

Schutze (1920) pointed out the futility of grouping *Salmonellas* on clinical and zoological grounds, and showed the value of serological methods of classification. By means of absorption tests he was able to divide the organisms included in the *Aertryeke* group into a number of types. Bruce White (1926, 1929 a.b.), by adopting Schutze's types as a primary basis for study, unified the *Salmonella* taxonomy by comparison of such representative strains as he could find available. He identified these various strains and introduced a system of labelling for their different antigenic components. Kauffmann (1929 a.b., 1930 a.b.c., 1931, 1934, 1935 a.b.c., 1937) continued and extended the work initiated by Bruce White, but used a different system of labelling. Lovell (1932 a) correlated the formulae presented by these two workers by giving the equivalent numbers and letters used in the two systems. In order to obviate the confusion that was bound to occur from the existence of two separate systems of antigenic labelling the *Salmonella* Sub-committee of the International Society of Microbiology (1934) adopted Kauffmann's terminology for general use.

In the study of the specific-phase—non-specific-phase variation of Andrewes (1922, 1925) the presence of two well-defined, but mutually convertible, types of organisms was recognised within the limits of a species. This phenomenon explained several of the factors concerned with the cross-agglutinations observed in a number of different types of *Salmonella*. But investigations on the antigenic structure of bacteria were actually commenced by Smith and Reagh (1903) when they studied motile and non-motile strains of the hog-cholera bacillus. They were the first to describe flagellar and somatic agglutination as two distinct processes and to show that the same organism may contain two agglutinable substances, which have the property of producing two corresponding agglutinins in animals. They found that animals inoculated with motile strains yielded a serum which agglutinated the homologous motile organisms at a dilution of over 1:10,000 but barely affected the non-motile organisms at a 1:500 dilution. Sera prepared against non-motile forms had a titre of only 100 to 500 for both motile and non-motile strains. They recognised two types of agglutination, (1) large, loose, rapidly-appearing flocculent clumps of flagellated (motile) organisms, and (2) small, compact, dense, slowly-forming (somatic) granules of non-motile organisms. They associated the agglutinins in the sera prepared with the non-motile organisms (somatic antigen) with the bodies of the bacilli and not with the flagella. On absorbing the sera made with the motile strain with non-motile bacteria, the somatic agglutinins alone were removed, the flagellar agglutinins remaining behind.

About the same time Joos (1903) described two kinds of agglutinogen and two corresponding agglutinins in *S. typhi*. He also observed two forms of clumping associated with two different agglutinogens, apparently corresponding to the flagellar and somatic agglutination of Smith and Reagh. Moreover, Joos noticed that heating at 60° to 62° C. destroyed the antigen responsible for the large loose floccules but not the flagellar agglutinins, while this temperature had no effect on the antigen forming the small granules, but destroyed the agglutinin produced by it. Soon afterwards Beyer and Reagh (1904), also working with the hog-cholera bacillus, found that the flagellar agglutinable substance was greatly damaged by heating at 70° C. for more than 20 minutes, while the somatic substance was not affected; but the heating did not destroy the agglutinogenic property of the flagellar substance. Moreover, these workers showed that heating at 70° C. destroyed the somatic but not the flagellar agglutinins.

The importance of these findings was not fully realised until Weil and Felix (1917) observed that variation in the growth characters of *Proteus* X19 was associated with very striking serological differences. The one variant, termed by them the "H" (Hauch) form, grew as a spreading film on ager and gave rise to a marked, loose flocculer agglutination with its own serum; while the other variant, the "O" (ohne Hauch) form, grew as circular clumps with is own serum. They called the agglutinable substance present in the "O" form, "O" receptors and the material responsible for the large floccules of the "H" forms, "H" receptors. They showed that the "H" forms contained both receptors,

while the " O " forms contained only the " O " receptor. Sera of rabbits inoculated with the " H " variant of *Proteus X*19 contained agglutinins for both " H " and " O " forms, while rabbits injected with the " O " variant, produced agglutinins for the " O " form alone. When the " O " variant was heated at 100° C. or exposed to dilute acids or to pure alcohol its agglutinative power remained unaltered, but when the " H " form was similarly treated or grown on phenol-agar it lost its power of agglutinating in large, loose floccules but retained the property of forming small granules.

These results showed the complete analogy between the motile and non-motile forms of the hog-cholera bacillus described by Smith and Reagh and the " H " and " O " forms of Weil and Felix. Soon afterwards Braun and Schaeffer (1919) demonstrated that the " H " antigen occurs only in cultures of motile organisms, while the " O " antigen is present in both motile and non-motile cultures.

Later Weil and Felix (1920) demonstrated the presence of similar antigens in organisms of the typhoid-paratyphoid group, an observation subsequently confirmed by Gruschka (1923), Schiff (1923), Bruce White (1925) and others. Bruce White (1926) advised the use of the term " H " antigen for the labile, flocculating flagellar form, and the term " O " antigen for the stable granular form; the corresponding agglutinins he referred to as " H " and " O " agglutinins respectively. It is now conventional to attach the label " H " to the heat-labile flagellar antigens, and the label " O " to the heat-stable somatic antigens.

A further advance with flagellar and somatic agglutination was made by Orcutt (1924a) when she confirmed the work of Smith and Reagh (1903) by using motile and non-motile strains of the hog-cholera bacillus derived from a single strain, originally motile. She employed a suspension of flagella as an agglutinogen and, by using rabbits, produced a serum containing only flagellar but no somatic agglutinins. This serum agglutinated motile strains to a titre of 1:5,000, but failed to flocculate non-motile strains at 1:40, while antisera prepared with the washed bodies agglutinated both motile and non-motile strains up to 1:1,000. Orcutt (1924b) also found that heating the free flagella at 70° C. destroyed their agglutinating power without materially altering their agglutinogenic property. On the other hand neither heating at 70° C. nor at 120° C. destroyed the agglutinating and absorbing properties of the somatic antigen. The somatic agglutinins were partly destroyed at 70° C. and completely at 75° C.; but the flagellar agglutinins, although unaffected at 70° C., were partly impaired at 75° C. The work of Craigie (1931) on the distribution of the " H " and " O " antigens in the bacterial body confirmed the results obtained by Orcutt.

Weil, Felix and Mitzenmacher (1918), while working with typhoid and paratyphoid organisms, found both " H " and " O " agglutinins in the sera of patients as well as in the sera of rabbits inoculated with whole bacilli. When bacterial suspensions heated at 100° C. were inoculated into rabbits agglutinins were formed which caused small granular flocculation of the " O " forms. These observations were subsequently confirmed by Bruce White (1926).

Andrewes (1922) found that the same culture of a pure growth of a motile *Salmonella* often contained two sets of individual bacilli with entirely different "H" antigens, the one specific for the particular race, or for a few races, while the other had wide affinities for a whole group of allied races of *Salmonella*. By picking a number of single colonies from an agar plate he succeeded in separating these two variants, which he referred to as the *specific* and the *group* phases; but on sub-cultivation, especially in fluid media, he found that each of the two phases usually mutated rapidly into organisms of both types. For the purpose of examining this phenomenon Andrewes (1925) advised the use of *specific* and *group* sera, prepared by absorbing the agglutinins not required from a serum which contained both. White (1925) showed that these phases were concerned purely with changes in the flagellar antigen, the somatic antigen being the same in both phases. Scott (1926a) showed that a strain of *thompson* occurring in a quasi-group phase could be changed into a type (specific) phase. In order to suppress the excess of group antigen he grew the strain in a powerful group serum, viz. media containing 15 parts nutrient broth and 1 part of a strong group serum. After 24 hours the supernatant fluid in the tube became clear, while a thick deposit collected at the bottom. After centrifuging the culture, another tube with group-serum-broth was inoculated and a drop was plated for individual colonies. The procedure was repeated after every 6 hours, plating a drop at each time. After a few passages a pure culture with a new phase was obtained and the deposit was no longer formed in the tube. By employing Wassén's (1935) modification of Scott's method Bruner and Edwards (1939 *a* and *b*) and Edwards and Bruner (1939) were able to demonstrate additional phases in a number of organisms that were previously regarded as monophasic.

Schutze (1922), Bruce White (1925, 1926, 1929), Kauffmann (1929a, 1930a,b,c 1935a, 1935b, 1935c, 1937 etc.) and others have pointed out that the somatic as well as the flagellar antigen of *Salmonellas* may be multiple, the somatic antigen being generally regarded as the connecting link between different races of species.

Ficker (1903) and Dreyer (1909) used broth cultures extensively as agglutinating suspensions for routine diagnosis. But the agglutination obtained should be regarded as an "H"-agglutination because liquid cultures generally contain bacteria which are more motile and better supplied with flagella for "H"-agglutination than solid cultures. Moreover, Dreyer advised the use of dead cultures killed by the addition of 0·1 per cent. formalin and exposure at 37° C. for some days. Pyper (1923), on the other hand, found that bacterial suspensions containing formalin are unsuitable for purposes of routine diagnosis—he succeeded in detecting many more positive cases of typhoid fever with the complement fixation test than with a Widal test in which he was using formalised suspensions. Later Felix and Olitsky (1928) showed that for somatic agglutination the antigen must be kept free from formalin and carbolic acid as either of these inhibit somatic agglutination in the presence of "H"-antigen. Thus, by using a formalised antigen for his test,

Pyper succeeded in detecting only those cases in which the serum contained " H " agglutinins. Those cases containing " O " agglutinins, but no " H ", failed to react.

Bien and Sonntag (1917) succeeded in killing the motile forms and in destroying the flagella by heating the organisms in 30 per cent. alcohol at 37° C.: thus leaving an almost pure " O " suspension. Braun and Schaeffer (1919) found that the addition of 0·1 per cent. phenol to agar suppressed the development of flagella and, therefore, the production of the corresponding labile antigen.

At present the agglutination test is generally regarded as a very reliable aid to the recognition and classification of pathogenic bacteria. For classification both somatic and flagellar antigens must be employed, although these are not equally important in all families. In *Bacillus proteus*, for example, the flagellar antigen can be used for distinguishing large groups, while the somatic antigen is far more specialised. In Salmonellas, on the other hand, some of the somatic antigens are very widely distributed in the group, while the flagellar antigen is much more specific (Weil and Felix, 1920, Bruce White 1926).

Both Schutze (1922) and Bruce White (1925, 1926) maintain that no reliable and safe antigenic relationship can be arrived at by means of absorption tests, unless cross-absorption and cross-agglutination methods are carried out with both strains of bacteria and their sera employed in the test, i.e., unless the complete mirror test is performed. Bruce White obtained a number of so-called " Schottmuller " strains, isolated from calves and described by Christiansen (1914) as *Paratyphus-B* (Schottmuller). By means of proper absorption tests Bruce White (1926) found these organisms to be typical *S. typhi-murium*.

The absorption of agglutinins from sera was first employed by Bordet (1899), Eisenberg and Valk (1902), Castellani (1902) and Joos (1903). Subsequently this method was extensively used by several workers for the antigenic analysis of different groups of bacteria. Boycott (1906) was one of the first workers to distinguish between *Paratyphosus B* and *Bact. Aertrycke* by its use; Bainbridge (1909) and O'Brien (1910) also adopted it for the differentiation of paratyphoid organisms, while Schutze (1920, 1922) and Bruce White (1925, 1926, 1929 a and b) made extensive use of absorption tests for the classification of *Salmonellas*. For the study of the antigenic analysis of bacteria absorption tests now play a most important and indispensable part. More detailed information on the subject of agglutination will be found in a review given by Arkwright (1931).

SALMONELLA INFECTION OF CALVES.

(1) Introduction.

In Europe, especially in Holland, Denmark and Germany, *Salmonella* infection in cattle has assumed considerable proportions in certain localities, where it tends to recur year after year in an enzootic form, causing very heavy losses among young stock. The incidence of *Salmonella* infection in adults is generally regarded as

sporadic. *Bact. enteritidis* of Gaertner is the organism commonly incriminated as the cause of calf mortality, but as this labelling frequently includes a number of closely allied serological types most of the records referring to it are incomplete and unreliable. Moreover, the members of this group cause disease in man as well as in animals, but it is seldom possible to recognise the exact type of organism involved, as a reliable antigenic description of the organism is hardly ever available. When Smith and Scott (1930) studied some of the organisms isolated from cases of calf diarrhoea and labelled *Bact. enteritidis Gaertner*, they found that these belonged to the *dublin* type.

According to Jensen (1913) a form of calf diarrhoea (Kälberruhr) has been known in Europe for more than a century. Obich (1865) was probably the first to regard the disease as infectious, but it was left to Franck (1876) to prove it. The first bacteriological study, however, was made by Jensen (1891) when he investigated a serious outbreak of Kälberruhr in Denmark; but he could not completely distinguish the bacteria obtained from the normal intestinal contents of healthy calves from those of Kälberruhr. Later Thomassen (1897) described an outbreak of calf diarrhoea in Holland associated with a bacteraemia and caused by organisms which were called " pseudotyphoid bacilli ". Soon afterwards Poels (1899) studied a disease in calves which he ascribed to *pseudocolibacilli*. He distinguished *pseudocolibacilli* from ordinary virulent *B. coli* by virtue of their higher virulence for small animals, their greater motility and their inability to ferment lactose. Later Jensen (1903, 1913) described diseases in young cattle and in calves under the term " paracolibacillosis ". The organisms recovered from the tissues of affected animals fermented glucose, dulcite, mannite, maltose, xylose, rhamnose and sorbite, but not lactose or saccharose. Jensen stated that cases of meat-poisoning as well as some outbreaks of paratyphoid in man could be traced to the consumption of the meat of calves suffering from this disease. He divided the organisms into three serological groups (1) those which correspond to Gaertner's bacillus and which comprise the majority of strains, (2) those which resemble *paratyphi-B* and (3) a few strains which resemble neither Gaertner nor *paratyphi-B*.

Mohler and Buckley (1902) reported a spontaneous enzootic in cattle due to a bacillus of the *enteritidis* group. They obtained the causal organism in pure culture from the internal organs of affected animals. Schmitt (1908) isolated Gaertner-like bacilli from calves affected with septicaemia, diarrhoea and pneumonia. He regarded this disease (calf paratyphoid) as probably identical with *pseudobacillosis* of Poels and *paracolibacillosis* of Jensen. Soon afterwards Luxwolda (1913), Warnecke (1914) and Douma (1916) described cases of *enteritidis Gaertner* infection in Holland, Christiansen (1915) regarded *paracoli bacilli* as identical with bacteria of the *enteritidis-paratyphus-B* group. Meyer, Traum and Roadhouse (1916) investigated an outbreak of infectious diarrhoea among a group of hand-reared calves, from 1 to 4 days old. They incriminated as the cause *Bact. enteritidis* isolated from the blood and internal organs of the affected calves.

Miessner and Kohlstock (1812), Lutje (1926) and Lehr (1927) described outbreaks of paratyphoid disease in adult cattle. Two children became infected after receiving milk from a sick cow studied by Lutje. Lehr noticed that the agglutination titre of the sera of infected animals varied from 1:100 to 1:20,000; from the faeces of some animals, the sera of which had a titre of 1:100 to 1:200, he isolated *Gaertner bacilli* and he found the milk of a cow that excreted *Gaertner bacilli* in the faeces to be infected.

Sometimes there exists a definite relationship between the disease in adult cattle and calves. Bourmer and Doetsch (1928) described several cases of Gaertner infection in both cows and calves. A number of adult animals excreted the bacilli with the faeces, and the milk of one particular cow that had to be emergency slaughtered caused infection in man. They also described an outbreak of paratyphoid involving more than 80 people who had partaken of cheese prepared from the milk of an apparently healthy cow which was discharging *Gaertner bacilli* with her faeces. Kinloch, Smith and Taylor (1926) described a widespread outbreak of acute enteritis affecting 497 persons in Aberdeen. Milk was found to be the cause of the disease and the source of infection was traced to a cow with an indurated udder which later developed septicaemia. *Gaertner bacilli* were isolated from the faeces and the vomit of a number of patients, from the infected milk and from the udder and flesh of the cow. In order to determine whether *Gaertner bacilli* are excreted with the milk Standfuss and Wilken (1933) carefully examined the milk of two cows that were discharging large numbers of *Gaertner bacilli* in the faeces. The results were entirely negative and these workers came to the conclusion that when paratyphoid bacilli occur in the milk it is due entirely to contamination.

Rimpau (1937) studied an outbreak of acute gastro-enteritis in 80 persons of an institution due to *typhi-murium (Breslau)*. The vehicle of infection was ice-cream, and it was found that the cream used originated from a herd in which there was a calf discharging *typhi-murium*.

A detailed study of the incidence of paratyphoid in calves and adult cattle was made by Pröscholdt (1931). Calves were found to be far more susceptible to infection than adults and *Gaertner bacilli* were considered to be the most important pathogen for calves, while adult cattle infected with this organism were regarded as the principal source of meat-poisoning. Pröscholdt described two outbreaks of *Gaertner* infection spreading from adult cattle to calves. Agglutination tests carried out sometimes revealed a titre of 1:100 in healthy animals, a titre of 1:200 being regarded as suspicious, and one of 1:400 as positive. Out of 465 cases tested by Pröscholdt, 404 were positive for *Gaertner* and only 61 to *typhi-murium*. Rievel (1933) kept 4 infected carriers under observation for 18 months. In some animals the agglutination titres were as high as 1:3,200. Periodically the organisms could not be detected in the faeces, and the presence of *Gaertner bacilli* could not be demonstrated in the milk at any time.

Pallaske and Lommatzsch (1933) were unable to recover *Gaertner bacilli* from the organs of more than 47 out of 79 cases which showed pathological changes of paratyphoid. By cultivating suspected material for long periods in enrichment media they were able

to detect bacteria in a large number of cases. Out of 43 outbreaks of paratyphoid in cattle, Francke, Standfuss and Wilken (1934) found 25 due to *dublin*, 11 to *typhi-murium* and a few to *rostock*. In Italy Strozzi (1934) found *S. enteritidis* var. *dublin* as the most important causal agent of calf paratyphoid. Karsten (1933) also made a comparative study of the incidence of *Gaertner* infection in adult cattle and calves. He considered that adult cattle, unlike calves, could discharge paratyphoid bacilli with the faeces for long periods, and he emphasised the danger of such dischargers to all animals that come in contact with them. Clarenburg (1933), on investigating an outbreak of paratyphoid infection, found apparently healthy calves and cows discharging Gaertner bacilli in the faeces. On slaughtering the calves three weeks later he discovered typical necrotic foci in the liver and kidneys, but failed to isolate the organisms from the intestinal contents or internal organs; but the titre of the serum at first negative was now found to be 1:200 and 1:400. With regard to meat inspection, Clarenburg (1934) showed that it is extremely difficult to obtain *Gaertner bacilli* from the muscles of some animals in which organ cultures have yielded positive results. He considered the use of enrichment media (e.g. tetrathionate broth) essential for the recovery of *Gaertner bacilli* from the muscles.

Weber (1936) regards the walls of the stomach and intestines as predilection sites for *S. enteritidis*, and therefore attaches considerable importance to the bacteriological examination of the mucosa of all suspected cases; he claims to have succeeded in detecting many carriers by placing scrapings from the intestinal mucosa in enrichment media, when the cultivation of faeces yielded negative results. He also noticed that *Gaertner bacilli* are frequently excreted intermittently in the faeces and that a negative serological test may be obtained even when an animal discharges bacilli with the faeces. Klimmeck (1936), on studying a number of herds of cattle for paratyphoid carriers, found 47 adults and 39 calves positive; of the adults 23 discharged *Gaertner* and 23 typhi-murium, while 33 calves excreted *Gaertner* and only 5 *typhi-murium*. A very small percentage of the adult carriers gave a positive agglutination reaction, while the proportion in calves was still smaller. Knoth (1936) made a differential study of 561 strains of *Gaertner bacilli* obtained from slaughter animals, using arabinose and rhamnose broth, Bitter's whey and Stern's glycerine-fuchsin broth for his identification. He included 1 strain (0·2 per cent.) in the Jena type, 12 (2·1 per cent.) in the Rostock type, 20 (3·6 per cent.) in the Ratin type and 528 (94·1 per cent.) in the Kiel type.

In East Africa Daubney (1927) investigated a very destructive form of calf paratyphoid associated with lung lesions, necrotic foci in the liver, haemorrhagic enteritis, tumor splenis and bacteraemia. He obtained organisms of the *Salmonella enteritidis* type from the blood, internal organs and faeces of affected calves. In India Shirlaw (1935) investigated a highly fatal disease in calves caused by a member of the *Salmonella enteritidis* group. Calves ranging from 4 to 120 days old were affected. A tentative diagnosis of *S. enteritidis* was made purely on biochemical grounds. Hygienic factors were regarded to play an important rôle in the genesis of the disease.

In domestic mammals calf paratyphoid is by far the most serious and most common form of *Salmonella* infection. Of the 104 calf strains studied by me, 2 were found to be *typhi-murium*, 3 *enteritidis* and ninety-nine *enteritidis* var. *dublin*—the latter described first by Bruce White (1929). The strain described by Bruce White was isolated by Biggar from a man affected with septicaemia following an operation on his kidney. Smith and Scott (1930) recognised this organism as the cause of three cases of continued fever in man studied by them. They considered that several of the cases of so-called " Gaertner septicaemia " encountered in man were in reality due to infection with the *dublin* type of organism. Some old laboratory strains obtained from outbreaks of food-poisoning, septicaemia and meningitis, and labelled *Bact. enteritidis*, were examined by them and found to be of the *dublin* type. Six strains isolated from outbreaks of calf dysentery in Denmark which were included in the paracolon group (*B. paracoli*) of Jensen (1913) were also recognised as belonging to the *dublin* type. Smith and Scott pointed out that in the majority of cases where this organism had been isolated from man, milk was incriminated as the cause; they regarded it as having a special association with bovine animals and concluded that cows' milk was the common vehicle of human infection. Bosworth and Lovell (1931) described three outbreaks of *dublin* infection in calves in Great Britain, where *Salmonella* infection is generally regarded to be very rare. A little later Smith (1934) recorded two fatal cases in children due to infection with *dublin*. The organisms were obtained from the blood, cerebro-spinal fluid, throat swabs and pus from the pleural sac of one or other of the patients.

In South Africa, Hutcheon (1893) referred to a disease of calves in the Eastern Province of the Cape that can probably be identified with " lewersiekte " of Otto Henning (1894). Hutcheon believed that the infection was spread from farm to farm by means of the faeces of infected calves.

Otto Henning (1894) described the disease under the name of " yellow liver " or " lewersiekte ". Subsequently calf diarrhoea was reported from different parts of the country. In 1920 I (Henning, 1932) investigated an outbreak near Estcourt in Natal and found lesions of necrotic foci in the liver and acute enteritis, but the etiology remained obscure until Viljoen and Martinaglia (1926, 1928) and Martinaglia (1929) incriminated *Salmonella enteritidis*, obtained from the organs of affected calves as the cause of the malady. They regarded this organism as a frequent secondary invader affecting mostly calves whose vitality had been lowered by factors such as improper feeding, bad hygiene, piroplasmosis, and anaplasmosis. Martinaglia (1929) described outbreaks of *Salmonella* infection in horses, fowls and canaries as well as in calves. He discussed the bacteriology, symptomatology, pathology and diagnosis of the disease caused by a number of different strains, and classified the organisms almost entirely on their biochemical characters, no attempt being made to give the antigenic structure of the bacteria described. As a result of the work of Andrewes, Schutze, Bruce White, Kauffman and others, reliable analytic methods of serological comparison are now available so that I have been able to devote my time largely to the study of the antigenic structure of different strains of *Salmonella*

isolated from domestic animals in this country. But, for the sake of comparison, the biochemical characters of the organisms are also given. (Table 25.)

During the last three years no less than 102 outbreaks of calf paratyphoid were recorded in South Africa and in the majority of these the losses were considerable; from these outbreaks I have obtained 102 different strains of *Salmonella*. According to information received from different parts of the country it is quite evident that outbreaks occur which are never reported. In many cases the farmer inoculates his calves with paratyphoid vaccine as soon as he suspects the disease, and the inoculation frequently protects the animals against infection. In other instances the vaccination has little or no effect in protecting calves that are exposed in grossly infected areas or in premises harbouring a particularly virulent strain of the organism. At one time it was thought that these apparent breakdowns in immunity occurred only when the vaccine was prepared from a stock strain of *Salmonella* (*dublin*), but it was subsequently found that even vaccines prepared from local strains could not produce an immunity strong enough to resist a natural infection.

The disease is always most severe in very young calves, but it may affect calves up to 4 months old. All affected calves discharge large numbers of bacilli with their faeces resulting in their wholesale dissemination. The scourge usually commences on a farm with a few cases of acute diarrhoea, and during the ensuing years the incidence of the disease may increase to an alarming extent, depending upon the conditions under which the animals are kept. In some outbreaks the infection becomes so severe that the majority of the calves reared on the place succumb to the disease. With the increase in the number of cases of paratyphoid the locality becomes more and more heavily infected resulting in the creation of a vicious circle. Farms which contain the greatest number of cattle are generally the worst infected.

The habit of kraaling calves, or of kraaling the cows while the calves are admitted during the milking, or any procedure which permits calves under conditions where they have to come in contact with infected manure, favours infection. It is not known how long the manure in infected premises will remain infective; all the available evidence suggests that the infection persists for a matter of years. In 1934 I inoculated a young bovine with a virulent culture of *Salmonella dublin* (strain 154). After a severe reaction the animal recovered, but remained a carrier and discharged the organisms in its faeces for several months afterwards. Some of the infected faeces were collected, spread in a thin layer over a Petri dish and dried in the incubator for 48 hours; the dried manure was scraped out, bottled and placed on a shelf in the laboratory. Periodically this manure was tested for the presence of *dublin*; this was done by inoculating some manure in an enrichment medium, like tetrathionate broth, and by spreading some of the growth obtained on MacConkey's lactose bile-salt agar. After 1,069 days the last test was made and the manure was found to be as badly infected as at the first test. Whether the organisms will survive for as long a period under natural conditions in the kraal or stable manure remains to be proved, but the

fact that, under certain conditions, *dublin* bacilli can remain alive in the manure for nearly three years is an indication that they are very resistant and that dry manure from infected premises must be regarded as very dangerous. The possibility of calves obtaining the infection from the manure under natural conditions should, therefore, be emphasised. Moreover, when cows are milked in stables or kraals with the floors covered with manure, dry or moist, contamination of the milk with manure may lead to the dissemination of *dublin* through the milk; a number of European workers (see above) have shown that the milk of cows discharging paratyphoid bacilli with the faeces may be contaminated with these bacilli, and that when milk is infected, the infection is always obtained from the faeces and not from the udder.

My observations agree with those of Daubney (1927) and Viljoen and Martinaglia (1928), viz., that exposure of calves to unfavourable conditions and to diseases like piroplasmosis and anaplasmosis predispose them to infection. I have studied several outbreaks in which the calves were so badly infested with ticks that their resistance must have been lowered considerably. Sometimes there was no doubt that the mortality could be attributed either to piroplasmosis, anaplasmosis, gonderiosis, or heartwater, but in other cases the calves were infected with *dublin* as well as one or more of the tick-borne diseases. The rôle played by the latter in predisposing calves to paratyphoid infection must, therefore, be considerable. In some of the outbreaks studied by me I consider paratyphoid as a disease *per se*, but in many I regard the tick-borne disease as the primary cause of illness and the *dublin* infection as secondary. Moreover, the hygienic conditions under which the animals are kept also play a very important part in the genesis of the disease—particularly when they are frequently exposed to conditions that bring them in contact with infected manure.

Prophylactic measures for combating calf paratyphoid, therefore, should entail the systematic eradication of ticks as well as the application of rigorous hygienic measures in all premises where calves are raised.

All excreta and infected carcases should be properly disposed of, and healthy calves should be removed from the infected premises to clean surroundings. Vaccination, although a useful method of prophylaxis, cannot be relied upon solely; its value is greatest when it is used in conjunction with the application of suitable hygienic and tick eradication measures. But, as vaccination against calf paratyphoid forms the subject of another paper which is being prepared in collaboration with other workers at Onderstepoort, it will not be discussed here.

From these records it is clear that *Salmonellas* are common pathogens of calves in different parts of the world, generally setting up symptoms of septicaemia, acute diarrhoea, pneumonia, and meningitis with lesions of haemorrhagic enteritis, broncho-pneumonia, tumor splenis, necrotic foci in the liver and kidneys, and meningitis. In the vast majority of outbreaks described, *S. enteritidis* is incriminated as the cause of the disease; but, apart from the work of Bruce White (1929), Smith and Scott (1930), Bosworth and Lovell (1931),

Smith (1934), Kauffmann (1935b, 1935c) and a few others, the identification of the organism was not based on its serological characters. On the basis of a series of agglutination absorption tests carried out with all the strains of *Salmonella* obtained from calves in South Africa, I have been able to recognise the organism responsible for each outbreak. The results of these tests are given in Tables 1, 2 and 3.

(2) The Technique Employed.

The material studied was obtained from different parts of the country. In most cases it was composed of organ specimens (liver and spleen) sent to the laboratory in 50 per cent. glycerine; sometimes fresh faeces or faeces sent in glycerine were submitted for examination. Occasionally a sick animal was available for investigation. In addition several cultures made from fowls by Mr. J. D. W. A. Coles, Chief of the Department of Poultry Diseases at Onderstepoort, were studied. These are described in Section VI. Most of the material was obtained from places 100 to 800 miles away from the laboratory so that it was not possible to visit more than one or two infected farms. As routine preventive inoculation of all calves in areas infected with paratyphoid was generally carried out, it was not possible to obtain sick calves for observation that had not been previously inoculated with paratyphoid vaccine.

For the identification of *Salmonella* types the technique advised by Scott (1934) and modified by me was ususally employed. Specimens of suspected material (blood, liver, spleen, faeces) were spread directly on MacConkey's lactose bile-salt agar in Mason tubes (Mason 1933)—Scott used Petri plates. Generally it is advisable to dilute some of the material in saline or broth before it is spread on the MacConkey. In this way isolated colonies will be obtained more easily. In addition material (especially faeces) is inoculated into an enrichment medium, e.g. tetrathionate broth or 1 per cent. peptone water containing brilliant green (1 in 150,000). After 18 to 24 hours incubation the Mason tubes are examined and the enriched cultures are spread on dry MacConkey agar. The characteristic pale, finely structured *Salmonella* colonies are picked from the tube which is frequently crowded with colonies of lactose-fermenting *B. coli;* sometimes colonies of late lactose fermenting or non-lactose fermenting *B. coli*, *B. pyocyaneus* and *B. proteus* are seen—these should be avoided and should not be confused with *Salmonellas*. The suspected *Salmonella* colonies are now subjected to an agglutination test. A portion of a suspected colony is picked and emulsified in a loopful of group serum (e.g. European *cholerae-suis* serum) and in a loopful of type serum (e.g. *enteritidis* serum) on a glass slide, the dilution of the serum depending on the titre—about 1 in 50 if the titre is 1:5,000. A number of the suspected colonies are emulsified each in two separate loopfuls of diluted sera (group and type); the amount of serum carried over from the one to the other drop is too small to confuse the reaction.

Some of the colonies may agglutinate with one or other of the two drops of serum; while others may fail to agglutinate with either, or may exhibit a mere trace of agglutination. Organisms which

occur in the specific phase will react with their own type sera, while those that happen to be in the non-specific phase will agglutinate with a group serum. When a reaction occurs a characteristic flocculation is seen which is readily distinguished from non-specific salt agglutination of Rough variants. Moreover, in a positive test flocculation will occur only in the one drop and not in the other, whereas in the case of salt agglutination clumping will be observed in both. A good hand lens and a dissecting microscope are very useful during the fishing for colonies as well as for the study of the reaction. Colonies that have given a positive reaction are picked, subcultured and studied further.

" Pure " type-specific sera can be prepared by inoculating rabbits with 6 to 8-hours old broth cultures of the organism in the specific phase. But as these sera always contain a certain amount of group agglutinin, preliminary absorption of the latter with another *Salmonella* containing the same group phase, but another type phase, is recommended. If *typhi-murium* serum, for example is absorbed with a mixture of *paratyphi-B* and *cholera-suis*, the group agglutinins will be removed leaving a " pure " type serum-dilution. If the organisms used for the absorption contain the same somatic antigen (e.g. *paratyphi-B and typhi-murium*) the " O " agglutinins will also be removed; thus preventing them from interfering with the reaction. The " pure " type serum will contain only type agglutinins, but neither " O " nor group agglutinins. For routine diagnosis a set of representative type-specific sera should be available, e.g. *paratyphi-B*, *typhi-murium*, *cholera-suis*, *newport*, *thompson*, *potsdam*, *bovis-morbificans*, *typhi*, *enteritidis* and *L2* sera. If a suspected colony gives a characteristic reaction with only one of these sera, a preliminary diagnosis is made and the culture obtained from it is studied further by means of agglutination absorption tests. If group serum is used, colonies occurring in the group phase will be detected. Occasionally more than one type *Salmonella* is present in the culture (mixed infection), but the second organism is not likely to be missed as long as a reasonable number of colonies is examined.

Sometimes, when diphasic *Salmonellas* are studied, there may be some difficulty in demonstrating the existence of specific-phase colonies, if colonies in the group phase predominate. On repeated sub-cultivation of the latter, however, an occasional colony occurring in the specific phase may be detected. But in cases like European *choleraesuis*, where the organism occurs permanently (?) in the group phase, phase dissociation will not readily take place.

For the acceleration of phase dissociation Scott (1934) recommends the use of broth containing approximately 15 per cent. group serum. Group colonies cultured in this medium yield a culture with a clear supernatant fluid and a dense deposit after 18 hours' incubation. On repeated sub-cultivation in group serum-broth, a turbid supernatant fluid may ultimately be obtained. If this turbid culture is now plated, most of the colonies resulting will be in the specific phase. Sometimes as many as 10 or 12 passages may be necessary before the phase dissociation becomes apparent.

Scott's technique was improved by Wassén (1935) and Bruner and Edwards (1939a and b).

The differentiation of monophasic organisms, like *enteritidis* and the members of its subgroups, can be carried out on similar lines. The specific serum is absorbed so that only the agglutinin factors not present in the sera of the other types are left. For example, by absorbing *enteritidis* serum with the type *moscow*, agglutinin factors *g.o.* of the Kauffmann-White Schema (1934) are removed, leaving factor *m* which is exclusively present in *enteritidis*. Several of the other members can be purified by absorption with *enteritidis* which removes factors *g.o.m.*

A pure culture of the strain studied was obtained either by picking single colonies from three successive generations of the culture on agar plates, or by single-celling the culture according to the method described by Mason (1936). Saline and thermo-agglutination tests, as well as the shape of individual colonies were studied for evidence of roughness. Unless indisputably smooth colonies could be obtained the culture was discarded. Only a few strains isolated from organ material (liver or spleen) were found to be completely rough; but several cultures obtained from faeces of infected or carrier animals turned out to be rough. All the strains studied behaved morphologically and culturally like typical *Salmonellas*.

Preparation of antigens.—For the preparation of agglutinating suspensions the technique employed is that described by Lovell (1932). For "O" suspensions a smooth strain of the organism is grown on agar contained in Mason tubes (Mason, 1933) for 24 hours at 37° C., the inoculum used being obtained from agar slope cultures. The growth is washed off with 95 per cent. alcohol and heated in a waterbath at 56° C. for 2 hours. After the suspension has been centrifuged and the alcohol poured off, the deposit is re-suspended in about one cubic centimeter of distilled water, before it is made up in saline to the opacity required. For preserving the antigen, Bruce White advises the addition of 66 per cent. of glycerine to the thick suspension in distilled water; this mixture is diluted in saline when required for use, the density of the antigen being approximately a thousand million organisms per cubic centimeter.

"H" suspensions are prepared by growing a motile strain of the organism in broth at 22° C. for 18 hours or at 37° C. for 6 to 8 hours. Frequently the culture is merely left standing on the laboratory bench over-night, during which period a suitable density is usually obtained. In the case of diphasic members of the group it is essential to pick colonies in both the specific and non-specific phases, and to prepare broth suspensions from each one. In order to obtain well separated colonies a small amount of inoculum is spread on fairly dry agar in Mason tubes and incubated over-night. Next morning about a dozen or more suitable colonies are selected and numbered; half of each colony is picked and inoculated into broth, and then transferred to the incubator for about 5 to 6 hours, while the Mason tubes are placed in a refrigerator to prevent further

growth and dissociation of the colonies. Alternatively the colonies are picked and each one is seeded into duplicate tubes of broth. After incubation the one tube is stored in the refrigerator, while the duplicate is tested. It is seldom necessary to incubate the broth tubes for more than 5 or 6 hours; if a suitable density is obtained the cultures are killed by the addition of formalin to a concentration of 0·25 per cent. and heating at 57° C. for two hours. Two parallel rows of Dreyer tubes are now placed in a rack; to each of the tubes in the one row 0·5 c.c. type serum dilution is added, and to each of the tubes of the other row a similar amount of diluted group serum is added; this is followed by the addition of 0·5 c.c. of the suspension from each of the broth cultures to a tube of serum dilution in each row. The rack is placed in a waterbath at 55° C. The cultures that are agglutinated by the type serum occur in the type phase and have been obtained from colonies in that phase, while the suspensions that flocculate with group serum have been obtained from group phase colonies. The kind of antigen, type or group, required can now be prepared by inoculating broth with the remaining half of the colony in the Mason tube, meanwhile stored in the refrigerator. In order to reduce the lag phase in the growth of the cultures the broth tubes are placed in a water-bath at 40° C. for about 10 minutes before incubation.

Group and type phase colonies may also be recognised by testing them in droplets of group and type serum on a glass slide according to the method described by Scott (1934).

" H " suspensions are made up to a density of approximately 500 million per cubic centimeter and " O " antigens up to roughly 1,000 million per cubic centimeter.

Agglutinating sera are prepared by injecting rabbits intravenously with killed bacteria 4 or 5 times at 3 or 4 day intervals. For mixed " H " and " O " sera, the antigen used is a saline suspension of an eighteen hours old agar culture. The organisms are also killed by the addition of formalin to make a concentration of 0·25 per cent. and heating at 57° C. for two hours. For the preparation of type and group sera the organism in the required phase is grown in broth for approximately 6 hours and killed before injection. But the type sera obtained always contain a certain amount of group agglutinins which should be removed by means of an organism occurring in the group phase, or by one which has the same group but a different type phase. Group phase sera are also seldom " pure ", but purification is far more difficult on account of the presence of some similar group factors in all group antigens.

For the preparation of " O " antisera the antigen consists of a boiled saline suspension of an eighteen hours old agar culture. The first dose given is usually about 100 to 200 million bacteria suspended in 1 c.c. of saline. Subsequent doses can be gradually increased until a final dosage of approximately 500 to 1,000 million bacteria is reached. The administration of larger doses does not appear to be justified. It is seldom necessary to give more than five or six injections; too many injections are liable to produce sera of titres too high for easy absorption work.

Agglutination tests are carried out in Dreyer tubes placed in a water-bath at 55° C., the lower half of the tubes being immersed in water. The agglutination of " H " suspensions results in the formation of coarse, loosely arranged floccules within a very short time, reaching its maximum in about 2 to 4 hours. The clumping of " O " suspensions occurs more slowly and is characterised by the formation of fine granules; this is best seen after the tubes have been standing in the water-bath overnight.

Saline dilutions of the serum to be tested are generally made in a series of dilution tubes. From these the serum dilutions are transferred to Dreyer tubes in 0·5 c.c. amounts. A similar amount of antigen is added to each tube. For " H " agglutination the tubes are read after standing for about 2 hours in the water-bath and for " O " agglutination the readings are taken on the following morning.

For absorption tests the absorbing organism is grown on agar in Mason tubes for about 24 hours. The agar is poured fairly thick into the Mason tubes so as to furnish a good growth. After the agar has properly set the Mason tubes are placed flat in a cupboard for about 3 or 4 days in order to allow most of the water of condensation to evaporate; alternately the tubes are put in the incubator overnight. Unless some of the water of condensation is evaporated, the surface of the agar will be too moist, and the excessive fluid on the surface of the agar will interfere with the subsequent removal of the growth. The seed material is either a fresh agar or broth culture of the organism. If the surface of the media in the Mason tubes is still moist a loopful of inoculum from the agar slant is preferred, but if the surface is dry a couple of drops from the broth culture should be used. As a rule, however, a thicker growth is obtained if a large amount of inoculum is used. The seed material is spread by means of a blunt, slightly bent Pasteur pipette flamed before use. After 24 hours' incubation a fairly thick homogeneous growth will be obtained on the surface of the agar. By using Mason tubes instead of Petri plates for culturing the organism the risk of contamination is reduced to a minimum; whereas contamination of Petri plates kept in the incubator overnight is not uncommon.

The serum to be tested is diluted to the concentration required; when the " H " titre is about 1:5000 a serum dilution of 1:25 or 1:50 is recommended. The desired amount of serum dilution is measured into a thick centrifuge tube. By means of a Pasteur pipette, approximately 25 c.m. long, with an open loop at the capillary end, the growth in the tubes is scraped off and emulsified with the serum dilution along the inside of the centrifuge tube; but great care should be taken that all the clumps of bacteria are properly broken up, so that the organisms are well distributed throughout the liquid. The suspension is now placed on the bench for an hour or more and shaken every now and then so as to ensure thorough mixing of the bacteria and the serum. Although the absorption is usually complete after an hour of two on the bench, the suspension is preferably kept in the refrigerator overnight and centrifuged the next morning at about 2,000 revolutions per minute for one hour. The clear supernatant fluid is removed with a pipette

and tested. Sometimes, especially with high titre sera, better results are obtained if the absorption is performed in stages; i.e. part of the antigen is mixed with the serum dilution at first, while the rest is emulsified in the same fluid after it has been centrifuged an hour or two later.

(3) Serology.

In Table 1 are given the results of agglutination and absorption tests that were obtained with cultures 154, 217 and 216 on the one hand and *dublin* (Knox) and *enteritidis* M.7. on the other hand. The tests carried out with cultures 154 and 216 were complete bilateral (mirror) absorption tests, while the one performed with culture 217 was a unilateral absorption. Complete mirror absorption tests were also carried out with cultures 170, 171, 173, 175, 198, 203, 295 and 430. The results obtained were identical with those given for culture 154 (Table 1) and, with the exception of those relating to culture 430 (Table 3), are not recorded separately in this paper.

Cultures 418 and 290 reacted the same way as culture 216 but the records of their tests are not given; they are regarded as identical with culture 216 serologically. The results obtained with culture 190 are given in Table 2; according to a one-sided absorption test performed with culture 502 its antigenic structure is apparently similar to that of culture 190 (see below). Unilateral absorptions were also carried out with the other 87 calf strains against *dublin* (Knox) serum; the results obtained with these show that they are identical with culture 217 and are therefore not recorded here.

The records of Table I show that *Salmonella enteritidis* var. *dublin* (Knox) absorbed all the agglutinins (" O " and " H ") from 154 serum as well as from the homologous serum, while culture 154 exhausted both its own serum and *dublin* serum; *enteritidis* M.7., while completely removing all the agglutinins from the homologous serum, absorbed only the " O " agglutinins from 154 serum, leaving its " H " titre practically unchanged; in the same way, culture 154 exhausted only the " O " agglutinins from *enteritidis* serum without materially reducing the " H " titre of the latter. These results, therefore, demonstrated that culture 154 has the same " O " antigen as *enteritidis* and *dublin*, and an " H " antigen similar to that of *dublin*. Although culture 154 was agglutinated by *enteritidis* serum up to full titre, and although *enteritidis* was fully flocculated by 154 serum, *enteritidis* could not appreciably lower the " H " titre of 154 serum and culture 154 failed to remove the " H " agglutinins from *enteritidis* serum.

According to the *Salmonella* Sub-committee of the Nomenclature Committee of the International Society of Microbiology, *S. enteritidis* var. *dublin* is composed of the following antigenic factors:— " O ", IX; and " H " *gp* " O " factor XII has been added subsequently by Kauffmann (1935*b*). As strain 154 is identical with *dublin* its antigenic structure is made up of the same components. Mirror-absorption tests performed with strains 170, 171, 173, 175, 198, 203, 295 yielded the same results as strain 154; they are, therefore, also

TABLE 1.

Dublin (Knox) serum absorbed by *dublin* (Knox)
154 serum absorbed by *dublin* (Knox), and strain
216 serum absorbed by strain 216 and *enteritidis*
Enteritidis M.7. serum absorbed by strain 216 a

Antigen.	*Dublin* (Knox) Serum Absorbed by *Dublin* (Knox).	*Dublin* (Knox) Serum Absorbed by Strain 154.	*Dublin* (Knox) Serum Absorbed by Strain 217.	*Dublin* (Knox) Serum Absorbed by Strain 216.	*Enteritidis* M. 7 Serum Absorbed by *Enteritidis* M. 7.	*Enteritidis* M. 7 Serum Absorbed by Strain 154.
Dublin Knox "O"	0	0	0	0	—	—
Dublin Knox "H"	0	0	0	6,400	—	—
Strain 154 "O"	0	0	—	—	0	0
Strain 154 "H"	0	0	—	—	0	0
Strain 216 "O"	0	—	—	0	0	—
Strain 216 "H"	0	—	—	0	0	—
Enteritidis "O" M. 7.	—	—	—	—	0	0
Enteritidis "H" M. 7	—	—	—	—	0	6,400
Strain 217 "O"	0	—	0	—	—	—
Strain 217 "H"	0	—	0	—	—	—

0 = less than 1 in 100.

97-98b

TABLE 1.

by *dublin* (Knox), and strains 154, 216 and 217.
(Knox), and strain 154 and *enteritidis* M.7.
216 and *enteritidis* M.7. and *dublin* (Knox).
by strain 216 and *enteritidis* M.7. and strain 154.

Enteritidis M. 7 Serum Absorbed by Strain 216.	154 Serum Absorbed by *Dublin* (Knox).	154 Serum Absorbed by *Enteritidis* M. 7.	216 Serum Absorbed by *Dublin* (Knox).	216 Serum Absorbed by *Enteritidis* M. 7.	154 Serum Absorbed by Strain 154.	216 Serum Absorbed by Strain 216.	*Dublin* (Knox) Serum Un-absorbed.	*Enteritidis* M. 7 Serum Un-absorbed.	154 Serum Un-absorbed.	216 Serum Un-absorbed.
—	0	—	0	—	0	0	1,600	—	1,600	800
—	0	—	0	—	0	0	12,800	—	25,600	6,400
—	0	0	—	—	0	—	1,600	800	1,600	—
—	0	12,800	—	—	0	—	12,800	12,800	25,600	—
0	—	—	0	0	—	0	1,600	800	—	800
0	—	—	3,200	0	—	0	12,800	12,800	—	6,400
0	—	0	—	0	0	0	—	800	1,600	800
0	—	0	—	0	0	0	—	12,800	25,600	6,400
—	—	—	—	—	—	—	1,600	—	—	—
—	—	—	—	—	—	—	12,800	—	—	—

0 = less than 1 in 100.

97-98a

97-98b

identical with *dublin* and possess the same antigenic factors. The experiments carried out with strain 217 and the other 87 strains not recorded in Tables 1 and 3 also demonstrated that these strains resemble *dublin* antigenically. In Table I it was shown that strain 217 exhausted *dublin* serum as completely as this was done by *dublin* (Knox).

With regard to strain 216 the results in Table 1 show that it completely absorbed *enteritidis* M.7. serum as well as the homologous serum, whereas *enteritidis* M.7. exhausted agglutinins from both 216 serum and its own serum. On the other hand, strain 216 failed to reduce the " H " titre of *dublin* (Knox) serum but removed all its " O " agglutinins. *Enteritidis* M.7. and strain 216 must, therefore, be regarded as identical, both containing the same antigenic factors viz. " O ", IX (XII), and " H " gom. Complete reciprocal absorption of the " O " agglutinins of *dublin* (Knox) and strain 216 sera was effected by cultures of these two organisms on account of the existence of identical " O " factors in them; but the " H " antigenic components, although cross-agglutinating to full titre with the two sera, were not sufficiently related to reduce the " H " titres of the sera. The cross-agglutination observed between the " H " antigens of strain 216 and *dublin*, of strain 154 and *enteritidis* M.7. and the corresponding sera, took place by virtue of the presence of factor *g* in the antigenic complexes of both types. Cross-absorption removed only this factor, leaving the other components undisturbed, hence the high " H " agglutinin titre of the absorbed sera.

The antigenic structure of strain 190 was found to differ completely from that of both *dublin* and *enteritidis*, but it was readily agglutinated by *typhi-murium* " O " and type sera as well as by a group serum (e.g. *cholerae-suis* var. Kunzendorf serum). In Table 2 the records of an absorption test between strain 190 and *typhi-murium* are given. The results show that strains 190 not only removed all the type, group and " O " agglutinins from the homologous serum but also from *typhi-murium* (Glasgow) serum, while *typhi-murium* (Glasgow) exhausted all the agglutinins from its own serum as well as from 190 serum. It is evident, therefore, that the antigenic structure of strain 190 and *typhi-murium* (Glasgow) are identical. According to the Salmonellas Sub-committee of the International Society of Microbiology, the following antigenic components have been assigned to *typhi-murium*:— " O " IV, V and " H "-specific, *i*, " H "-non-specific, 1, 2, 3. According to Table 2 the same assignment should be allotted to strain 190.

A one-sided absorption test was performed with another diphasic strain of *Salmonella* (*culture* 502) also isolated from a calf that had died from paratyphoid. *Typhi-murium* (Glasgow) serum was used for the test. The result was that *culture* 502 completely removed all the " O " type and group agglutinins from *typhi-murium* serum. *Culture* 502 therefore contained the same antigenic components as *typhi-murium* (Glasgow).

The results recorded above clearly demonstrate that *Salmonella enteritidis* var. *dublin* is by far the most common cause of calf paratyphoid in South Africa. Of the 102 strains studied only two proved to be *S. typhi-murium*, three were classified as *S. enteritidis* and 97 were grouped under *S. enteritidis* var. *dublin*.

TABLE 2.

Typhi-murium (Glasgow) serum absorbed by *typhi-murium* (Glasgow) and by strain 190.

Strain 190 serum absorbed by *typhi-murium* (Glasgow) and by strain 190.

Antigen.	*Typhi-murium* Serum Absorbed by *Typhi-murium* (Glasgow).	*Typhi-murium* Serum Absorbed by Strain 190.	*Typhi-murium* Un-absorbed.	190 Serum Absorbed by *Typhi-murium* (Glasgow).	190 Serum Absorbed by Strain 190.	190 Serum Un-absorbed.
Typhi-murium "O"	0	0	1,600	0	0	1,600
Typhi-murium type	100	100	100,000	0	0	6,400
Typhi-murium group..	0	0	25,600	0	0	3,200
Strain 190 "O"..	0	0	1,600	0	0	1,600
Strain 190 type...	100	100	100,000	0	0	6,400
Strain 190 group..	0	0	25,600	0	0	3,200

0 = less than 1 : 100.

The antigenic properties of another strain, culture 430, isolated from the blood of a Native should also be recorded. The Native with a number of others had partaken of the meat of a calf which was suspected to have died from paratyphoid. Several of the Natives became violently ill and one, a woman, died from septicaemia. The blood of this woman was submitted to me for investigation and a *Salmonella*, strain 430, was isolated from it after enrichment in tetrathionate broth. Unfortunately no meat or part of the suspected carcass was available for bacteriological study (Henning 1938).

TABLE 3.

Antigen.	*Dublin* Serum Absorbed by *Dublin*.	*Dublin* Serum Absorbed by Strain 430.	*Dublin* Serum Un-absorbed.	430 Serum Absorbed by *Dublin*.	430 Serum Absorbed by Strain 430.	430 Serum Un-absorbed.
Dublin "O".....	0	0	1,600	0	0	800
Dublin "H".....	100	100	25,600	100	100	50,000
430 "O".....	0	0	1,600	0	0	800
430 "H".....	100	100	25,600	100	100	50,000

0 = less than 1 : 100.

Cultures of strain 430 were readily agglutinated by *dublin* " H " and " O " sera; cross-agglutination and cross-absorption tests were, therefore, carried out as shown in Table 3.

The results show that strain 430 absorbed all agglutinins (" O " and " H ") from dublin serum as well as from the homologous serum, while *dublin* in the same way completely exhausted both 430 serum and its own serum. The presence of the small residues of unabsorbed agglutinins in both the *dublin* and 430 sera can be attributed to the high titres of the sera used for the test.

Strain 430 should therefore be regarded as another strain of *Salmonella enteritidis* var. *dublin*, containing the following antigenic components:— " O ", IX (XII) and " H " *gp*.

For fermentation reactions see Table 25.

SALMONELLA INFECTION OF SHEEP.

In a recent paper (Henning 1936) I pointed out that *Salmonella* infection is not very common in sheep and that food-poisoning in man associated with mutton is comparatively rare. It is true that shortly after the Great War a very severe outbreak of food-poisoning was described in Germany by Fickinger (1919) and by Bruns and Gasters (1920). The source of the infection was traced to sheep, several of which were emergency-slaughtered in order to save the carcasses for human food. Organisms described to be of the " Paratyphosus B " type were isolated from the suspected mutton as well as the stools of the patients; but Bruce White (1929) regarded the organisms incriminated as *S. typhi-murium*. Severe outbreaks of *Salmonella* infection in sheep have also been described in America. Jordan (1925) reported an extensive epizootic of dysentery in lambs in Colorado and found the causal agent to be *S. typhi-murium*, while Newsom and Cross (1924, 1930, 1935) investigated several outbreaks of gastro-enteritis in lambs caused by the same organism; Newsom and Cross regarded the long railway journeys the lambs had to make and the long periods of fasting as predisposing factors; *typhi-murium* were obtained in pure culture from the heart blood and spleen of the affected lambs. The most common pathogenic *Salmonella* for sheep, however, is *S. abortus ovis*. This organism has been described by several workers in Europe, but it has not yet been recorded in South Africa; it was first described by Schermer and Ehrlich (1921), and later by Stephan and Geiger (1922), Bosworth and Glover (1925), Miessner and Baars (1927), Lovell (1931), Bosworth (1933) and Lesbouyries *et al.* (1933).

Although several cases of suspected paratyphoid in sheep have been reported from time to time very little is really known of the incidence of the disease in South African sheep. So far only two authentic cases of *Salmonella* infection in sheep have been studied in this country; both strains have been isolated by Dr. J. H. Mason at Onderstepoort, and handed to me for identification. The

serological characters of the one were recently described in full (Henning, 1936). As its " O " antigen was shown to differ from the somatic antigen of all previously described *Salmonellas*, it was admitted to species rank in compliance with the recommendations of the *Salmonella* Sub-committee of the Nomenclature Committee of the International Society of Microbiology (1934); in accordance with the suggestions of the Sub-committee, this organism was called *Salmonella onderstepoort*.

Serology.—The conclusions drawn regarding the specificity of *S. onderstepoort* were based on the following information condensed in Tables 4, 5A and 6.

" O " agglutination.—Cross-agglutination tests were performed with the heat-stable " O " antigens and " O " sera of the *Salmonella* types given in the Kauffmann-White schema of the Salmonella Sub-committee, as well as with the two newer types *S. aberdeen* (Smith, 1934) and *S. poonae* (Bridges and Scott, 1935). The results are recorded in Table 4; negative reactions are not given. Although *Onderstepoort* serum agglutinated *Senftenberg* " O " suspensions to nearly full titre, *Senftenberg* serum barely agglutinated *Onderstepoort* " O " antigen at a dilution of 1:100. *Onderstepoort* serum also gave a trace of flocculation with the " O " antigens of *Paratyphi-A* and *Enteritidis*. But the titre of *Onderstepoort* serum remained unaltered after absorption with either *Senftenberg*, *Paratyphi-A* or *Enteritidis*. On the other hand, *Onderstepoort* did not appreciably reduce the titre of *Senftenberg*. These results clearly show that *Onderstepoort* possesses an " O " antigen which does not correspond to that of any other *Salmonella* previously described.

" H " agglutination.—An *Onderstepoort* culture was plated on a Mason tube so as to obtain a number of individual colonies. These were tested against several type sera and against *cholerae-suis* (European) group serum. Some colonies were flocculated by the type sera of *Reading*, *Newport*, *Anatum*, while others were agglutinated by *cholerae-suis* group serum. These type-phase and group-phase colonies were now sown into separate tubes (or flasks) of broth and grown at room temperature for 18 hours, or at 37° C. for 5 to 6 hours; the cultures were killed by formalin (0·25 per cent.) and heat at 57° C. for 2 hours, as described above. On the other hand, *Onderstepoort* serum agglutinated to full titre broth cultures of the type phases of *newport*, *reading* and *anatum*, and broth cultures of the group phases of *cholerae-suis*, *reading*, *sendai*, *paratyphi-C*. A strong agglutination was obtained between *onderstepoort* serum and those antigens containing factors *e.h* of the Kauffman-White schema; when antigens containing only factor *e*, but not *h*, (e.g. *potsdam* and *brandenburg* were used the agglutination titre was much lower (Table 5A). *Onderstepoort* type serum (titre 1:6,400), also gave a low agglutination with the " H " antigens of *moscow* (1:400), *senftenberg* (1:400), *rostock* (1:200) and *derby* (1:200) but not with *dublin*; *moscow* and *derby* sera also gave a weak flocculation (1:100) with *onderstepoort* type suspension, but *senftenberg*, *derby* and *dublin* sera had no effect on it.

TABLE 4.—"*O*" *Agglutination.*

Sera.

Somatic Antigens.	*Onderste-poort.*	*Senften-berg.*	*Para-typhi*—A.	*Chole-rae suis.*	*Enteri-tidis.*	*Onderste-poort* Absor-bed by *Onderste-poort.*	*Onderste-poort* Absor-bed by *Senften-berg.*	*Onderste-poort* Absor-bed by *Para-typhi*–A	*Onderste-poort* Absor-bed by *Chole-rae suis.*	*Onderste-poort* Absor-bed by *Enteri-tidis.*	*Senften-berg* Absor-bed by *Onderste-poort.*	*Anatum.*	*London.*
Onderstepoort	6,400	100	50	0	± 50	0	6,400	6,400	6,400	6,400	0	0	0
Senftenberg	3,200	3,200	—	—	—	0	0	—	—	—	1,600	—	—
Paratyphi—A	100	—	800	—	—	0	—	0	—	—	—	—	—
Choleraesuis	10	—	—	1,600	—	0	—	—	0	—	—	—	—
Anatum	0	—	—	—	—	—	—	—	—	—	—	1,600	—
London	0	—	—	—	—	—	—	—	—	—	—	—	800
Enteritidis	± 50	—	—	—	1,600	0	—	—	—	0	—	—	—

0 = less than 1 : 50.

TABLE 5A.

"H" Antigens (Type).	UNABSORBED TYPE SERA.					ABSORBED TYPE SERA.							
						Onderstepoort Absorbed by.			*Reading* Absorbed by.		*Newport* Absorbed by.		*Branden-burg* Absor-bed by *Onderste-poort* Type.
	Onderste-poort.	*Reading.*	*Newport.*	*Branden-burg.*	*Potsdam.*	*Onderste-poort* Type.	*Reading* Type.	*Newport* Type.	*Onderste-poort* Type.	*Reading* Type.	*Onderste-poort* Type.	*Newport* Type.	
Onderstepoort	6,400	3,200	6,400	800	1,600	0	200	400	50	50	50	50	0
Reading	3,200	3,200	—	—	—	0	50	—	400	50	—	—	—
Newport	3,200	—	6,400	—	—	0	—	50	—	—	800	50	—
Brandenburg	200	—	—	6,400	—	0	—	—	—	—	—	—	6,400
Potsdam	400	—	—	—	12,800	—	—	—	—	—	—	—	—
Anatum	3,200	—	—	—	—	—	—	—	—	—	—	—	—

0 = less than 1 : 50.

On performing absorption tests (Table 5A) the specific phase of either *reading*, *newport or anatum* lowered the titre of *onderstepoort* type serum from 6,400 to 200, while the specific phase of *onderstepoort* was not able to exhaust the type sera of *newport* and *reading* completely. These results show that, although the type factors *e.h.* of *reading*, *newport* and *anatum* are fairly well represented in *onderstepoort*, complete absorption could not be effected. Whether this is due to the presence or absence of a minor extra factor, or due to the existence of a small residue of group agglutinin in the absorbed sera, remains to be seen.

The non-specific phase serum of *onderstepoort* (titre 1:25,600) agglutinated various group antigens up to different titres (Table 6). On absorbing *onderstepoort* group serum with *binns* or *newport* (group factors 1, 2, 3) the titre for *onderstepoort* antigen was reduced from 25,600 to only 12,800, and for the group phases of *L2* and *anatum* (group factors 1, 4, 6) the reduction was from 1,600 to 400. *L2* and to 3,200, removing all group agglutinations for *binns* as well as for *L2* and *anatum*. When this partly absorbed serum was further absorbed by monophasic *cholerae-suis* the titre was further reduced to 800. By absorbing unabsorbed *onderstepoort* group serum with *cholerae-suis* the titre was lowered from 25,600 to 800, and simultaneously all the group agglutinations for *binns*, *anatum* and *L2* were exhausted. The group phases of *reading* and *sendai* (factors 1, 4, 5) also lowered the titre of *onderstepoort* group serum from 25,600 to 800.

These results suggest that the reduction in the titre of *onderstepoort* serum effected by the group phases of *binns* and *newport* was caused by their group factor 1; that the reduction produced by *L2* and *anatum* can be ascribed to their group components 1 and 4; and that the almost complete absorption brought about by the non-specific phases of *cholerae-suis*, *reading* and *sendai* shold be attributed to their group factors 1, 4, 5. It is evident that *onderstepoort* contains group factors 1, 4, 5 and not 2, 3, 6. The unabsorbed agglutinins left after absorbing *cholerae-suis* serum with *onderstepoort* can be ascribed to group factor 3 contained in *cholerae-suis;* but the presence of the residue left after absorbing *reading* group serum with *onderpoort* cannot be explained, nor is it clear why *cholerae-suis*, *reading* or *sendai* failed to exhaust *onderstepoort* serum completely unless *onderstepoort* contains an extra group factor.

According to the information recorded above *Salmonella onderstepoort* possesses an " H " specific antigen which corresponds largely to the factors *e.h.* of *reading*, *newport* and *anatum*, and it contains a non-specific antigen which is very closely related to that of *reading* and *paratyphi-C* (factors 1, 4, 5). But *onderstepoort* failed to exhaust completely the specific sera of *reading*, *newport* and *anatum*, or the non-specific sera of *reading* or *cholerae-suis* (European). On the other hand, the specific phases of *reading*, *newport* or *anatum* could not absorb all the specific agglutinations from *onderstepoort* type serum, while the group phases of *reading*, *paratyphi-C* or *cholerae-suis* did not remove all the group agglutinations from *onderstepoort* group serum. It is not quite clear how to explain the residue of unabsorbed agglutinins left after these absorptions; it is possible

that the specific serum contained a small amount of group agglutinin after absorption with organisms in the specific phase, or that the specific phase of *onderstepoort* possesses some factor that is lacking in the type factors *e.h.* of *reading*, *newport* and *anatum*, or that the specific factors *e.h.* contain some component that is not present in the type phase of *onderstepoort*.

The "O" antigen of *onderstepoort* exhibited characters which do not correspond to those that have been described for any other member of the *Salmonella* group of bacteria and the numeral XIV has been assigned to this new "O" factor.

Kauffmann (1937) would not accept my assignment of specific factors *e.h.* to *onderstepoort*. He agrees that factor *e* is common to *onderstepoort*, and organisms like *eastbourne*, which contain components *e.h.* and he claims to have succeeded in completely exhausting the specific agglutinins from *onderstepoort* serum by means of a strain of *eastbourne* which occurs only in the specific phase, while *onderstepoort* failed to remove all the specific agglutinins from *eastbourne*,. He does not agree, therefore, that *onderstepoort* contains factors *e.h.* and he assigns specific factor *e* . . . to *onderstepoort*.

In my previous paper (Henning, 1936) I made the following conclusions:—"A new type of *Salmonella* has been described, which it is proposed to name *Salmonella onderstepoort*. The 'H' specific antigen corresponds largely to factors *e.h.* of *reading*, *newport* and *anatum*. Although cross-agglutination to full titre occurred, complete cross-absorption could not be affected. Apparently the specific factors *e.h.* contain some component which is lacking in the type phase of *onderstepoort*, while the specific phase of *onderstepoort* possesses some factor in addition to *e.h.*" I proposed the following antigenic components for *onderstepoort*:—

> "'O' antigen XIV.
>
> 'H' antigen (specific) *e.h.* but there is probably some small portion of *e.h.* which is lacking in *onderstepoort* and apparently *onderstepoort* contains a small addition factor which is lacking in *e.h.*
>
> 'H' antigen (non-specific) 1, 4, 5 plus an additional factor which does not occur in *S. cholerae-suis*, *S. anatum* or *Binns*".

In view of Kauffmann's findings I repeated some of the tests which I had previously performed with *onderstepoort*. Unfortunately I did not have available a strain of *eastbourne* which occurs only in the type phase, and all the strains of *reading*, *newport* and *anatum* of my collection were definitely diphasic. Even the strains of *newport* var. *Kottbus* labelled "specific phase" and a strain of *chester* also labelled "specific" were found to contain both phases. I had to rely, therefore, on my available strains for the tests.

The results obtained are given in Table 8, and they confirm my previous findings. The specific phase of *newport* var. *Kottbus* reduced the titre of *onderstepoort* type serum from 3,200 to 200, while the type phase of *newport* lowered it to 400, and *anatum* var. *Muenster* decreased it to 300. On the other hand, the type phase

of *onderstepoort* reduced the titre of the specific agglutinins of *newport* var. *Kottbus* serum from 3,200 to 200, of *newport* from 2,000 to 400 and of *Muenster* from 12,800 to 1,600. Accordingly, the specific phase (factors e.h.) of *newport* and *newport* var. *Kottbus* could not remove a small residue of agglutinins from *onderstepoort* serum, and *onderstepoort* failed to exhaust a small residue of agglutinins from both *newport* and *newport* var. *Kottbus* sera.

TABLE 7.

Antigen.	*Typhi-murium* Serum Absorbed by *Typhi-murium*.	*Typhi-murium* Serum Absorbed by Strain 234.	*Typhi-murium* Serum Un-absorbed.	234 Serum Absorbed by *Typhi-murium*.	234 Serum Absorbed by Strain 234.	234 Serum Un-absorbed.
Typhi-murium "O"	0	0	1,600	0	0	800
Typhi-murium type	200	200	100,000	0	0	6,400
Typhi-murium group	100	100	50,000	0	0	3,200
234—"0"	0	0	1,600	0	0	800
234—type	200	200	100,000	0	0	6,400
234—group	100	100	50,000	0	0	3,200

The specific and non-specific "H" agglutinins of *typhi-murium* could not be completely exhausted on account of the high titre of the unabsorbed serum.

0 = less than 1 : 100.

Moreover, *onderstepoort* type antigen was barely agglutinated by the serum of *abortus-equi* (factors *enx*) at 1:400, while the homologous titre was 1:6400; in the same way *onderstepoort* serum just flocculated *abortus-equi* "H" antigen at 1:200. When absorption tests were performed *abortus-equi* could not appreciably reduce the titre of *onderstepoort* serum and *onderstepoort* had no effect in lowering the titre of *abortus-equi* serum. Factor *e* of *abortus-equi* is, therefore, not well represented in *onderstepoort*.

It is evident from these results that the specific phases of *onderstepoort* and *newport*, although not entirely alike, have a great deal in common. The specific phase (factors *e.h.*) of *newport* is well represented in *onderstepoort*, and the specific phase of *onderstepoort* has a great deal in common with that of *newport*. If the component shared by *onderstepoort* and *newport* is represented by specific factor *e*, then *abortus-equi* should be expected to lower the titre of *onderstepoort serum* for the type phase of newport. According to Table 8 *abortus-equi* failed to reduce the titre of *onderstepoort* serum for the specific phases of both *onderstepoort* and *newport*.

The specific phase of *onderstepoort*, therefore, contains a factor in addition to the small one which it shares with *abortus equi*. This factor comprises most of the *eh* of *newport*, but it does not correspond to the entire *eh*.

Antigens.	Unobserved Group Sera.				
	Onderste-poort.	*Reading.*	*Newport.*	*Binns.*	*Kunzen-dorf.*
Onderstepoort	25,600	25,600	6,400	12,800	3,200
Reading	25,600	25,600	—	—	—
Newport	800	—	12,800	—	—
Binns	800	—	—	25,600	—
Kunzendorf	25,600	—	—	—	3,200
Paratyphi—C	25,600	—	—	—	—
Sendai	12,800	—	—	—	—
London	1,600	—	—	—	—
Anatum	1,600	—	—	—	—

TABLE 6.—*Absorbed Group Sera.*

ONDERSTEPOORT, GROUP SERUM ABSORBED BY.									
Binns.	*London.*	*Binns+ London.*	*Binns+ London+ Kunzendorf.*	*Kunzendorf.*	*Paratyphi-C.*	*Reading.*	*Newport.*	*Sendai.*	*Anatum.*
12,800	3,200	3,200	800	800	800	400	6,400	800	3,200
—	—	—	—	—	—	0	—	—	—
—	—	—	—	—	—	—	50	—	—
0	0	0	0	0	—	—	—	0	—
—	—	—	0	0	—	—	—	50	—
—	—	—	—	—	0	—	—	—	—
—	—	—	—	—	—	—	—	0	—
400	0	0	0	0	—	—	—	0	—
—	—	—	—	—	—	—	—	—	0

0 = less than 1 : 50.

			Absorbed Sera.			
Anatum.	*Anatum*+ *Binns.*	*Anatum*+ *Binns*+ *Kunzendorf.*	*Reading* Absorbed by *Onderstepoort.*	*Newport* Absorbed by *Onderstepoort.*	*Binns* Absorbed by *Onderstepoort.*	*Kunzendorf* Absorbed by *Onderstepoort.*
3,200	3,200	800	0	50	0	0
—	—	—	400	—	—	—
—	—	—	—	6,400	—	—
—	0	0	—	—	6,400	—
—	—	0	—	—	—	400
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
0	0	0	—	—	—	—

← 107-108b 107-108c

TABLE 8.

Type Antigens.	*Onderstepoort* s.a.b. *Newport* var. *Kottbus*.	*Onderstepoort* s.a.b. *Newport*.	*Onderstepoort* s.a.b. *Anatum* var. *Muenster*.	*Newport* var. *Kottbus* s.a.b. *Onderstepoort*.	*Newport* s.a.b. *Onderstepoort*.	*Anatum* var. *Muenster* s.a.b. *Onderstepoort*.	*Onderstepoort* Serum.	*Newport* Serum.	*Newport* var. *Kottbus* Serum.	*Anatum* var. *Muenster* Serum.	*Abortus equi* s.a.b. *Onderstepoort*.	*Abortus equi* Serum.	*Onderstepoort* Serum a.b. *ab. equi*.
Onderstepoort.....	200	400	800	0	0	0	3,200	2,000	3,200	12,800	0	400	3,200
Newport var. *Kottbus*.....	0	—	—	200	—	—	3,200	—	3,200	—	—	—	—
Newport.....	—	0	—	—	400	—	3,200	2,000	—	—	—	400	3,200
Anatum var. *Nuenster*.....	—	—	0	—	—	1,600	3,200	—	—	12,800	—	—	—
Abortus equi.....	—	—	—	—	—	—	200	200	—	—	6,400	6,400	0

s. = serum; a.b. = absorbed by; 0 = less than 1 in 50.

The fact that *onderstepoort* " O " serum (titre=6,400) agglutinates *senftenberg* " O " suspension nearly up to full titre (1:3,200) shows that *onderstepoort* also contains an " O " factor which is present in *senftenberg;* but, *onderstepoort* " O " serum barely agglutinates *paratyphi-A* (factors 1, 11) at 1:100 and it fails to agglutinate *anatum* and *London* (factors III, X). It can be assumed, therefore, that factors II and III which are also contained in *senftenberg*, are either entirely absent or so poorly represented in *onderstepoort* that they can be disregarded. Hence it is probable that the additional factor contained in *onderstepoort* and shared by *senftenberg* is factor XIX of Kauffmann (1937).

The following antigenic analysis can, therefore, be assigned to *onderstepoort*:—

O=XIV, (XIX).

H.specific=the greater part, but not the whole of *eh* of *newport*.

H.non-specific=1, 2, 4, 5.

The second strain of *Salmonella* (*culture* 234) obtained from sheep was also tested against various " O ", type and group sera. It was found to be diphasic and was readily agglutinated by " O " sera of group B of the the Salmonella Sub-committee, by the type serum of *typhi-murium* and the group serum of *cholerae-suis* (European). Absorption tests were, therefore, carried out between culture 234 and *typhi-murium* (Glasgow) (Table 7). Culture 234 was first plated on Mason tubes and individual colonies tested for type and group phases as described above; type and group antigens were prepared from the colonies identified.

The results of Table 7 show that *typhi-murium* removed all " O " type and group agglutinins from 234 serum, as well as from the homologous serum, while culture 234 completely exhausted both *typhi-murium* serum and its own serum. Accordingly culture 234 must be regarded as identical with *typhi-murium*, and its antigenic structure should be made up of the same components, viz. " O " antigen IV, V, " H " specific antigen *i*, " H " non-specific antigen 1, 2, 3. For fermentation reactions see Table 25.

SALMONELLA INFECTION OF PIGS.

Salmonella infection is fairly common in pigs. Although Salmon and Smith's (1885) interpretation of the significance of *S. cholerae-suis* as an etiological factor of swine fever is no longer accepted, there can be no doubt that this organism is an important pathogen for pigs and a frequent cause of food-poisoning in man. On comparing the hog-cholera bacillus with other members of the *Salmonella* group Smith and Moore (1894) found that it fermented dextrose, but no lactose or sucrose ,and that it was highly pathogenic for rabbits in very small doses. Kruse (1896) described the hog-cholera bacillus under the name of *Bacillus suipestifer* and, according to him, Selander regarded this organism as the cause of

Danish swine fever. Preisz (1898) also found *Bact. suipestifer* pathogenic for pigs and he incriminated it as the cause of " Schweineseuche " (swine-fever) and " Schweineseptikaemie " in Germany.

After Salmon's isolation of the hog-cholera bacillus from the blood and internal organs of most of the cases of swine-fever studied by him, this organism was universally accepted as the cause of this malady until de Schweinitz and Dorset (1904) pointed out that a disease indistinguishable from hog-cholera could be readily produced by injecting healthy pigs with morbid material and body fluids, that had been proved to be free from organisms. Later Dorset, Bolton and McBryde (1905) showed that, while hog-cholera could be most readily transmitted by means of inoculations of blood and serum from diseased pigs, the use of cultures of the hog-cholera bacillus only sometimes produced a disease resembling hog-cholera. Whereas pigs infected by means of morbid material from diseased ones could easily transmit swine-fever to in-contact pigs, those that were infected with culture remained practically innocuous for other pigs. They further showed that the causal agent of hog-cholera was contained in the filtrates of the blood and body fluids of sick animals, and that these filtrates, although entirely free from *Bact. choleraesuis*, were nevertheless highly infective. They regarded the hog-cholera bacillus merely as an accessory factor in the production of disease.

Bainbridge (1908) divided the members of the paratyphoid group into four sub-groups, viz. (1) *Paratyphosus-A;* (2) *Paratyphosus-B* which was indistinguishable in its cultural characteristics from *aertrycke* and *suipestifer,* but which could be differentiated by means of absorption tests; (3) *aertrycke* and *suipestifer* which were regarded as strains of the same organism indistinguishable from one another; and (4) *enteritidis* (Gaertner) sub-group easily differentiated from the preceding by means of absorption tests. Later Bainbridge (1911) and Bainbridge and O'Brien (1912) divided paratyphoid bacteria into two groups of separate organisms; the first group they regarded as identical with *B. suipestifer,* and the second similar to *Paratyphosus-B.* These workers used agglutination and absorption tests for their identification. The source of *Paratyphosus-B* was considered to be from cases of paratyphoid fever and carriers, while *suipestifer* was apparently obtained from contaminated food and cases of food-poisoning. Savage (1912) on the other hand, like Bainbridge (1908), considered that food-poisoning bacilli of the *aertrycke* type were indistinguishable from *suipestifer.*

Dammann and Stedefeder (1910) succeeded in infecting healthy pigs either by feeding or by inoculating cultures of *B. suipestifer* and transmitted swine-fever by means of filtered material. Glässer (1909) found a type of *B. suipestifer* as the cause of disease in young pigs and called the organism *Bac. paratyphi-suis.*

Dammann and Stedefeder described *B. suipestifer* (Voldagsen) as the cause of a disease in young pigs, resembling swine-fever clinically, and known as Ferkel-typhus (suckling pig disease). Although Glässer's bacillus was frequently regarded as identical with

the Voldagsen bacillus it is now known that they differ from each other in that the former is diphasic, while the latter is monophasic, occurring only in the group phase like European *cholerae-suis*. Both of them differ from *suipestifer* culturally; but serologically *Glässer* is indistinguishable from the American variety, whereas *Voldagsen* resembles the European type.

Jordan (1917), basing his division on the study of recently isolated strains, also divided paratyphoid organisms into four groups:—

(1) *Paratyphosus-A* which fermented arabinose rapidly and dulcite slowly, xylose being left unaltered; litmus milk was turned alkaline only after some time.

(2) *Paratyphosus-B* which rapidly fermented arabinose, dulcite and xylose, and turned litmus milk alkaline in a very short period.

(3) *Suipestifer* which fermented xylose rapidly, but arabinose and dulcite slowly or not at all, i.e. not sooner than after 24 hours incubation.

(4) *Enteritidis* which was indistinguishable from the *Paratyphosus-B* group culturally but not serologically.

Jordan and Victorson (1917) used lead acetate agar for the differentiation of the types of paratyphoid bacilli. All *enteritidis* strains and most *Paratyphosus-B* strains were found to blacken this medium, while all their *suipestifer* strains and typical *Paratyphosus-A* failed to do so. According to Bruce White (1926) Schutze found that the Hirschfeld bacillus and European *suipestifer* could be distinguished from the American variety by the fact that they readily blackened lead acetate.

In the course of an investigation of swine-fever Uhlenhuth and Hübener (1909) encountered a bacterium which they called *Paratyphosus-C* bacillus. They found that culturally it was indistinguishable from *B. suipestifer*, but that it was not agglutinated by either *suipestifer* or *Gaertner* serum, while its own serum was without effect on the hog-cholera bacillus. They claimed to have isolated this bacterium from the organs of swine-fever pigs, from sausages and from human, pig and calf excreta, and regarded it as similar to the organism concerned with calf dysentery. Heimann (1912) isolated a strain of the so-called *paratyphosus-C* bacillus from cases of food-poisoning at Hildesheim, following the consumption of infected pork obtained from emergency-slaughtered pigs, but Andrewes and Neave (1921) did not regard the tests employed by Heimann as sufficiently reliable for the recognition of the organisms. Bruce White (1926), on the other hand, identified some of the Hildesheim strains as European hog-cholera bacilli.

During the Great War, and subsequently, several closely related organisms were isolated from cases of paratyphoid fever, especially in Eastern Europe. In 1915 Neukirk (1918) encountered an outbreak of disease in the Turkish army and called the causal organism *Erzindjan bacillus*. Subsequently several other workers observed a similar type of organism in different localities. Weil and Saxl (1917) isolated them from a number of Russian prisoners suffering from

paratyphoid (Walhynian strain). Weil studied another type from Albania, while Dienes and Wagner (1918), on investigating an outbreak of disease among a group of Russian prisoners, encountered several strains which were agglutinated by Voldagsen serum. They regarded their organism as identical with Weil's strain, Neukirk's *erzindjan* strain and Uhlenhuth's *paratyphosus-C* bacillus. Hirschfeld (1919) also investigated an enteric-like disease in the Serbian army and called the causal organism *Bacillus paratyphosus-C*, in flagrant disregard of the original usage of this term by Uhlenhuth and Hübener (1909). Mackie and Bowen (1919) and Macadam (1919) observed a similar type of organism in Mesopotamia, while Garrow (1920) found it in East Africa. Schutze (1920, 1921) identified an organism isolated in India in 1914 as *paratyphosus-C* and divided the *Salmonella* group of organisms into two sub-groups:—(1) *Enteritidis* (Gaertner) and (2) *Paratyphosus-B*. He further divided the latter into four serological types, viz. Schottmuller, mutton, Hirschfeld and hog-cholera bacilli. In his mutton type he included the bacillus of swine typhus or animal *paratyphosus-B* (*typhi-murium*).

By means of agglutination and absorption tests Bruce White (1926) showed that the Hirschfeld strain and Weil's Albanian strain were identical diphasic organisms, while one of the cultures described by Weil and Saxl proved to be a typical *newport* strain. The *erzindjan* strain of Neukirk was also found to be a true Hirschfeld bacillus.

Tenbroeck (1920 a and b) regarded Hirschfeld's bacillus as serologically identical with the American hog-cholera bacillus, but different culturally; whereas the former fermented dulcite and arabinose and produced hydrogen sulphide, the latter failed to do so. Unlike the hog-cholera bacillus Hirschfeld's organism did not prove to be very pathogenic for rabbits. When these animals were first injected with live Hirschfeld bacilli they were resistant to subsequent inoculations of virulent *cholerae-suis*. Tenbroeck placed far more reliance on serological tests than on biochemical reactions, and on account of its serum reactions he placed the Hirschfeld bacillus in the hog-cholera group.

Andrewes and Neave (1921) noticed that the Glässer and Voldagsen strains resembled each other culturally but not serologically; Voldagsen serum was completely exhausted of all agglutinins by Glässer, while the specific agglutinins present in Glässer serum were almost entirely unaffected by saturation with Voldagsen. Glässer and Voldagsen did not produce much hydrogen sulphide, whereas this gas was readily formed by (European) *suipestifer* and *paratyphosus-C of Hirschfeld*. Andrewes and Neave divided the hog-cholera group of organisms into two sub-groups:—

Group 1 comprising American *suipestifer*, Glässer's *typhi-suis* and Hirschfeld's *paratyphosus-C*, while group 2 was composed of European *suipestifer* and the Voldagsen strain. They showed that any member of group 1 could exhaust all agglutinins from the serum of any member of group 2, while group 2 strains could not materially reduce the titre of the sera of group 1 strains for members of group 1, although the sera were completely exhausted for the members of group 2.

The various porcine strains of *Salmonella* of the hog-cholera type and those closely related human strains to which, regardless of its original usage, the term *Bacillus paratyphosus-C* is frequently applied, form a group of organisms with very close serological affinities. Although different workers have contributed towards the study of the composition of this group it was Bruce White (1926) who finally divided the members into four well-defined types:—

1. Eastern or Hirschfeld bacillus.
2. American hog-cholera bacillus.
3. European hog-cholera bacillus.
4. Glässer-Voldagsen (Ferkeltyphus) bacillus.

The differential features of these organisms were described by Bruce White (1926) and by Nabarro, White, Dyke and Scott (1929). The specific phases of the diphasic members of the group, viz. Hirschfeld bacillus, American hog-cholera bacillus, and the Ferkel-typhus bacillus (Glässer strain) are indistinguishable; the non-specific phases of the last two are identical, while the European hog-cholera bacillus and the Voldagsen strain differ from them only in so far as they lack any trace of specific phase antigen. The Hirschfeld bacillus differs from all these by the deficiency of its non-specific phase in some of the antigenic components. They all differ from each other biochemically. The Hirschfeld bacillus ferments mannite, dulcite, arabinose, but not rhamnose; the hog-cholera bacillus (both European and American) ferments mannite and rhamnose, but not dulcite and arabinose; while the Ferkel-typhus strains (Glässer-Voldagsen) ferment only arabinose and rhamnose. All strains, excepting the American hog-cholera bacillus, produce hydrogen sulphide. Recently Bruner and Edwards (1939b) have shown that a specific phase may be obtained from monophasic European *choleræ-suis*.

Tenbroeck (1920 a and b) expressed his surprise at the comparative infrequency of paratyphoid in man caused by the hog-cholera bacillus, while Savage and Bruce White (1925) also remarked upon the rarity of *suipestifer* food-poisoning in man. They considered that the slight virulence of the organism for man and the massive doses required for setting up an infection are responsible for the low incidence of the disease. According to Krüger (1932b), it was declared by Uhlenhuth (1926), at a meeting of the German Society of Microbiology, that *suipestifer* bacilli could not be regarded as very pathogenic for man: and it was stated by Ostertag that, although thousands of swine-fever pigs were slaughtered for human consumption, mass infection of man did not occur. Kruger considered that *suipestifer* frequently lives as a saprophyte in the human body, setting up an infection only when the resistance has been lowered by conditions like appendicitis. Nevertheless, *Salmonella* infection of porcine origin has been known to cause serious disease in man. Indeed, the number of human cases of infection with *choleræ-suis* recorded during recent years cannot be treated as insignificant. Apart from the number of outbreaks of paratyphoid fever in man in Eastern Europe, due to the Hirschfeld

bacillus, several cases are reported from time to time where the hog-cholera bacillus has been incriminated as the cause of the disease. Krumwiede, Provost and Cooper (1922) recorded an outbreak of paratyphoid fever in four members of a family after eating tapioca pudding. One of the patients died and *S. cholerae-suis* was isolated from the liver. It was thought that the source of the infection was pork that contaminated the pudding. Scott (1926) described four outbreaks of infection due to the European hog-cholera bacillus, involving over a hundred persons. In all the cases the source of the infection was traced to prepared meats. A fatal case of septicaemia in man caused by "*Bacillus* (*Salmonella*) *suipestifer*" (America) and resembling typhoid fever was studied by Bauer and McLintock (1929), while two cases of human infection with the America hog-cholera bacillus were reported by Nabarro, Bruce White, Dyke and Scott (1929). Another case of American *suipestifer* causing disease in man in England was described by Boycott and McNee (1936); the organisms were obtained from blood culture, but although they were diphasic they resembled the European type culturally.

Clayton, Milne and Menton (1930) recorded an outbreak of acute gastro-enteritis in eight persons following the ingestion of pork pie. Three of the cases ended fatally; from the intestines of these patients as well as from the stools of the other five, American *suipestifer* was isolated. Another case of *cholerae-suis* infection of man was described by Branham, Motyca and Devine (1930). Kuttner and Zepp (1932, 1933) reported eleven cases of *suipestifer* infection, mostly in children. Of these ten were due to the European variety, and only one was caused by the American type; *Bact. suipestifer* was obtained by blood culture from all the patients All the cases recovered excepting one which ended fatally.

In Germany Köbe (1930) described two strains of *suipestifer* obtained from cases of meat-poisoning following the ingestion of pork. The patients showed symptoms of septicaemia with gastro-enteritis, as in typhoid fever. Krüger (1932a) reviewed several outbreaks of paratyphoid fever in which *Bact. suipestifer* was incriminated as the causal agent.

Giglioli (1930) studied a number of cases of quinine resistant fever in British Guiana and found Hirschfeld bacillus in 72 out the 77 patients examined. The organisms isolated corresponded both culturally and serologically with Hirschfeld's bacillus. More recently D'Hooghe (1932) and Mattlet (1932) described a number of fatal cases of paratyphoid fever in the Belgian Congo where they incriminated Hirschfeld's bacillus as the cause, while Tenbroeck, Li and Yii (1931) recorded five cases of infection in man caused by the same organism in Peiping (China). Materna and Januschke (1925) incriminated *cholera-suis* as the cause of purulent meningitis in a man, while Ravitch and Washington (1937) described several cases of *suipestifer* septicaemia in Negro children.

In South Africa Greenfield and Judd (1936) and Henning and Greenfield (1937) have described an outbreak of food-poisoning following the ingestion of pork infected with *S. bovis-morbificans* (Basenau).

The organism was originally described by Greenfield and Judd (1936) as a new *Salmonella—suipestifer var. Afri. Aust.* But later Henning and Greenfield (1937) showed that it is not distinguishable from *bovis-morbificans*. Basenau. Cultures of the organism were tested against various " O " and " H " sera. These were agglutinated by " O " sera of organisms containing factors VI and VIII of the Kauffmann-White schema, by group sera and by the type sera of *heidelberg and bovis-morbificans.* The organism was, therefore, regarded as diphasic.

A culture was plated so as to yield several well-separated colonies after 24 hours incubation; a number of fresh colonies were picked into broth and incubated for 5 to 6 hours at 37° C. The cultures obtained were tested against a pure group serum, e.g. *Kunzendorf* serum, and also against the type sera of *heidelberg* and *bovis-morbificans.* The colonies that occurred in the group phase agglutinated with *Kunzendorf* serum, while those that occurred in the type phase were flocculated only by *heidelberg* and *bovis-morbificans* sera. Group, type and " O " suspensions were now prepared and tested against a number of sera (Table 9). It will be noticed that *heidelberg* serum agglutinated both the group and type antigens, but not the " O " antigen, that *kunzendorf* flocculated the group antigen, but neither the type nor the " O " antigen, and that *bovis-morbificans* serum agglutinated all three antigens up to a very high titre.

After suitable antisera were prepared against *Afri. Aust.* absorption tests were performed (Table 9). On absorbing *Afri. Aust.* serum with *heidelberg* all agglutinins for the type phases of both *heidelberg* and *Afri. Aust.* were exhausted, but there was hardly any reduction of the group agglutinins (from 6,400 to 3,200), and all the " O " agglutinins remained. When this partly absorbed serum was re-absorbed by *Kunzendorf* a marked reduction of group agglutinins (from 3,200 to 400) was effected, but the " O " titre remained unaltered; Kunzendorf also reduced the group titre of unabsorbed *Afri. Aust.* serum from 6,400 to 400, but it had no ecect on the type agglutinins. On the other hand, *Afri. Aust.* removed all the type, but very little of the group agglutinins from *heidelberg* serum, and it did not reduce the " O " titre. Moreover, *Afri. Aust.* absorbed most of the group agglutinins (from 3,200 to 200) from *Kunzendorf* serum without reducing its " O " titre appreciably.

On absorbing *bovis-morbificans* serum with *Afri. Aust.* and *Afri. Aust.* serum with *bovis-morbificans* all the type, group and " O " agglutinins for both organisms were completely exhausted.

It will be observed that *Kunzendorf* did not completely exhaust the group agglutinins from *Afri. Aust.* serum and that *Afri. Aust.* failed to remove all the group agglutinins from *Kunzendorf* serum. This occurrence cannot be explained as *Afri. Aust.* and *bovis-morbificans* have the same group antigenic factors, and, according to the Kauffmann-White schema, the group antigens of *Kunzendorf* and *bovis-morbificans* are identical. There was barely any " O " agglutination between *Afri. Aust.* and *Kunzendorf*, indicating that the somatic factor VI of *Kunzendorf* is either absent or poorly represented in *Afri. Aust.*

TABLE 9.—(After Henning and Greenfi

Antigen.	UNOBSERVED SERA.					
	Afri. Aust Serum Un-Absorbed.	*Heidelberg* Serum Un-Absorbed.	*Kunzendorf* Serum Un-Absorbed.	*Newport Kottbus* Serum.	*Muenchen* Serum.	*Bovis-morbi-ficans* Serum. Un-absorbed.
Afri. Aust. " O "	800	0	0	1,600	800	1,600
Afri. Aust. Type	6,400	6,400	0	—	—	6,400
Afri. Aust. Group	6,400	3,200	3,200	—	—	25,600
Heidelberg " O "	0	800	—	—	—	—
Heidelberg Type	6,400	6,400	—	—	—	—
Heidelberg Group	1,600	12,800	800	—	—	—
Kunzendorf " O "	50	—	800	—	—	—
Kunzendorf " H "	3,200	—	3,200	—	—	—
Bovis-morbificans " O "	800	—	0	—	—	1,600
Bovis-morbificans Type	3,200	—	—	—	—	6,400
Bovis-morbificans Group	6,400	—	—	—	—	25,600
Newport (Kottbus) " O "	—	—	0	1,600	—	—
Muenchen " O "	—	—	0	—	800	—

0 = less than 1 : 50.

Henning and Greenfield.)

Absorbed Sera.								
Afri. Aust Serum Absorbed with *Afri. Aust.*	*Afri. Aust* Serum Absorbed with *Kunzendorf.*	*Afri. Aust* Serum Absorbed with *Heidelberg.*	*Afri. Aust* Serum Absorbed with *Bovismorbificans.*	*Kunzendorf* Serum Absorbed with *Afri. Aust.*	*Heidelberg* Serum Absorbed with *Afri. Aust.*	*Bovismorbificans* Serum Absorbed with *Afri. Aust.*	*Bovismorbificans* Serum Absorbed with *Bovismorbificans.*	*Afri. Aust* Serum Absorbed by *Heidelberg* and then by *Kunzendorf.*
0	400	800	0	—	—	0	0	400
0	6,400	0	0	—	—	0	0	0
0	400	3,200	0	0	—	0	0	400
—	—	—	—	—	800	—	—	—
—	—	0	—	—	—	—	—	—
—	—	0	—	—	6,400	—	—	—
—	—	—	—	800	—	—	—	—
—	0	—	—	200	—	—	—	—
0	—	—	0	—	—	0	0	—
0	—	—	0	—	—	0	0	—
0	—	—	0	—	—	0	0	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—

ess than 1 · 50

← 117-118a 117-118b

These results clearly show that *S. suipestifer* var. *Afri. Aust.* of Greenfield and Judd has the same type antigen as *heidelberg* and *bovis-morbificans* (factor *r*) and a group antigen that corresponds largely with that of *Kunzendorf* and entirely with that of *bovis-morbificans* (factors 1, 3, 4, 5), while its somatic " O " antigen resembles that of *bovis-morbificans* (factors VI, VIII). Moreover, since *Afri. Aust.* removes all agglutinins, type, group and " O ", from *bovis-morbificans* serum, and *bovis-morbificans* exhausts all agglutinins from *Afri. Aust.* serum there can be no doubt that the two organisms are identical.

TABLE 10.

Antigen.	*Typhi-murium* s.a.b. *Typhi-murium.*	*Typhi-murium* s.a.b. 192.	192 s.a.b. *Typhi-murium.*	192 s.a.b. 192.	*Typhi-murium* s. Unabsorbed.	192 s. Unabsorbed.
Typhi-murium " O "	0	0	0	0	800	1,600
Typhi-murium " H " type	100	100	0	0	100,000	6,400
Typhi-murium " H " group	0	0	0	0	25,000	3,200
192—" O "	0	0	0	0	800	1,600
192—" H " type	100	100	0	0	100,000	6,400
192—" H " group	0	0	0	0	25,000	6,400

0 = less than 1 : 100 ; s. = serum ; a.b. = absorbed by. The " H " type titer of *typhi-murium* was so high (1 : 100,000) that it was impossible to remove a small residue (1 : 100) of the agglutinin.

The only other record of a *Salmonella* obtained from a pig is that of Robinson and Martinaglia (1932) when they described an organism isolated from a pig at Onderstepoort. A description of the antigenic structure of this organism, strain 192, was not attempted by them, but its antigenic analysis was subsequently performed by me (Table 10). It was noticed that strain 192 was agglutinated far better by *typhi-murium* than by *cholerae-suis* serum. The agglutination obtained with the latter serum was purely floccular, while with the former the agglutination was both granular and floccular, suggesting the existence of a closer relationship between strain 192 and *typhi-murium* than between it and *cholerae-suis*. Moreover, strain 192 was found to be diphasic; its type phase colonies were agglutinated by *typhi-murium* type serum, while the group phase colonies were flocculated by both *typhi-murium* and *cholerae-suis* group sera. Accordingly agglutination and absorption tests were performed as shown in Table 10, mixed " O " and " H " type and group sera being used for the tests. The results show that *typhi-murium* removed all agglutinins (" O ", " H " type and " H " group) from 192 serum as well as from the homologous serum; *culture* 192 also completely exhausted both *typhi-murium* serum and its own serum. Accordingly it was evident that *culture* 192 and

typhi-murium were composed of the same antigenic structure, and that they both contained the following antigenic factors of the Kauffmann-White schema:—" O " IV, V, " H " specific *i*, " H " non-specific 1, 2, 3.

In 1933 Dr. Robinson and myself isolated another strain of *Salmonella* (*culture* 168) from the blood of pigs suffering from a septicaemic disease in the Cape Province. This organism was readily agglutinated by *cholerae-suis* serum and was found to occur entirely in the group phase. Accordingly, absorption tests were conducted as shown in Table 11.

The results show that *culture* 168 removed all agglutinins from *cholerea-suis* (European) serum as well as from the homologous serum, and that *cholerae-suis* (European) exhausted both 168 serum and its serum. Absorption tests were also carried out with the diphasic American hog-cholera bacillus and its serum. It was found that *culture* 168, while completely removing all the " O " and group agglutinins from *cholerae-suis* serum, left the " H " specific titre almost unaltered. On the other hand *cholerae-suis* (America) completely exhausted all the agglutinins (" O " and " H " non-specific) from 168 serum. The results, therefore, showed that *culture* 168 was devoid of an " H " specific antigen and that it contained the same antigenic components as *cholerae-suis* (European), viz. the following factors of the Kauffmann-White scheme:—" O ", VI, VII. " H " non-specific 1, 3, 4, 5. " H " specific *nil*.

Six other strains, cultures 365, 380, 381, 382, 383, 384, isolated from the blood of pigs during an outbreak of swine fever in the Transvaal were also studied serologically. With strain 365 complete mirror absorption tests were carried out as in the case of *culture* 168 (Table 11) and exactly similar results were obtained. With the other five strains one-sided absorption tests were performed, using both European and American hog-cholera sera. Whereas all the agglutinins (" O " and " H " non-specific) were removed from European hog-cholera serum, the " H " specific agglutinins of the American *suipestifer* serum were unabsorbed. All six strains were found to be monophasic.

These results, therefore, clearly show that strains 365, 380, 381, 382, 383, 384 resemble *cholerae-suis* (European) antigenically, containing the same antigenic factors assigned to strain 168.

For fermentation tests see Table 25.

Murray (1934) cites several different workers who have isolated *cholerae-suis* from the faeces of a small percentage of apparently healthy pigs. He states, however, that he has been unable to demonstrate the presence of *suipestifer* in normal pigs.

SALMONELLA INFECTION OF EQUINES.

In horses infection with *S. abortus-equi* is undoubtedly the most common disease caused by the genus *Salmonella*. More than forty years ago Kilborne (1893) and Smith (1893) studied an outbreak of

TABLE 11.

Antigen.	*Cholerae-suis* (European) s.a.b. *Cholerae-suis* (European).	*Cholerae-suis* (European) s.a.b. 168.	168 s.a.b. *Cholerae-suis* (European).	168 s.a.b. 168.	168 s.a.b. *Cholerae-suis* (America).	*Cholerae suis* (America) s.a.b. 168.	*Cholerae-suis* (America) s.a.b. *Cholerae-suis* (America).	*Cholerae-suis* (European) Unabsorbed.	168 Serum Unabsorbed.	*Cholerae-suis* (America) Serum Unabsorbed.
Cholerae-suis (European) " O ".	0	0	0	0	—	—	—	800	1,600	—
Cholerae-suis (European) " H ".	0	0	0	0	—	—	—	6,400	12,800	—
168—" O ".....	0	0	0	0	0	0	0	800	1,600	800
168—" H ".....	0	0	0	0	0	0	0	6,400	12,800	6,400
Cholerae-suis (America) " O "...	—	—	—	—	0	0	0	—	1,600	800
Cholerae-suis (America) "H' type	—	—	—	—	0	3,200	0	—	0	3,200
Cholerae-suis (America) " H " group.....	—	—	—	—	0	0	0	—	12,800	6,400

0 = less than 1 : 100.
s. = serum.
a.b. = absorbed by.

abortions in mares and isolated a non-lactose fermenting organism of the hog-cholera group from the vaginal discharges of the affected animals. This organism was found to be pathogenic for rabbits and was regarded as the cause of the abortions; on cultivation it formed a membranous growth with wrinkled edges on the agar.

Subsequently several different workers investigated outbreaks of infectious absortion in mares caused ostensibly by the same bacterium studied by Kilborne and Smith. Good and Corbett (1913) studied a very serious epizootic in Kentucky due to organisms of the *enteritidis* hog-cholera group, which produced nearly 100 per cent. abortions. Intravenous inoculations of cultures of this organism caused abortions in mares within 10 days. About the same time Meyer and Boerner (1913), de Jong (1913), Dassonville and Riviere (1913), van Heelsbergen (1914) and Schofield (1914) also described epizootics of abortion in mares due to Bact. abortus-equi. Later MacFadyean and Edwards (1917) discussed the relationship of infectious abortion in mares and joint-ill in foals, while Miessner and Berge (1917) and Murray (1919) also incriminated *abortus-equi* as the etiological agent of outbreaks of abortion in mares.

Apart from causing abortions in equines this organism has been found responsible for pyaemic arthritis, joint-ill, abscessation and tendo-vaginitis. While studying the etiology of infectious arthritis in colts in America, Good and Smith (1914) isolated from the pus of the joints a bacterium which resembled the causal agent of infectious abortion in mares; but from the affected synovia of one foal they obtained *streptococci* only. In the outbreaks of pyaemic arthritis in foals investigated by Schofield (1914) Gram-negative bacteria were isolated in pure culture from the synovia of the affected joints—in a few cases only, the culture yielded a mixed growth of Gram-negative bacteria and *streptococci*. The former were regarded as closely related to the bacterium of contagious abortion in mares. In Germany Miessner and Berge (1917) ascribed the cause of a severe epizootic of abortion in a stud to a paratyphoid organism, which was isolated from the stomach and intestines of dead foetuses; they pointed out that the majority of the foals which were born alive on the affected farm developed joint-ill, but *streptococci* were regarded as the most important etiological agent, paratyphoid organisms being obtained from only one case. In a comprehensive study of contagious abortion in mares and joint-ill in foals, MacFadyean and Edwards (1917) found *Bact. abortus-equi* as the most common cause of the two diseases. They isolated this organism from the heart-blood and internal organs of several of the aborted foetuses, and also from the joints of a number of foals affected with joint-ill. Some of the horses that were immunised with *abortus-equi* for the purpose of serum production developed arthritis. Magnusson (1919) on the other hand, considered an organism, which he called *Bact. viscosum equi*, as the most common cause of joint-ill in foals.

In South Africa Martinaglia (1929) described several cases of tendo-vaginitis in adult horses due to *abortus-equi* following horse-sickness immunisation. Out of twelve cases studied in 1922, nine yielded pure cultures of *abortus-equi*, while in the remaining three a mixed infection of this organism and a streptococcus was found.

One animal, a stallion, was affected with orchitis due to *abortus-equi*. In 1925 a similar condition appeared in mules, also after immunisation against horsesickness.

Seymour (1936) also incriminated *abortus-equi* as the cause of an outbreak of pyosepticaemia in foals, while Fujimura and Hoshi (1936) described outbreaks of contagious abortion and cases of abscessation in equines due to this organism. Moreover, they reported a case of *abortus-equi* infection in man.

Although the antigenic structure of the organism incriminated in these outbreaks is not clearly given, there seems to be very little doubt that *abortus-equi*, or a very closely related bacterium, was responsible for most of the cases. The strains isolated by Martinaglia were described as actively motile; but only one of these, *culture* 219, was kept. When this strain was finally received by me it was found to have lost all its properties of motility. After preparing antisera, agglutination and absorption tests were performed with this organism and *abortus-equi WH2*. The results of these tests showed conclusively that *culture* 219 and *abortus-equi WH2* had the same somatic antigen; culture 219 removed all the " O " agglutinins from *abortus-equi WH2* serum as well as from the homologous serum, while antigen; *culture* 219 removed all the " O " agglutinins from both sera. As *culture* 219 was non-motile its serum was devoid of " H " agglutinins and it left the " H " agglutinins of *abortus-equi WH2* serum unaltered.

Apart from *abortus-equi* infection, other types of *Salmonella* are sometimes responsible for outbreaks of disease in solipeds. Thus, Moulin and Amichau (1918), Combes (1918) and Urbain, Stocanne and Chaillot (1929) described epizootics in horses due to paratyphoid bacilli. Graham, Reynolds and Hill (1919) studied a virulent outbreak in a shipment of horses and mules due to *enteritidis*. Meissner incriminated *typhi-murium* as the cause of a disease in foals and obtained this organism as well as *abortus-equi* from mares that had aborted. Moreover, Lutje (1930) isolated both *enteritidis* and *typhi-murium* from equines affected with abortion, and he obtained *enteritidis* from foals exposed to infection with calves. Standfuss (1925) and Lehr (1928) isolated paratyphoid organisms from horses that were slaughtered in emergency. Other workers like Baumann and Gratzl (1931) and Arnberger (1931) described outbreaks of gastro-enteritis in horses due to *typhi-murium*, while Edwards (1934) investigated an epizootic of infectious colitis in 3 to 7 months old foals caused by the same organism. Cernozubov, Pilipovie and Stavel (1937) claim to have isolated five strains of *typhi-murium* and four of *paratyphi-B* from diseases in horses.

But apart from causing diseases in equines *Salmonella* infection of horses may lead to serious outbreaks of food-poisoning in countries where horse flesh is used for human food. Thus, during the Great War and the years immediately following more than 25 per cent. of all outbreaks of meat-poisoning in Germany were traced to horse meat, on the other hand, the incidence of gastro-enteritis from this source has been very low during recent years (Meyer 1934, 1936). In 1923, Meyer recorded 19 outbreaks and in 1932 only one that could be ascribed to this cause. Kuppelmayr (1924) described 47 outbreaks

of food-poisoning, involving 5,440 cases and causing 63 deaths, all traced to the consumption of infected horse meat. Elkeles (1925) recorded 61 outbreaks of meat-poisoning in Germany during 1923, involving 3,093 persons and causing 20 deaths; the majority of these cases were due to horse-meat. Glage (1916) studied an epizootic of food-poisoning due to horse-meat; 392 persons were affected and there were 2 deaths. Organisms of the *paratyphus-B* group were isolated from the suspected meat and from the stools of the patients. Infection of man following the consumption of horse-meat was also described by Muller (1921). Clarenburg (1931) described two outbreaks of food-poisoning in Holland where the cause of infection was horse-meat. Uhlenhuth (1925) isolated *typhi-murium* from patients who were suffering from acute gastro-enteritis following the consumption of horse-meat, while Kauffmann and Silberstein (1934) obtained *anatum* var. *Muenster* from a person who had developed food-poisoning after a meal containing raw horse-meat. Several other outbreaks of *Salmonella* food-poisoning in man resulting from the ingestion of horse-meat has been studied in the Reichsgesundheitsamt in Germany. Many of these have followed the consumption of meat from animals slaughtered in emergency.

Recently my colleague, Mr. R. Clark, investigated an outbreak of purulent arthritis in foals in the Orange Free State (Henning and Clark, 1938). He obtained pus from the affected joints of one foal and made cultures on agar slants; the growths obtained were submitted to me for identification. These were plated on MacConkey's bile-salt agar and yielded pure cultures of a non-lactose fermenting bacterium which looked like a *salmonella*. Several of the single colonies obtained were tested against various " O ", type and group sera. They were all agglutinated by the " O " sera of group B of the Kauffmann-White schema, and it was at first thought that the organism was probably *abortus-equi*. But, on further testing, it was found that some of the colonies were agglutinated by *typhi-murium* type serum, while others were flocculated by a pure group serum, like that of *cholerea-suis* var. *Kunzendorf*. The organism (*culture* 478) therefore was diphasic. Accordingly, antisera were prepared against it for the purposes of carrying out agglutination and absorption tests.

These tests (Table 12) show that *typhi-murium* removes all " O ", " H "-type and " H- "-group agglutinins from 478 serum, while 478, although completely exhausting the " H " agglutinins from *typhi-murium* serum, reduced the " O " titre of the serum from 800 to approximately 200. *Culture* 478 also absorbed all the type and group agglutinins from *aberdeen* serum without altering the " O " titre, and *aberdeen* exhausted all the " H " agglutinins from 478 serum, but failed to redule its " O " agglutinin content. On the other hand, *culture* 478 removed all the agglutinins (" O ", type and group) from both *storrs* and *copenhagen* sera, while both *storrs* and *copenhagen* completely exhausted 478 serum. The results of these tests, therefore, showed that *culture* 478 is identical with *Salmonella typhi-murium* var. *storrs* (Edwards, 1935), or *S. typhi-murium* var. *copenhagen* (Kauffman, 1934), containing the following antigenic formula:—" O " IV, " H "-specific *i*, " H "-non-specific 1, 2, 3. The fermentation reactions of culture 478 are given below (Table 25).

Strains of *Salmonella typhi-murium* devoid of "O" factor V were first described by Landsteiner and Levine (1932) when they studied the *Binns* strain of Schutze. Later Kauffmann (1935a) recorded 16 variants of *typhi-murium* which contained "O" factor IV, but not factor V, and he called these variants *typhi-murium* var. *Copenhagen*. About the same time Jungherr and Wilcox (1934) obtained from pigeons a strain of *typhi-murium* which reacted atypically with maltose; an antigenic analysis of this organism made by Edwards (1935) showed that it is lacking in "O" factor V. Edwards called the variant *typhi-murium* var. *Storrs*. Hohn and Hermann (1937) also recorded an outbreak of disease in pigeons due to the IV-variant of *typhi-murium*, while Hoffmann and Edwards (1937) studied an infection in rabbits caused by the same type of organism. Moreover, several cases of infection in man due to strains of *typhi-murium* devoid of "O" factor V have been described by Zahn (1935).

Both Edwards and Kauffmann found that the IV-variants exhibited biochemical reactions that are not typical for *typhi-murium* and that strains from different localities did not always react in the same way.

Edwards (1938) points out that all the recorded outbreaks of disease due to IV-variants of *typhi-murium* have occurred in man, pigeons and rabbits; no IV-variants were found among *typhi-murium* cultures obtained from horses, sheep, guinea-pigs, rats, mice, turkeys, chickens, ducks and canaries. The strain of *typhi-murium* var. *Copenhagen* (Storrs) described by me is, therefore, the first record of this organism obtained from a horse.

SALMONELLA INFECTION OF BIRDS.

Infection of birds with different types of *Salmonella* is much more varied and widespread than in mammals, and the losses sustained through this group of organisms are probably far greater than those resulting from any other cause. Epizootics in fowls due to *S. gallinarum* are extremely common in some countries; in South Africa, fowl typhoid is without doubt the most serious infectious disease of fowls, while in Europe and America *Pullorum* disease seems to be more important. Epizootics due to *Salmonellas* other than *gallinarum* and *pullorum*, although less common, may nevertheless be responsible for serious losses in all species of domestic birds. It is with a discussion of these diseases that this part of my paper is chiefly concerned. Although relatively few outbreaks of paratyphoid in pigeons, ducks and geese have been recorded in South Africa, my discussion will not be complete unless the literature relating to disease in these birds is duly reviewed. Moreover, *Salmonella* infection, other than that due to *gallinarum* and *pullorum* occurs apparently more frequently in them than in gallinaceous birds. The extensive literature relating to fowl typhoid and *pullorum* disease is not discussed in this paper; it has been fully reviewed by a number of different workers.

Salmonella infection is most common, and also most serious, in very young birds. Adult birds usually suffer from a chronic form of the disease with lesions in the ovary, testes, joints, liver and spleen; whereas in young birds septicaemia and enteritis with changes in the internal organs are more frequently observed. The infection may be the cause of serious losses in the affected flocks and of food-poisoning in man, either through the medium of infected meat or eggs. Several different species of birds may be affected, and a number of different types of *Salmonella* have been incriminated as etiological agents.

In a recent review Schaaf (1936) mentioned *typhi-murium*, *enteritidis*, *anatum*, *cholera-suis* and *abortus-equi* as the causes of paratyphoid in birds; while Edwards (1936, 1937) has found *oranienburg* as the cause of an infection in quail and *Senftenberg* responsible for a disease in turkeys. Recently I have recorded an outbreak in chickens due to *Salmonella amersfoort* (Henning, 1937). *Typhi-murium* seems to be the most common cause, with *enteritidis* next in importance; the other organisms are only rarely found.

Natural infection usually occurs by means of foods or water contaminated with the excreta of infected animals or birds; but transmission may also take place through the medium of the egg which has obtained the infection in the ovary or oviduct, or which has been contaminated by means of infected faeces. Sometimes the embryo is dead in the shell as a result of the infection, but generally the newly hatched birds develop the disease during the first few days of life. There are several predisposing factors like bad hygiene, improper feeding and infestation by parasites which favour infection; the dirty habits of water birds, probably account for the frequency of paratyphoid in ducks and geese, as well as the number of outbreaks of food-poisoning that result from the ingestion of food-stuffs containing their eggs or meat as ingredients.

Lerche (1936) considers that 5·7 per cent. of the duck eggs sold in Germany are infected with *Salmonellas*. Frequently the shell is contaminated with infected faeces and under favourable conditions the organisms penetrate from the shell into the interior of the egg; but although the yolk is an excellent culture medium, the albumen of the fresh egg is strongly bactericidal (Lachtschenko, 1909, Rettger and Sperry, 1912, and Scott, 1930). This germicidal action, however, deteriorates when the egg becomes stale and when it is exposed to warm, moist weather for more than two weeks, the organisms may penetrate into the interior and increase in number; this increase occurs only when some yolk has diffused into the albumen. The most dangerous source of infection is food which contains duck eggs as an ingredient, and in which the organisms can readily multiply, e.g. creams, custards, puddings and " Hackfleisch " that have not been sufficiently heated during the preparation; mayonnaise is too acid for bacterial growth and is, therefore, less dangerous. The danger of eating duck eggs in the raw state is obvious, but even frying or boiling may not be sufficient to kill the organisms. Lerche (1936) considers that after 5 minutes boiling the temperature of the yolk of a duck's egg may not be much more than

Antigen.	478 Serum Absorbed by :—					
	478.	*Storrs.*	*Copenhagen*, 659.	*Typhi-murium.*	*Abortus-equi.*	*Aberdeen.*
78—" O "	0	0	0	0	0	800
78—type	0	0	0	0	12,800	0
478—group	0	0	0	0	3,200	0
Storrs—" O "	0	0	—	—	—	—
Storrs-type	0	0	—	—	—	—
Storrs-group	0	0	—	—	—	—
Copenhagen " O "	0	—	0	—	—	—
Copenhagen-type	0	—	0	—	—	—
Copenhagen-group	0	—	0	—	—	—
Typhi-murium " O "	0	—	—	0	—	—
Typhi-murium-type	0	—	—	100	—	—
Typhi-murium-group	0	—	—	100	—	—
Abortus-equi " O "	—	—	—	0	0	—
Abortus-equi " H "	—	—	—	—	0	—
Aberdeen " O "	—	—	—	—	—	0
Aberdeen type	—	—	—	—	—	0
Aberdeen group	—	—	—	—	—	0

0 = Less than 1

TABLE 12.

ABSORBED SERA.

Storrs Serum Absorbed by :—		*Copenhagen* 659 Serum Absorbed by :—		*Typhi-murium* Serum Absorbed by :—		*Abortus-equi* Serum Absorbed by :—		*Aberdeen* Serum Absorbed by :—
478.	*Storrs.*	478.	*Copen-hagen.*	478.	*Typhi-murium.*	478.	*Abortus-equi.*	478.
0	—	0	0	0	0	0	0	0
0	—	0	0	0	0	—	—	0
0	—	0	0	0	0	—	—	0
0	0	—	—	—	—	—	—	—
0	0	—	—	—	—	—	—	—
0	0	—	—	—	—	—	—	—
—	—	0	0	—	—	—	—	—
—	—	0	0	—	—	—	—	—
—	—	0	0	—	—	—	—	—
—	—	—	—	200–400	0	—	—	—
—	—	—	—	100	100	—	—	—
—	—	—	—	100	100	—	—	—
—	—	—	—	—	—	0	0	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	400
—	—	—	—	—	—	—	—	100
—	—	—	—	—	—	—	—	0

0 = Less than 1 : 100. — = No test carried out.

Unabsorbed Sera.

478.	*Storrs.*	*Copenhagen.*	*Typhimurium.*	*Abortus-equi.*	*Aberdeen.*
800	1,600	1,600	800	800	0
12,800	6,400	12,800	25,600	—	50,000
3,200	25,600	6,400	25,600	—	6,400
800	1,600	—	—	—	—
12,800	6,400	—	—	—	—
3,200	25,600	—	—	—	—
800	—	1,600	—	—	—
12,800	—	12,800	—	—	—
3,200	—	6,400	—	—	—
800	—	—	800	—	—
12,800	—	—	25,600	—	—
3,200	—	—	25,600	—	—
1,600	—	—	—	1,600	—
—	—	—	—	—	—
0	—	—	—	—	400
12,800	—	—	—	—	50,000
3,200	—	—	—	—	6,400

← 127-128b 127-128c

40° C., while Bruns and Fromme (1934) state that after boiling an egg in the shell for 3½ minutes the temperature in the interior is only 28° C., but after 5 minutes boiling it rises to about 65° C.

(1) Pigeons.

The first record of a disease in birds caused by a *Salmonella* is given by Moore (1895) in his description of a severe epizootic in pigeons due to a bacillus of the hog-cholera group. The organism was recovered from the heart-blood and internal organs of affected birds. Salmon (1904) also described a rapidly fatal disease in pigeons, due to an organism of the " enteritidis group ". Another outbreak of pigeon paratyphoid was described by Zingle (1914), when he investigated a mortality among military birds at Strassburg. Organisms of the *Paratyphoid-B* group (*typhi-murium*?-) were obtained in pure culture from the heart-blood and organs of diseased birds, but it was not quite clear whether this infection was primary or secondary as the birds were affected simultaneously with pigeon-pox. The invasion of the body by paratyphoid organisms under certain abnormal conditions is explained by Cash and Doan (1931). They have found that latent infections with *typhi-murium* become seemingly active under adverse conditions.

Subsequently several other workers described outbreaks of *Salmonella* infection in pigeons. Thus, Reitsma (1924) studied an epizootic in Holland, as a result of which the pigeons developed an ulcerative enteritis and became very much emaciated; a pure culture of an organism, labelled " *B.paratyphus-B* " (*typhi-murium*) was obtained from the liver of the affected birds. Sahaya and Willems (1927) recorded a chronic and an acute form of the disease affecting adult and young birds respectively. The adult pigeons were usually afflicted with a severe arthritis and swelling of the joints, associated with softening and atrophy of the pectoral muscles, while young birds suffered mostly from acute enteritis. A *Salmonella*, which was not identified, was isolated from the pus of the joints in the chronic cases and from the heart-blood of the young birds. On investigating the cause of a serious epizootic among a group of young squabs, Beaudette (1926b) found *typhi-murium* in the heart-blood, internal organs and unabsorbed yolk of the young birds. The sick birds showed nervous symptoms, like incoordination of movements and convulsions, and diarrhoea; the lesions were swelling of the liver and lungs, catarrhal enteritis and inflammation of the proventriculus. The birds had been kept under very unhygienic conditions, which were regarded as a predisposing factor.

Several outbreaks of paratyphoid in pigeons from widespread areas in Germany were studied by Beck and Meyer (1927). The cause was ascribed to *typhi-murium* (Breslau) and the disease affected old birds as well as young ones. Beck (1929) considered that the etiological agent of pigeon paratyphoid resembled *typhi-murium* (Breslau) serologically, and that adult birds were much less susceptible than young ones and that the latter could be readily infected parenterally or otheriwse. Berge (1929) regarded paratyphoid as one of the most important diseases of pigeons in Germany —of 193 birds examined by him 22·6 per cent. were found to be

infected with *typhi-murium* (Breslau). Young birds commonly suffer from an acute form of the disease, but in older birds the condition is generally chronic and the symptoms may last for several weeks; the joints are swollen and there is paralysis of the muscles of locomotion and flight. Emmel (1929) also found *Schottmuller* (*typhi-murium*?) in practically pure culture in the exudates obtained from the swollen joints of pigeons examined by him.

By examining a flock of over 8,000 pigeons suffering from weakness of the wings and swelling of the joints Brunett (1930) found a straw coloured exudate in the joint swellings and abnormalities in the ovaries, resembling those of *pullorum* disease. *Typhi-murium* was isolated from the joints as well as from the ovaries.

Recently Jungherr and Wilcox (1934) investigated the cause of a disease in a flock of about 1,500 pigeons in which there was an annual loss of about 20 per cent. They incriminated an atypical non-maltose fermenting variant of *typhi-murium* as the etiological agent. In some cases *typhi-murium* could not be obtained from reacting squabs, while at other times the organisms were isolated from birds that failed to react serologically. Edwards (1935b) studied the same variant from three widely separated areas and found the "O" antigen, like that of *abortus equi*, lacking in factor V of the Kauffmann-White schema. The variant was noticed to be non-maltose fermenting and negative to the Bitter test; it appeared to be similar to *typhi-murium var. Copenhagen* of Kauffmann (1935a). Edwards labelled the organism *S. typhi-murium var. Storrs*. A similar organism, obtained from a case of purulent arthritis in a foal, is described by me above.

Lesbouyries and Verge (1932) described pigeon paratyphoid in France and Cernaianu and Popovici (1933) in Rumania, while Ismail Abu Bakr Khalifa (1935) studied an epizootic in Egypt due to *typhi-murium*. More recently Shirlaw and Ganapathy Iyer (1937) have recorded an outbreak of pigeon septicaemia in India caused by what they called a "Gaertner infection". Soon after a number of birds had been inoculated with fowl-pox vaccine they developed symptoms of acute enteritis and fever from which they died. It is not possible to recognise the type of *Salmonella* incriminated from the description given.

That infection of pigeons with *Salmonella* may lead to serious outbreak of food-poisoning in man is illustrated by the description of Clarenburg and Dornickx (1932) of an epizootic which involved 20 persons in the military hospital at the Hague. The source of the infection was traced to pudding made largely from pigeons' eggs. *S. typhi-murium* was isolated from the pudding, and from the blood, faeces and urine of some of the patients; the sera of the affected persons also agglutinated cultures of the *Salmonella* found. On investigations, it was ascertained that the flock of pigeons from which the eggs originated were suffering from paratyphoid. Moreover, *typhi-murium* was recovered from eggs laid by these birds.

Although several outbreaks of a Septicaemic disease in pigeons have been reported in South Africa from time to time the cause has remained obscure until recently when Henning and Haig (1938)

studied an epizootic of squabs in which a *Salmonella* was found to be the cause. (The outbreak was studied after the completion of this paper.)

The affected birds suffered from loss of appetite, acute diarrhoea with green evacuations and rapid loss of condition. The most important lesions observed were enlargement of the spleen and liver and acute catarrhal enteritis. The affected flock was composed of over 200 birds, of which 24 have died from the disease. Heart-blood, spleen and liver cultures yielded a pure growth of a non-lactose fermenting, Gram-negative motile bacterium (culture 548). On testing this bacterium against various " O ", type and group sera of different groups of *Salmonella*, it was agglutinated by " O " sera containing factor IV of the Kauffmann-White Schema, by type sera containing factor *i* and by group sera. This suggested that the organism is related to *typhi-murium*. Agglutination and absorption tests were, therefore, performed with different varieties of *typhi-murium*. The results are given in Table 13.

The results of Table 13 show that *typhi-murium* absorbed all the agglutinins, " O " type and group, from 548 serum as well as from its own serum, but that culture 548 merely reduced the " O " titre of *typhi-murium* serum from 3,200 to 1,600. Culture 548 removed all the " O " agglutinins from its own serum but failed to exhaust a small portion of type and group agglutinins from both its own and *typhi-murium* serum. This is attributed to the reduction of its motility which occurs on subcultivation on solid agar.

When 548 serum was absorbed with either *typhi-murium* var. *Storrs* or *typhi-murium* var. *Copenhagen* all the " O " agglutinins were removed for *typhi-murium*, *typhi-murium* var. *Storrs*, *typhi-murium* var. *Copenhagen* and for itself.

According to these results, therefore, culture 548 contains the same type and group antigens as *typhi-murium* and the same " O " antigen as *typhi-murium* var. *Copenhagen* (Storrs). Its antigenic formula should be " O "=IV, type *i*, group 1, 2, 3.

An outbreak of pyo-arthritis in foals caused by the IV-variant of *typhi-murium* is described on page 124.

(2) Canaries.

Canaries seem to be particularly susceptible to *Salmonella* infection. They usually contract a very virulent form of the disease which may account for very severe losses in both young and adult birds. Joest (1906) was probably the first to draw attention to the occurrence of a disease in canaries caused by the enteric group of bacteria. Another early record of an epizootic apparently due to a *Salmonella* is that of Gilruth (1910). A bacterium isolated from the heartblood was found to be pathogenic for mice, rabbits, guinea-pigs and canaries. About the same time Pfeiler (1911) incriminated an organism of the *Paratyphi-B* group, obtained from blood culture, as the cause of a virulent outbreak of diarrhoea among a group of well-bred canaries. A somewhat similar outbreak was recorded by Lutje (1924).

TABLE 13.

Antigen.	548 Serum Unabsorbed.	*Typhi-murium* Serum Unabsorbed.	548 Serum* Absorbed by 548.	548 Serum Absorbed by *Typhi-murium*.	*Typhi-murium* Absorbed by 548.	548 Serum* Absorbed by *Typhi-murium* var. *Storrs*.	548 Serum Absorbed by *Typhi-murium* var. *Copenhagen*.
548—" O ".....	1,600	3,200	0	0	0	0	0
548 type.....	6,400	100,000	100	0	100	—	—
548 group.....	100,000	50,000	200	0	200	—	—
Typhi-murium—" O ".....	1,600	3,200	0	0	1,600	0	0
Typhi-murium type.....	6,400	100,000	100	0	100	—	—
Typhi-murium group.....	100,000	25,000	200	0	200	—	—
Typhi-murium var. *Storrs* " O "...	1,600	—	—	—	—	0	0
Typhi-murium var. *Copenhagen* "O"	1,600	—	—	—	—	0	0

* Strain 548, although undoubtedly motile, lost a great deal of its motility when it was grown on solid agar. It, therefore, failed to absorb all the " H " agglutinins from its own serum as well as from *Typhi-murium* serum.

Later Beaudette (1926 a), Beaudette and Edwards (1926), and Harkins (1926) also described virulent epizootics in canaries in which organisms of the *Paratyphoid-B* group were incriminated as the etiological agent. Beaudette and Edwards (1926) studied two outbreaks in which birds of all ages were affected with severe diarrhoea associated with an increase in the amount of urates excreted; an organism which resembled *typhi-murium* serologically was obtained from the heart-blood and internal organs. The premises into which the birds studied by Harkins were introduced were well-kept and clean, and none of the local birds became affected; but the imported canaries arrived in soiled wooden cages, which probably played a predisposing part in setting up the infection.

In South Africa, Martinaglia (1929) recorded two outbreaks of paratyphoid in canaries in which *typhi-murium* was the cause, and in 1933 I investigated a very virulent epizootic in an aviary comprising about 200 well-bred canaries. The most important symptoms were drowsiness and diarrhoea, and the course of the disease was always very rapid, with a mortality of over 95 per cent. The most important lesions observed were hydro-pericardium, enteritis, tumor splenis and swelling of the liver. A gram-negative, non-lactose fermenting organism was obtained in pure culture from the heart-blood and spleen of all the birds examined. Cultures of this organism were readily agglutinated by *typhi-murium* serum and the organism was found to be di-phasic. A mixed serum was prepared by injecting a rabbit five times with a suspension of the canary strain (culture 176) in saline. Agglutination and absorption tests were then performed as shown in Table 14.

TABLE 14.

Antigen.	*Typhi-murium* S. a.b. *typhi-murium.*	*Typhi-murium* S. a.b. 176.	*Typhi-murium* S. unab.	176 S. a.b. *Typhi-murium.*	176 S. a.b. 176.	176 S. unab.
Typhi-murium " O ".....	0	0	1,600	0	0	800
Typhi-murium " H " type	0	0	50,000	0	0	12,800
Typhi-murium "H" group	0	0	25,000	0	0	6,400
176 " O ".....	0	0	1,600	0	0	800
176 type.....	0	0	50,000	0	0	12,800
176 group.....	0	0	25,000	0	0	6,400

S = serum; a.b. = absorbed by; unab. = unabsorbed:
0 = no agglutination at 1 in 100.

The results of Table 14 show that *culture* 176 removed all agglutinins (" O ", type and group) from *typhi-murium* serum as well as from 176 serum; while *typhi-murium* completely exhausted both 176 serum and the homologous serum. Culture 176, therefore, resembles *typhi-murium* serologically and contains the same antigenic factors; it should be regarded as *typhi-murium*.

On investigating the source of the infection, I found that losses commenced to occur soon after the owner had changed the food supply. Several samples of grain used by the owner for feeding were obtained and inoculated into enrichment media, e.g. tetrathionate broth. After 24 hours' incubation a loopful from each tube was spread on to a Mason tube of MacConkey's bile-salt agar. A few translucent, non-lactose fermenting colonies were observed on one of the tubes; some of these were picked and tested against drops of a *typhi-murium* serum dilution on a glass slide; the result was a coarse floccular agglutination and *typhi-murium* was suspected. The remainder of two of the positive colonies was subcultivated until a pure culture (*culture* 177) was obtained. Culture 177 was found to be diphasic, and, like *culture* 176, it exhausted all agglutinins from the serum of *culture* 176, as well as from the serum of *typhi-murium* Glasgow. Accordingly, *culture* 177, like *culture* 176, should be regarded as a strain of *typhi-murium*. A one-sided absorption was considered sufficient in the case of this test.

Although the presence of *typhi-murium* in the grain may explain the origin of the infection, the possibility of the grain becoming contaminated by attendants handling it after the outbreak among the canaries cannot be excluded. It may be of interest to mention that Jones and Wright (1938) described an outbreak of *typhi-murium* food-poisoning in man due to contamination of food with the excreta of mice.

Culture 153, obtained from one of a number of finches that were dying from a septicaemic disease in an aviary, was also studied. By testing it with the same method used for culture 176 it was found to be diphasic and to exhibit the same antigenic characters as cultures 176 and 177. It was, therefore, also labelled *typhi-murium*.

(3) Geese.

Outside Germany there is very little information available regarding the incidence of paratyphoid infection in geese. According to the classical monograph of Hubener (1910) seventeen outbreaks of food-poisoning traced to birds' meat have been recorded in Germany during the period 1903 to 1908; of these 14 outbreaks were due to goose meat, one to duck, one to fowl, and, in the case of one, the species of bird was not mentioned. Nine of the outbreaks were ascribed to paratyphoid organisms; of these, eight were caused by goose meat and one was due to the meat of a sick hen.

One of the first records of paratyphoid in geese is that of Pfeiler (1919) when he described a virulent epizootic among 9-week old birds with symptoms of septicaemia and swelling of the head and eyes. Heart-blood and organ cultures yielded a pure growth of an organism of the *Paratyphi-B* group (*typhi-murium*?). Earlier in the year cultures of *typhi-murium* were used for the eradication of mice and there was a suspicion that geese obtained the infection from the mice. Later Weissgerber and Müller (1922), Lutje (1924) and Burghoffer (1927) described similar epizootics among young geese. An organism which resembled both *Paratyphus-B* and *suipestifer* was obtained by Weissgerber and Müller from the heart-blood and