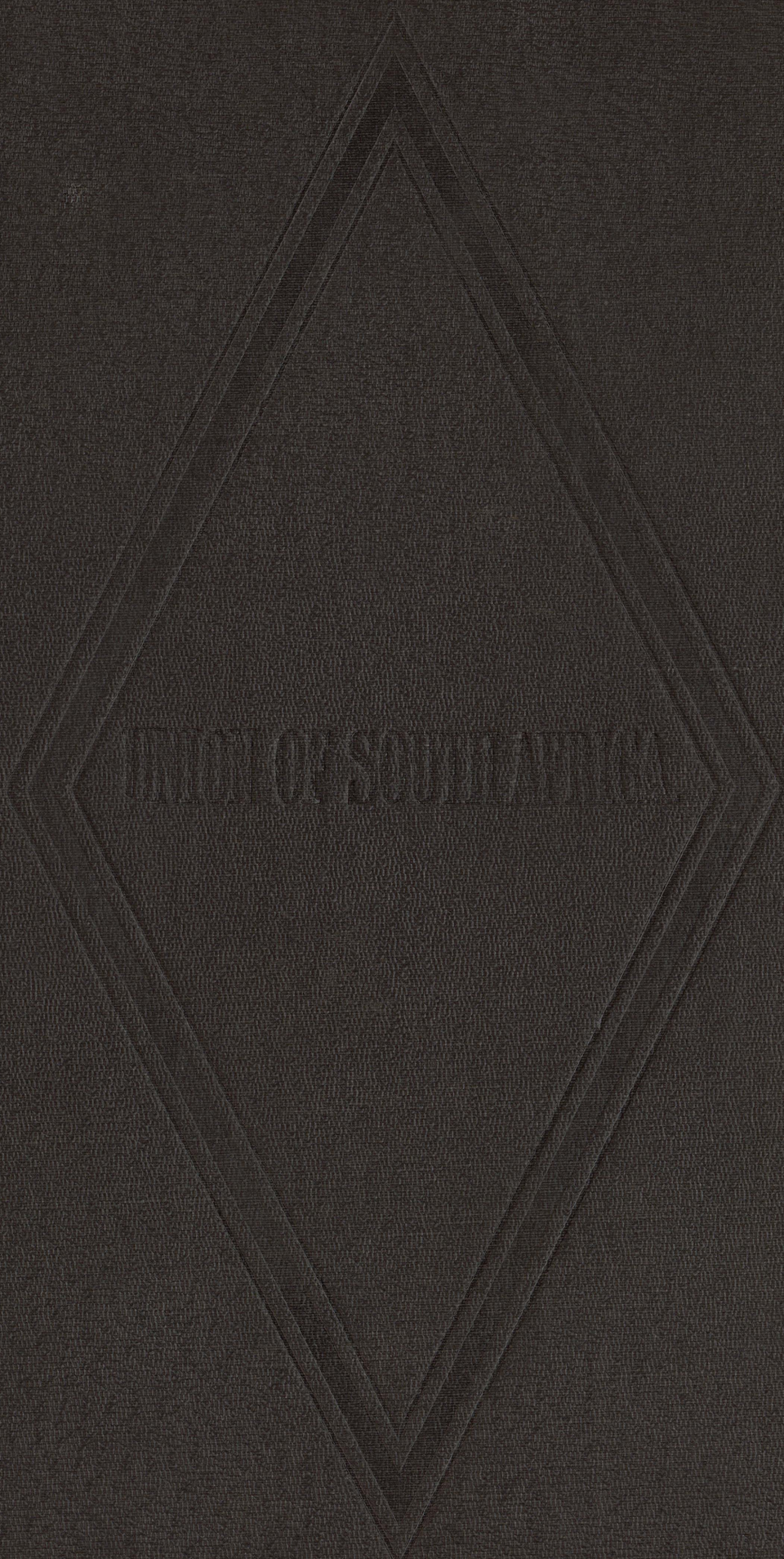

T H E S I S

for the D. Sc. DEGREE of the

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THE ANTIGENIC STRUCTURE OF SALMONELLAS OBTAINED FROM DOMESTIC ANIMALS AND BIRDS IN SOUTH AFRICA.

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:-

1. 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 lead 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 Salmonellas 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 ~~in~~ of ~~their~~ classification. By means of absorption tests he was able to divide the organisms included in the Aertrycke 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 also 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 agar and gave rise to a marked, loose floccular agglutination with its own serum; while the other variant, the "O" (ohne Hauch) form, grew as circular discrete colonies and agglutinated in fine, granular clumps with its 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 X19

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 1:5000, 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 1:1000. Orcutt (1924b) also found that heating at 70°C destroyed the agglutinating power ~~of~~ the free flagella 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. 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 ~~with~~ in which he was using formalised ~~emulsions~~ 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), Joos (1903), Eisenberg and Valk (1902), and Castellani (1902). 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 it 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. But Schutze (1921) and Krumwiede, Cooper and Provast (1925) emphasised the dangers of relying on unilateral absorptions alone as a basis for the identification of bacterial species. They recommended the use of reciprocal absorption tests for the recognition of the type or species. More recently a detailed review of the subject of agglutination has been given by Arkwright (1931).

11. 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. A small oval motile bacterium, resembling B. coli and found in the blood, internal organs and intestinal contents, was incriminated as the cause of the malady. By feeding milk containing a small amount of the organism in pure culture to calves he managed to set up the disease, while a subcutaneous inoculation of the organism produced a fatal septicaemia. Jensen, however, 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". The

affected calves also suffered from meningitis and epileptiform convulsions with cloudiness of the cerebro-spinal fluid. Soon afterwards Poels (1899) studied a disease in calves which he ascribed to pseudocolibacilli. Infection was supposed to occur either per os or through the umbilicus of the newly-born. Calves under a week old were considered to be the most susceptible. Poels 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. He showed that these bacilli may occur in the intestinal canal of adult cattle. Subsequently several observers have shown that a similar infection of the small intestine may occur in adult cattle, sometimes enzootically, with haemorrhagic enteritis as a common symptom. Later Jensen (1903, 1913) described diseases in young cattle and in calves under the term "paracolibacillosis". In calves the infection was generally associated with enteritis, sometimes a haemorrhagic enteritis, swelling of the mesenteric lymphatic glands, tumor splenis and sero-fibrinous exudations into the body cavities. Numerous bacilli were found in the blood, exudates, and organs. In older animals the infection usually assumed the form of a septicaemic enteritis or a pneumonia. The organisms 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 could be traced to the consumption of the meat of calves suffering from this disease and ~~that~~ which were slaughtered as a result of the enteritis. 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. Miessner and Kohlstock (1912) obtained Bact. enteritidis, Gaertner, from the organs of cows that had died from a croupous enteritis. 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: The disease studied by him affected mostly calves between 2 and 4 weeks old and lung lesions were very common. Meyer, Traum and Roadhouse (1916) investigated an outbreak of infectious diarrhoea among a group of hand-reared calves, from 1 to 4 days old. Bact. enteritidis was isolated from the blood and internal organs of the affected calves, and cultures of this organism produced the disease in experimental calves; one of the investigators working with the disease was also accidentally infected. They regarded Bact. enteritidis (Gaertner) as the common cause of meat-poisoning in man and considered that it should be distinguished from paracolon bacilli, the cause of calf diarrhoea.

Both Lutje (1926) and Lehr (1927) described outbreaks of paratyphoid disease in adult cattle. Lutje's animals showed lesions of tumor splenis, enteritis, necrotic nodules in the liver, septicaemia and sometimes pneumonia; two children that received milk from a sick cow became infected. Lehr found profuse mucous or bloody diarrhoea, abortion and retention of the afterbirth in some of the

animals studied by him. The agglutination titre of the sera of these animals varied from 1:100 to 1:20,000. From the faeces of some animals, the sera of which had with an agglutination titre of 1:100 to 1:200, Gaertner bacilli were isolated, and the milk of a cow that excreted Gaertner bacilli in the faeces was found 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 ~~that~~ 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 the 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 Prõscholdt (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. Prõscholdt described two outbreaks of Gaertner infection spreading from adult cattle to calves. The organisms were isolated from the blood, internal organs and faeces; it was thought that by disseminating the organisms with the manure and urine the carrier infected the pasture and stables. 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 Prõscholdt, 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 1:3200. 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 in 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 larger 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) also found S. enteritidis var. dublin as the most important causal agent of calf paratyphoid; the most common lesions described were tumor splenis and necrotic miliary nodules in the liver and spleen. 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. Clarenberg (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 in pure cultures from the blood and internal organs of affected calves, and he isolated it also from the faeces. The most important symptom recorded was severe diarrhoea, frequently preceded by constipation. A non-fatal form of the disease associated with haemorrhagic diarrhoea was produced in calves by feeding cultures of the organism. Like Viljoen and Martinaglia (1926, 1928) Daubney regarded exposure to redwater and gallsickness as predisposing ~~factors for~~ causes of paratyphoid infection. He found the agglutination titre of calves ~~that~~ which had been sick for more than 6 days to vary from 1:200 to 1:2500. Daubney also described a case of paratyphoid in a bull which he attributed to S. enteritidis and an outbreak of paratyphoid in dairy cattle where typhi-murium was the cause. In India Shirlaw (1935)

investigated a highly fatal disease in calves caused by a member of the Salmonella enteritidis group. The most important lesions described were septicaemia, tumor splenis, necrotic foci in the liver and spleen, haemorrhagic enteritis and pleuro-pneumonia. Calves ranging from 4 to 120 days old were affected. Shirlaw could not transmit the disease by contact or by feeding, but succeeded in infecting calves by inoculating virulent cultures. A tentative diagnosis of S. enteritidis was made on purely biochemical grounds, and Shirlaw found that enteritidis serum agglutinated the organism obtained from the lesions up to 1:5000, while typhi-murium serum was agglutinated to a titre of 1:2400. Hygienic factors were regarded to play an important rôle in the genesis of the disease.

In domestic mammalscalf 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. The most important symptoms were acute diarrhoea and sometimes pneumonia. The first outbreak occurred in a batch of 20 calves bought for experimental purposes. Three days after their arrival at the Laboratory at Cambridge most of the calves were noticed to be sick; 18 died and 2 recovered. The second was an outbreak of contagious pneumonia among a group of 3-weeks old calves which had been purchased; it spread rapidly to a number of very young locally bred calves. Although not in actual contact, the two groups of calves were housed in the same building and fed by the same attendant. There were 17 cases of which three died. The third outbreak was also observed at Cambridge among 10 calves bought from the dealer who supplied the first batch. The most outstanding symptom was diarrhoea; 8 of the calves died. A little later Smith (1934) recorded two fatal cases in children due to infection with dublin. (1) A 7-months old male infant showing symptoms of pyrexia, convulsions, broncho-pneumonia, hypertonia of the skeletal muscles and meningitis. Death occurred on the 34th day after admission to hospital and dublin was isolated from the blood and cerebral spinal fluid. (2) A 2-year old female suffering from enlargement and ulceration of the tonsils, broncho-pneumonia, oedema of the pharyngeal wall and pus in the left pleural sac; the breath had a foetid smell and the liver and spleen were enlarged; dublin was obtained from the blood, throat swabs and pus from the pleural sac.

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 through the medium of their faeces.

Soon afterwards 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 Eastcourt 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 organisms as a frequent secondary invader affecting mostly calves whose vitality had been lowered by factors such as ~~like~~ 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, Kauffmann 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 26).

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 10[illegible] 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 ~~which was~~ 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 numbers 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 virulentculture 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 dired 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 1069 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.

Like Daubney (1927) and Viljoen and Martinaglia (1928) I have also found that the exposure of calves to unfavourable conditions and diseases such as piroplasmosis and anaplasmosis, may predispose them to infection; but, in the majority of outbreaks studied by me, these conditions apparently did not play a very important part in the genesis of the disease; I have noticed that by far the most important factor in the production and spread of paratyphoid is the exposure of calves to conditions where they come in intimate contact with infected manure. Prophylactic measures for combating calf paratyphoid, therefore, should entail 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 measures. But, as vaccination against calf paratyphoid forms the subject matter 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 ~~the following~~ 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 usually 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 1:5000. A number of the suspected colonies are emulsified each in two separate loopsful 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 cholerae-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, cholerae-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 of 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 subcultivation of the latter, however, an occasional colony occurring in the specific phase may be detected. But in cases like European cholerae-suis, where the organism occurs permanently in the group phase, phase dissociation will not 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 subcultivation 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.

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 Salmonellae.

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 water-bath 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. 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 water-bath 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. 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 1000 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 1000 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 homogenous 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 or two on the bench, the suspension is preferably kept in the refrigerator overnight and centrifuged the next morning at about 2000 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 with those relating to culture 430 (Table 3), are not recorded separately in this paper.

TABLE L.

Dublin (Knox) serum absorbed by dublin (Knox), strains 154, 216 and 217.

154 serum " " " " and strain 154 and enteritidis M.7.

216 " " " strain 216 and enteritidis M.7. and dublin (Knox).

Enteritidis M.7. serum " " " " " " " " " strain 154.

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	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 unabsorbed	Enteritidis M.7. serum unabsorbed	154 serum unabsorbed	216 serum unabsorbed
Dublin Knox "O"	0	0	0	0				0		0		0	0	1600		1600	800
Dublin Knox "H"	0	0	0	6400				0		0		0	0	12800		25600	6400
Strain 154 "O"	0	0			0	0		0	0			0		1600	800	1600	
Strain 154 "H"	0	0			0	0		0	12800			0		12800	12800	25600	
Strain 216 "O"	0			0	0		0			0	0		0	1600	800		800
Strain 216 "H"	0			0	0		0		3200		0		0	12800	12800		6400
Enteritidis "O" M.7.					0	0	0		0		0	0	0		800	1600	800
Enteritidis "H" M.7.					0	6400	0		0		0	0	0		12800	25600	6400
Strain 217 "O"	0		0											1600	~~1600~~		
Strain 217 "H"	0		0											12,800	~~12800~~		

0 = less than 1 in 100

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 1 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", 1X; and "H" gp - "O" factor X11 has been added subsequently by Kauffmann (1935b).

As strain 154 is identical ~~to~~ with dublin its antigenic structure is made up of the same components. ~~But~~ Mirror-absorption tests performed with strains 170, 171, 173, 175, 198, 203, 295 yielded the same results as strain 154; they are, therefore, also identical with dublin and possess the same antigenic factors. The experiments carried out with strain 217 and the other ~~190~~ 87 strains not recorded in Tables 1 and 2 also demonstrated that these strains resemble dublin antigenically. In Table 1 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 shows that it completely absorbed enteritidis M.7. serum as well as the homologous serum, whereas enteritidis M.7. exhausted all 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", 1X, (X11) 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 strain 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 structures of strain 190 and typhi-murium (Glasgow) are identical. According to the Salmonellas Sub-committee of the International Society of Microbiology, the following antigenic omponents have been assigned to typhi-murium:- "O", 1V, 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).

TABLE 2.

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

Strain 190 " " " " " " " " " "

Antigen	Typhi-murium serum absorbed by typhi-murium (Glasgow).	Typhi-murium serum absorbed by strain 190.	Typhi-murium unabsorbed.	190 serum absorbed by typhi-murium (Glasgow).	190 serum absorbed by strain 190	190 serum unabsorbed.
Typhi-murium "O"	0	0	1600	0	0	1600
Typhi-murium type	100	100	100000	0	0	6400
Typhi-murium group	0	0	25600	0	0	3200
Strain 190 "O"	0	0	1600	0	0	1600
Strain 190 type	100	100	100000	0	0	6400
Strain 190 group	0	0	25600	0	0	3200

0 = less than 1:100

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.

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 ~~that~~ 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).

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.

TABLE 3.

Antigen	Dublin serum absorbed by Dublin	Dublin serum absorbed by strain 430	Dublin serum unabsorbed	430 serum absorbed by Dublin	430 serum absorbed by strain 430	430 serum unabsorbed
Dublin "O"	0	0	1600	0	0	800
Dublin "H"	100	100	25600	100	100	50000
430 "O"	0	0	1600	0	0	800
430 "H"	100	100	25600	100	100	50000

0 = less than 1:100

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 asanother strain of Salmonella enteritidis var. dublin, containing the following antigenic components:- "O", 1X,(X11)and "H" gp.

For fermentation reactions see Table 25.

111. 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-commitee, 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.

TABLE 4 : "O" AGGLUTINATION.

Somatic Antigens.	Sera. Onderstepoort.	Senftenberg.	Paratyphi-A.	Choleraesuis.	Enteritidis	Onderstepoort absorbed by Onderstepoort.	Onderstepoort absorbed by Senftenberg.	Onderstepoort absorbed by Paratyphi-A.	Onderstepoort absorbed by Choleraesuis.	Onderstepoort absorbed by Enteritidis.	Senftenberg absorbed by Onderstepoort	anatum	London
Onderstepoort	6400	100	50	0	±50	0	6400	6400	6400	6400	0	0	0
Senftenberg	3200	3200	-	-	-	0	0	-	-	-	1600		
Paratyphi-A	100	-	800	-	-	0	-	0	-	-	-		
Choleraesuis	10	-	-	1600	-	0	-	-	0	-	-		
anatum	0	-	-	-	-		-	-	-	-	-	1600	-
London	0	-	-	-	-		-	-	-	-	-	0	800
Enteritidis	±50	-	-	-	1600	0	-	-	-	0	-		

0 = less than 1:50

TABLE 5.A.

"H" antigens type.	Unabsorbed type sera. Onderstepoort.	Reading.	Newport.	Brandenburg.	Potsdam.	Absorbed type sera. Onderstepoort absorbed by Onderstepoort type	Onderstepoort absorbed by Reading type.	Onderstepoort absorbed by Newport type.	Reading absorbed by Onderstepoort type.	Reading absorbed by Reading type.	Newport absorbed by Onderstepoort type.	Newport absorbed by Newport type.	Brandenburg absorbed by Onderstepoort type.
Onderstepoort	6400	3200	6400	800	1,600	0	200	400	50	50	50	50	0
Reading	3200	3200	-	-	-	0	50	-	400	50	-	-	-
Newport	3200	-	6400	-	-	0	-	50	-	-	800	50	-
Brandenburg	200	-	-	6400	-	0	-	-	-	-	-	-	6400
Potsdam	400	-	-	-	12800	-	-	-	-	-	-	-	-
Anatum	3200	-	-	-	-	-	-	-	-	-	-	-	-

0 = less than 1:50

TABLE 6.

	Unabsorbed group sera					Absorbed group sera Onderstepoort group serum absorbed by													
	Onderstepoort.	Reading.	Newport.	Binns.	Kunzendorf.	Binns	London.	Binns + London	Binns + London + Kunzendorf	Kunzendorf	Paratyphi C	Reading	Newport	Sendai	Anatum	Anatum + Binns	Anatum + Binns + Kunzendorf	Reading absorbed by Onderstepoort.	Newport absorbed by Onderstepoort.	Binns absorbed by Onderstepoort	Kunzendorf absorbed by Onderstepoort.
Onderstepoort.	25,600	25,600	6,400	12,800	3200	12,800	3200	3200	800	800	800	400	6400	800	3200	3200	800	0	50	0	0
Reading	25,600	25,600	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	400	-	-	-
Newport	800	-	12,800	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-	6400	-	-
Binns	800	-	-	25,600	-	0	0	0	0	0	-	-	-	0	-	0	0	-	-	6400	-
Kunzendorf.	25,600	-	-	-	3200	-	-	-	0	0	-	-	-	50	-	-	0	-	-	-	400
Paratyphi C	25,600	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
Sendai.	12,800	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-
London.	1,600	-	-	-	-	400	0	0	0	0	-	-	-	0	-	-	-	-	-	-	-
Anatum.	1,600	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	-	-	-	-

0 = less than 1:50

"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 sewn 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 Kauffmann-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 1:6400) 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.

On performing absorption tests (Table 5A) the specific phase of either reading, newport or anatum lowered the titre of onderstepoort type serum from 6400 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 ~~the homologous~~ onderstepoort antigen was reduced from 25,600 to only 12800, and for the group phases of L2 and anatum (group factors 1, 4, 6) the reduction was from 1600 to 400. L2 and anatum reduced the titre of onderstepoort group serum from 25,600 to 3200, removing all group agglutinins 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 agglutinins 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 should 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 onderstepoort 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 agglutinins from onderstepoort type serum, while the group phases of reading, paratyphi-C or cholerae-suis did not remove all the group agglutinins 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 XlV 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 XlV.

'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 additional 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 oudersteepoort. 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.

TABLE 5 B.

Type Antigens	Onderste-poort s.a.b. Newport var. Kottbus.	Onderste-poort s.a.b. Newport.	Onderste-poort s.a.b. Anatum var. Muenster.	Newport var. Kottbus s.a.b. Onderste-poort.	Newport s.a.b. Onderste-poort.	Anatum var. Muenster s.a.b. Onderste-poort.	Onderste-poort serum.	Newport serum.	Newport var. Kottbus serum.	Anatum var. Muenster serum.	Abortus-equi s.a. Onderste-poort.	Abortus-equi serum.	Onderste-poort serum a.b. ab.-equi.
Onderste-poort	200	400	800	0	0	0	3200	2000	3200	12800	0	400	3200
Newport var Kottbus	0	-	-	200	-	-	3200	-	3200	-	-	-	-
Newport	-	0	-	-	400	-	3200	2000	-	-	-	400	3200
Anatum var. Muenster	-	-	0	-	-	1600	3200	-	-	12800	-	-	-
Abortus-equi	-	-	-	-	-	-	200	200	-	-	6400	6400	0

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

The results obtained are given in Table 5B, and they confirm my previous findings. The specific phase of newport var. Kottbus reduced the titre of onderstepoort type serum from 3200 to 200, while the type phase of newport lowered it to 400, and anatum var. Muenster decreased it to 800. On the other hand, the type phase of onderstepoort reduced the titre of the specific agglutinins of newport var. Kottbus serum from 3200 to 200, of newport from 2000 to 400 and of Muenster from 12800 to 1600. 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.

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 5B 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 mot correspond to the entire eh.

The fact that onderstepoort "0" serum (titre= 6400) agglutinates senftenberg "0" suspension nearly up to full titre (1:3200) shows that onderstepoort also contains an "0" factor which is present in senftenberg; but, onderste poort "0" serum barely agglutinates paratyphi-A (factors 1, 11) at 1:100 and it fails to agglutinate anatum and London (factors 111, X). It can be assumed, therefore, that factors 11 and 111 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 X1X of Kauffmann (1937).

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

0 = X1V, (X1X)

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 "0", type and group sera. It was found to be diphasic and was readily agglutinated by "0" sera of group B of 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 o

Mason tubes and individual colonies tested for type and group phases as described above ; type and group antigens were prepared from the colonies identified.

TABLE 7.

Antigen	Typhi-murium serum absorbed by Typhi-murium	Typhi murium serum absorbed by strain 234	Typhi-murium serum unabsorbed	234 serum absorbed by typhi-murium	234 serum absorbed by strain 234	234 serum unabsorbed.
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 - "O"	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