

the first record in which this organism has been incriminated as the cause of an animal disease. The affected birds showed symptoms of icterus, distension of the ureters with urates and derangement of the hock joints. Moreover, infection of turkeys with S. gallinarum is comparatively frequent. Two of the 149 outbreaks caused by this bacterium and recorded by me (vide infra) involved turkeys only.

(6) Fowls.

Virulent epizootics, like fowl-typhoid and bacillary white diarrhoea, are so common in gallinaceous birds that it is quite possible that some outbreaks due to other types of Salmonella have been mistaken for these diseases. In many outbreaks of paratyphoid the scourge affe^cts only very young birds in the same way as pullorum disease attacks chickens. Frequently the source of infection can be traced to eggs laid by infected hens, while in other epizootics older birds also suffer sever^ely, and the disease resembles fowl typhoid. The nature of the malady is revealed only when^a careful bacteriological examination of the dead birds is made.

One of the first records of a disease that can be interpreted as paratyphoid in fowls is the description by Mazza (1899) of an epizootic among these birds in Italy. The affected fowls showed haemorrhagic patches on the skin with discoloration of the combs and wattles. After death there were^{Mazza} observed serous exudates in the peritoneal cavity, swelling of the liver, spleen and kidneys, and occasionally haemorrhagic enteritis and hydropericardium. A motile, non-indol forming, glucose fermenting organism was obtained from the internal organs; it was found to be pathogenic for fowls and pigeons, and slightly for guinea-pigs; rabbits were^{not} susceptible. Moreover, the bacterium was distinguished from that of fowl cholera.

But there are very few early descriptions of paratyphoid in fowls, probably on account of the marked resistance of adult birds to infection. Reinholdt (1912) and others

have tried to infect fowls, geese, ducks and pigeons with enteritidis and Paratyphus-B (typhi-murium ?) both parenterally and per os; fowls proved to be the most resistant. Pfeiler and Rehse (1913) also found that fowls were not very susceptible to paratyphoid infection; they studied the outbreak on a farm of a chronic disease which occurred enzootically with a few deaths reported from time to time.

The main lesions recorded were hydropericardium and extensive swelling and mottling of the liver, due to the presence of numerous light-grey nodules. They recovered a bacterium from the internal organs which they placed in the Paratyphus-B group. Nevertheless, although adult fowls may not be very susceptible to natural or artificial infection with certain types of Salmonella, numbers of very virulent outbreaks of paratyphoid in chickens are reported periodically. Thus Spray and Doyle (1921) found that outbreaks of a very destructive disease in newly hatched chicks (2 to 4 days old) may be caused by organisms of the paratyphoid-B group, as well as by S. pullorum. Edwards (1929) investigated an epizootic affecting over 2000 very young chicks with a mortality rate of about 25 per cent: S. pullorum could not be detected in any of the birds examined, but there was a mixed infection of typhi-murium and anatum associated partly with coccidiosis. The disease resembled bacillary white diarrhoea in some respects; the birds were dull and listless and became progressively weaker until they died, but there was no diarrhoea. The tissues of the body were extensively oedematous, and the peritoneal cavity was distended with exudate. If the birds were as old as 7 days necrotic foci could generally be found in the liver. The organisms were isolated from the heart-blood and organs. By means of serological tests and post mortem examinations, no carriers could be detected in either the breeding stock or in the survivors.

Later five separate outbreaks of paratyphoid in birds were recorded by McGaughey (1932). Of these three occurred

in chicks, one in adult fowls and one in ducks. In one of the chicken epizootics typhi-murium was obtained from the heart-blood and internal organs of the dead birds; in another outbreak a non-motile strain of the typhi-murium Paratyphi-B group was isolated from the carcasses, while enteritidis was recovered from the third group of chickens. The clinical symptoms in all three outbreaks resembled those of pullorum disease; the mortality varied from 50 to 70 per cent, but no definite pathological changes were encountered. The disease in ^{the} adult fowls caused a large number of sudden deaths. McGaughey isolated typhi-murium from the internal organs of ^{one} the bird and pullorum from another, while the examination of six fowls gave entirely negative results; the blood of 16 live birds was positive for pullorum, and of one for both pullorum and typhi-murium. In the outbreak affecting the ducks several deaths occurred during the course of a few months. The ovary of one of the ducks examined resembled that of a case infected with pullorum, and S. enteritidis was obtained from its liver and ovary. It was stated above that Jansen (1936) isolated enteritidis var. Essen from the organs of diseased ducks, and also from the yolk sac of chickens that had been living in close association with ducks.

During the course of the routine diagnosis of chick diseases, Jungherr and Borden (1934) encountered 5 cases of paratyphoid infection. In two of these the causal agent was found to be typhi-murium var. storrs, in two atypical strains of cholerae-suis, and in one an atypical strain of L2.

In spring of 1936 Schalm (1937) investigated a pullorum-like disease that affected several batches of chicks on a Californian farm. The breeding stock had been healthy for a number of years and the farm was free from bacillary white diarrhoea, but deaths were reported in the chicks sold to five different farmers, and about 40 per cent losses were sustained in 4 to 10 days old birds. There were symptoms of acute diarrhoea, pasty vent, listlessness and retention of semi-coagulated yolk. Typhi-murium was isolated from the heart-

blood and organs, and chilling during shipment was considered to be a predisposing cause. The chicks that remained on the breeder's farm developed an apparently chronic form of the disease which affected fewer and much older birds. The latter usually developed pericarditis, but symptoms of diarrhoea and retention of the yolk were seldom observed. Twenty of the chronic cases were killed and examined at ages varying from 32 to 51 days, and from the pericardial fluid of six of these typhi-murium was obtained. In order to determine the method of infection, Schalm smeared faeces infected with typhi-murium on the shells of 16 eggs before placing them in the incubator; the result was that five of the chickens that hatched out were infected. It was thought, therefore, that infection of the chickens on the farm took place in the incubator after hatching by means of bacteria present in the faecal matter on the surface of the egg shells. Schalm could not infect 4-day old chickens either by feeding or by intravenous inoculations of cultures of typhi-murium.

According to Emmel (1936) different species of Salmonella may occur as facultative parasites in the alimentary canal of fowls; by examining the intestinal contents of a number of fowls suffering from enteritis due either to coccidiosis or to worm infestation he claims to have isolated aertrycke, paratyphi-A, paratyphi-B, enteritidis, typhi as well as pullorum. The account published by Emmel does not appear to be complete; there are no records given of the methods used for typing the strains, and it is not stated on what grounds the different strains ^{were} ~~have been~~ classified. To me it seems that Emmel's claims cannot be accepted, unless much more information is available than is presented in his thesis.

The incidence of food poisoning produced by fowl's meat is apparently much lower than that caused by foods prepared from duck's and goose meat and eggs. In a review

of outbreaks of food-poisoning due to bird's meat, Beller (1933) discussed several cases where the meat of aquatic birds was incriminated; he pointed out that fowl and pigeon meat intended for food are always well cooked so that food-poisoning cannot be readily set up even when the meat is infected. From 1923 to 1932 Meyer (1933) studied 50 outbreaks of food-poisoning caused by bird's meat. Three hundred people were affected and there were three deaths; 37 of the outbreaks were due to goose, 3 to duck, 7 to fowl, one to partridge, one to pigeon^{meat}, and in one case both goose and fowl^{meat} were incriminated. Although the type of Salmonella recovered was not determined in all outbreaks, typhi-murium was found to be by far the most common; enteritidis was incriminated in a small number of the cases, while "Paratyphus-B" and a newport-like organism were recovered from one outbreak. The bacteria were generally isolated either from the suspected food, or from the patients, or from both food and patients. As stated above, the importance of ducks as carriers of Salmonella infection lies rather in the eggs than in the meat.

Although S. gallinarum and S. pullorum are generally regarded as non-pathogenic for man, Kauffmann (1934) has recently described a strain of gallinarum (the Duisberg strain) which he isolated from the stools of patients that developed acute symptoms of gastro-enteritis after they had partaken of a salad. The organism resembled gallinarum serologically, and was pathogenic for chickens, but its fermentation reactions were atypical.

Apart from infection with gallinarum and pullorum, I have studied four outbreaks of Salmonella infection in fowls in South Africa.

1. The information relating to this outbreak has been

furnished by me in another paper (Henning, 1937).

In 1935 a farmer at Amersfoort in the Transvaal sustained serious losses amongst his chickens from what appeared to be an infectious disease. The disease was not investigated and the cause of the mortality remained unknown until the end of 1936, when the malady reappeared and a few affected birds were sent to Onderstepoort for examination. An apparently pure culture, obtained by Mr. J.D.W.A. Coles, head of the Poultry Diseases Section, from the heart blood of a 7-day-old chick, was handed to me for identification. The culture was plated and a few isolated colonies were picked. The cultures obtained from these were now tested against various agglutinating sera. It was found that the antigenic structure of the organism^s exhibited an entirely new combination of antigenic components; ^{for this reason} ~~and that it should~~, therefore, ^{the germ should} be 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). The name Salmonella amersfoort (Henning, 1937) was given to the organism - after the place of its origin.

Morphology and cultural characters. Morphologically, S. amersfoort resembles a typical Salmonella, and, like it, grows readily on ordinary laboratory media. It is Gram-negative and ~~is~~ actively motile. Saline and thermo-agglutination tests, as well as the shape of individual colonies, show that it is smooth.

Biochemical characters. S. amersfoort forms acid and gas in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose, and sorbitol; it forms hydrogen sulphide and renders litmus milk alkaline; it does not produce indol.

Pathogenicity. S. amersfoort is pathogenic for chickens and mice; 0.25 c.c. of a 24-hour-old broth culture, given intraperitoneally, kills a 6-week-old chicken in 4 days and 0.05 c.c. kills an adult mouse in 36 hours. S. amersfoort

was recovered from the heart blood and spleen in each case. But chickens dosed with 1 c.c. of the virulent broth culture remained apparently healthy.

While making an antigenic analysis of Salmonella amersfoort I noticed well-marked flocculation occurring between this organism and the sera of organisms that are generally regarded as not even remotely related antigenically. It is the study of the antigenic structure of this organism that forms the basis of the discussion below.

Bruce White (1929) described three forms of antigenic variation occurring in the genus Salmonella: (1) the "H" form - "O" form variation of Weil and Felix (1920), (2) the Smooth form - Rough form variation of Arkwright (1921), and (3) the specific phase - non-specific phase variation of Andrewes (1922). Later Kauffmann and Mitsui (1930) described a new type of phase variation, involving the specific phases of brandenburg, dar-es-salaam and potsdam; and they called this α - β -variation. A similar variation has been observed in a number of other types of Salmonella viz. abortus-bovis (Bernard, 1935), hvittingfoss and oslo (Tesdal, 1936, 1937), bispebjerg and typhi (Kauffmann, 1936a, 1936b), chester and schleissheim (Kauffmann and Tesdal, 1937). The antigenic structure of these organisms, according to Kauffmann and Tesdal (1937), is given in Table 20. The inagglutinable (containing Vi antigen) and agglutinable forms of S. typhi described by Felix and his co-workers (1934, 1935, 1936) may be regarded as another type of variation. Kauffmann (1935) introduced the term "V-form" and "W-form" to denote, respectively, the variant containing Vi-antigen and that devoid of it; while Craigie and Brandon (1936), Brown (1936), Scholtens and others showed the effect of bacteriophage on the V - W degradation.

Serology. For the study of the antigenic structure

of S. amersfoort, "O" sera, "H" specific and non-specific sera, and mixed "O" and "H" sera, prepared against a number of representative strains of Salmonella, were used. Sera prepared against S. amersfoort were also used. The sera and agglutinating suspensions were prepared according to the methods described above.

The tubes containing the serum dilutions plus the antigen were placed in the water-bath at 55°C and read after 2 hours, and again after 18 hours. In "H" agglutination the two readings usually corresponded, but in "O" agglutination the second reading generally gave a much higher titre.

Preliminary tests showed that amersfoort gave a well-marked fine granular agglutination with "O" sera containing factors VI and VII of the Kauffmann-White schema (cholerae-suis, newport, potsdam and others) while a distinctly coarse floccular agglutination was produced not only with "H" sera containing factors en or e (abortus equi, brandenburg, potsdam, dar-es-salaam or onderstepoort, newport, reading or anatum), but also with those containing factor d (stanley, muenchen and typhi). However, a much stronger agglutination was produced by sera containing factors en than with those containing factor e but not n.

The culture was again plated on Mason tubes to obtain a number of separate colonies for independent study. After 5 hours' incubation at 37°C broth cultures of these colonies were tested against Kunzendorf and Binns group sera as well as against the type sera of onderstepoort, newport, potsdam and typhi. The results are given in Table 15. It will be noticed that the majority of the cultures agglutinated with typhi serum (factor d), a number agglutinated with potsdam (factors enly), newport (factors eh) and onderstepoort.

(factors eh) sera, a few agglutinated incompletely with all four sera, being apparently intermediate forms, but no agglutination whatsoever was effected with Kunzendorf and Binns sera.

TABLE 15.

Thirty colonies grown in broth for 5 hours and tested against 5 different sera.

No. of colony	<u>Typhi</u> s.	<u>Newport or</u> <u>Onderstepoort</u> s	<u>Potsdam</u> s	<u>Kunzendorf</u> s	<u>Binns</u> s
1-16	++++	0	0	0	0
17-25	0	++++	++++	0	0
26-30	+	+	+	0	0

++++ = complete flocculation within 30 minutes.

+ = partial flocculation after 1 hour.

0 = no flocculation after 18 hours.

In headings to table s. = serum.

These results indicated (1) that the organism occurred only in the type phase and (2) that the culture used was either a mixed one or that it exhibited properties that have hitherto not been described in a member of the Salmonella group. In order to settle the matter of the purity of the strain, Dr. J.H. Mason kindly single-celled fresh cultures derived from a colony of each of the two types - i.e. from one colony agglutinating only with sera made against specific factor d and from another that flocculated solely with the anti-sera of specific factors en and eh. After plating the primary cultures obtained from the single cells a number of well-isolated colonies were again picked into broth tubes and incubated at 37°C for 5 hours - in order to reduce the lag phase in the growth the broth tubes were placed in a water-bath at 40°C for 10 minutes before transferring them to the incubator.

Four single cells (a b c and d) obtained from colony 1, Table 16B, were now cultivated separately in broth and plated. A number of colonies from each plate were picked into broth, incubated and tested against both d and en sera. The results are given in Table 17.

TABLE 16A.

Twenty-two colonies picked from the plate seeded with broth from the single cell obtained from colony 1, Table 15

No. of colony	<u>Typhi</u> serum	<u>Understepoort</u> or <u>potsdam</u> serum.
1 to 21	+ + + +	0
22	0	+ + + +

TABLE 16B.

Thirty colonies picked from the plate seeded with the broth culture from single cell of colony 17, Table 15

No. of colony	<u>Typhi</u> serum	<u>Understepoort</u> or <u>potsdam</u> serum	Saline control
1 to 28	0	+ + + +	0
29 and 30	+ + + +	0	0

TABLE 17.

Single cell	No. of colony	<u>Typhi</u> serum	<u>Understepoort</u> or <u>potsdam</u> serum
<u>a</u>	1 to 4	+ + + +	0
<u>b</u>	5 to 12	0	+ + + +
<u>b</u>	1 to 14	0	+ + + +
<u>b</u>	15	+ + + +	0
<u>c</u>	1 to 14	+ + + +	0
<u>c</u>	15	0	+ + + +
<u>d</u>	1 to 10	+ + + +	0

+ + + + = complete flocculation after 30 minutes.
0 = no flocculation after 18 hours

Therefore, these results clearly show that S. amersfoort is composed of two distinct "H" antigenic complexes, both of which occur in the specific phase; the second (d-) antigen, apparently corresponding to the α -phase of Kauffmann and Mitsui (1930), is agglutinated with the "H" serum of typhi and, as will be shown below, also with specific sera of other Salmonellas, stanley and muenchen, containing specific factor d, while the other component, the first (en-) antigen, apparently corresponding to the β -phase of Kauffmann and Mitsui (1930), is agglutinated solely with potsdam, onderstepoort, and other sera containing agglutinins for the type factors en and eh (vide infra). Sera containing agglutinins for factors en always give a much stronger flocculation than the anti-sera of factors eh. It has also been shown that single cells composed of either the one or other complex constantly give rise to daughter cells some of which resemble the parent cell antigenically, while others have adopted a new antigenic structure entirely different from that present in the parent. The latter daughter cells again give rise to offspring some of which resemble themselves, while others are like their parent. These mutations constantly proceed and cells containing either the one or other antigenic complex continually produce cells of both types, and neither the one nor the other type of cell has been found to breed entirely true.

On single-celling the growth obtained from each of the two types of colonies serially three successive times, both variants constantly appear in the cultures arising from the single cells.

The purity of the culture is therefore beyond dispute; it is the property of the bacterium of giving rise to two distinct types of variants in the specific phase that is responsible for the uncommon behaviour of the culture. The

organism apparently does not occur in the non-specific phase.

"O" agglutination. Cross-agglutination tests were carried out with the heat-stable "O" antigens and "O" sera of the different Salmonella types of the Kauffmann-White schema; also with S. aberdeen (Smith, 1934), S. poonae (Bridges and Scott, 1935) and S. onderstepoort (Henning, 1936). The reactions obtained are given in Table 18.

TABLE 18. "O" Agglutination.

"O" antigen

	<u>Amers- foort</u>	<u>Pots- dam</u>	<u>Muen- chen</u>	<u>Onder- ste- poort.</u>	<u>Branden- burg.</u>
Unabsorbed sera:					
<u>Amersfoort</u> s.	800	800	200	100	0
<u>Potsdam</u> s.	800	800	-	-	-
<u>Muenchen</u> s.	200	-	1600	-	-
<u>Brandenburg</u> s.	0	-	-	-	1600
<u>Onderstepoort</u> s.	50	-	-	800	-
Absorbed sera:					
<u>Amersfoort</u> s.a.b. <u>amersf.</u>	0	0	-	-	-
<u>Amersfoort</u> s.a.b. <u>potsdam</u>	0	0	-	-	-
<u>Amersfoort</u> s.a.b. <u>muenchen</u>	200	-	0	-	-
<u>Amersfoort</u> s.a.b. <u>brandenburg</u>	800	-	-	-	0
<u>Potsdam</u> s.a.b. <u>potsdam</u>	0	0	-	-	-
<u>Potsdam</u> s.a.b. <u>amersf.</u>	0	0	-	-	-
<u>Muenchen</u> s.a.b. <u>amersf.</u>	0	-	800	-	-

0 = less than 1:50

- = not tested

In this table s. = serum, s.a.b. = serum absorbed by.

The results show that amersfoort "O" as well as potsdam "O" sera are completely exhausted for the homologous "O" antigen by amersfoort. In the same way both sera are exhausted by potsdam. The somatic "O" antigen of amersfoort

must, therefore, be regarded as identical with that of potsdam, i.e. it is composed of factors VI, VII.

"H" agglutination. Flocculation, approximately equivalent in titre to that produced with the homologous antigen, was obtained with the specific sera of abortus equi, potsdam, brandenburg, dar-es-salaam, stanley, muenchen and typhi, but a much weaker agglutination resulted when the type sera of onderstepoort, newport, reading or anatum were used for the test. In the same way amersfoort "H" serum agglutinated the specific antigens of abortus equi, potsdam, brandenburg, dar-es-salaam, stanley, muenchen and typhi almost up to full titre, while its titre for type antigens containing factors eh was much lower.

On absorbing amersfoort "H" serum with the specific phase of either potsdam (factors enlv), brandenburg (factors enlv) or dar-es-salaam (factors enlw) the titre of the serum for one of the homologous specific antigens (en-), β -phase, was reduced from 6400 to approximately 800, while the titre for the other homologous specific antigen (d-), α -phase, as well as for stanley, muenchen and typhi (factor d) remained unaltered. When abortus equi (factors enx) was used for the absorption, the reduction in titre for the homologous en antigen (β -phase) was almost complete, but still no noticeable decrease in agglutinins, for the homologous d antigen (α -phase) was effected; a small residue, however, remained which caused an incomplete agglutination with the en-variant (β -phase) of amersfoort. The cause of this flocculation is discussed below.

On the other hand, when amersfoort "H" serum was absorbed with either stanley, muenchen or typhi (factor d) most of the agglutinins for the ^{one} second variant (d) α -phase, of amersfoort were removed, while the titre for the other homologous antigen (en)- β -phase, remained unaltered (Table 19)

TABLE 19.

"H" Specific Antigen.

	<u>Amers- foort d</u>	<u>Amers- foort en</u>	<u>Typhi</u>	<u>Stanley</u>	<u>muen- chen.</u>	<u>Pots- dam.</u>	<u>Brand- en- burg.</u>	<u>Dar-es- salaam.</u>	<u>Abor- tus equi</u>	<u>Onder- ste- poort.</u>	<u>New- port</u>	<u>Read- ing</u>	<u>Ana- tum</u>	<u>Pana- ma</u>	<u>London</u>
<u>Unabsorbed sera</u>															
<u>Amersfoort d.s.</u>	12,800	6400	12,800	12,800	12,800	1600	3,200	3,200	3,200	200	-	-	-	0	0
<u>Amersfoort en.s</u>	6,400	6400	6,400	6,400	6,400	3200	6,400	3,200	6,400	400	400	400	400	0	0
<u>Typhi s.</u>	12,800	0	12,800	12,800	12,800	-	-	-	-	-	-	-	-	-	-
<u>Stanley type s</u>	25,600	400	25,600	25,600	25,600	-	-	-	-	-	-	-	-	-	-
<u>muenchen type s.</u>	6,400	0	6,400	6,400	12,800	-	-	-	-	-	-	-	-	-	-
<u>Potsdam s.</u>	0	6400	-	-	-	6400	6,400	6,400	3,200	800	-	-	-	6400	1600
<u>Brandenburg s.</u>	0	6400	-	-	-	6400	12,800	3,200	3,200	400	-	-	-	6400	3200
<u>Dar-es-salaam s.</u>	0	1600	-	-	-	-	-	3,200	-	-	-	-	-	1600	-
<u>Onderstepoort s.</u>	0	400	-	-	-	200	-	200	200	1600	-	-	-	-	-
<u>Abortus-equi s.</u>	-	6400	-	-	-	1600	3,200	6,400	3,200	-	-	-	-	-	-
<u>Absorbed serum:</u>															
<u>Amersfoort d s.a.b. amersf.</u>	0	0	0	0	0	0	-	-	0	-	-	-	-	-	-
<u>Amersfoort d s.a.b. typhi</u>	400	6400	0	-	-	-	-	-	-	-	-	-	-	-	-
<u>Amersfoort d s.a.b. stanley</u>	400	3200	-	0	-	-	-	-	-	-	-	-	-	-	-
<u>Amersfoort d s.a.b. muenchen</u>	400	3200	-	0	0	-	3,200	3,200	-	-	-	-	-	-	-
<u>Amersfoort en s.a.b. potsdam</u>	6,400	800 ^x	-	-	-	0	-	0	800	-	-	-	-	-	-
<u>Amersfoort en s.a.b. brandenburg</u>	6,400	800 ^x	6,400	-	-	-	0	-	-	-	-	-	-	-	-
<u>Amersfoort en s.a.b. dar-es- salaam</u>	6,400	1600 ^x	-	6,400	-	-	-	0	-	-	-	-	-	-	-

<u>Amersfoort en</u> <u>s.a.b. abortus</u> <u>equi</u>	6,400	200	-	-	-	-	0	-	0	-	-	-	-	-	-
<u>Typhi s.a.b.</u> <u>amersf.</u>	0	0	800	-	-	-	-	-	-	-	-	-	-	-	-
<u>Typhi s.a.b. typhi</u>	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-
<u>Stanley s.a.b.</u> <u>amersf.</u>	0	0	-	400	400	-	-	-	-	-	-	-	-	-	-
<u>Stanley s.a.b.</u> <u>stanley</u>	0	0	-	0	-	-	-	-	-	-	-	-	-	-	-
<u>Muenchen s.a.b.</u> <u>amersf.</u>	0	0	-	0	100	-	-	-	-	-	-	-	-	-	-
<u>Potsdam s.a.b.</u> <u>amersf.</u>	-	0	-	-	-	6400 ^x	6,400	6,400	0	-	-	-	-	6400	5200
<u>Brandenburg s.a.b.</u> <u>amersf.</u>	-	0	-	-	-	-	6,400	-	-	-	-	-	-	6400	3200
<u>Dar-es-salaam s.a.b.</u> <u>amersf.</u>	-	0	-	-	-	800	-	1,600	-	-	-	-	-	1600	800
<u>Abortus equi s.a.b.</u> <u>amersf.</u>	0	0	-	-	-	0	0	-	0	-	-	-	-	-	-
<u>Potsdam s.a.b.</u> <u>amersf.</u> then by <u>panama</u>	-	-	-	-	-	100	100	-	-	-	-	-	-	0	0
<u>Brandenburg s.a.b.</u> <u>amersf.</u> , then by <u>london</u>	-	-	-	-	-	-	200	-	-	-	-	-	-	0	0
<u>Abortus equi s.a.b.</u> <u>potsdam</u>	-	400	-	-	-	-	0	-	200	-	-	-	-	-	-

0 = less than 1:50. x = partial flocculation, fluid remaining turbid.

In this table s. = serum, s.a.b. = serum absorbed by. - = not tested.

When either potsdam or brandenburg serum was absorbed with amersfoort, all agglutinins for amersfoort were removed, but the titre of the serum for panama (factors Iv) and london (factors Iv) was not affected. Moreover, the treated serum still agglutinated the homologous antigen although the flocculation was incomplete and the fluid remained turbid, due, no doubt, to the persistence of Iv agglutinins in the serum. Dar-es-salaam serum behaved in practically the same way, but amersfoort is apparently capable of removing all the agglutinins for the homologous antigen from abortus-equi serum.

On absorbing either stanley, muenchen or typhi serum with amersfoort, most of the agglutinins for the homologous "H" specific antigen were exhausted, muenchen serum being exhausted much more completely than either stanley or typhi serum, while all the agglutinins for the second variant of amersfoort (factor d-) were removed.

DISCUSSION.

These results show that Salmonella amersfoort contains two distinct antigenic complexes, the one, β -phase, corresponding to factors en of abortus-equi, potsdam, brandenburg and dar-es-salaam plus an additional factor, part of which apparently corresponds to factor x of abortus-equi; the other complex, α -phase, coincides largely with factor d of stanley, muenchen and typhi. The additional factor is probably responsible for the residue of agglutinins left for the first (en-) antigen, β -phase, after absorbing amersfoort serum with potsdam, brandenburg or dar-es-salaam; but, although factor x of abortus-equi apparently forms a part of this additional factor, there may be another component which is not present in abortus-equi. The fact that amersfoort exhausts all agglutinins from abortus-equi serum for itself as well as for the homologous specific antigen indicates that

Amersfoort contains all the specific antigenic components of abortus-equi i.e. factors enx; but since abortus-equi fails to exhaust amersfoort serum completely for the homologous first (en-) antigen it is possible that this antigen of amersfoort contains a minor factor in addition to the enx of abortus-equi.

After absorbing amersfoort serum with either stanley, muenchen or typhi, a small residue is left which still agglutinates the homologous second (d-) antigen α -phase, but not the specific antigen (factor d) of either stanley, muenchen or typhi. It is not quite clear to what this residue can be ascribed; whether it should be regarded as an extra factor in the second (d-) antigen, α -phase, in addition to factor d of stanley, muenchen and typhi, or whether it can be attributed to a trace of the first (en-) antigen, β -phase, present in the emulsion of the second (d-) antigen, α -phase, of amersfoort used for the test, is not certain. If the latter explanation holds it is likely that the agglutination occurring in amersfoort serum absorbed with abortus-equi is likewise due to an overflow of the second (d-) antigen, α -phase, in the emulsion of the first (en-) antigen, β -phase, of amersfoort.

Neither abortus-equi, potsdam, brandenburg, nor dar-es-salaam effected any reduction in the titre of amersfoort serum for the homologous second (d-) antigenic complex, α -phase, or for the type phases of stanley, muenchen and typhi. In the same way neither stanley, muenchen nor typhi absorbed an appreciable amount of agglutinins from amersfoort serum for the homologous first (en-) antigen, β -phase, or for abortus-equi, potsdam, brandenburg and dar-es-salaam.

When potsdam serum was absorbed by amersfoort all agglutinins for both amersfoort and abortus-equi were completely exhausted, but flocculation to nearly full titre was still effected with the specific phases of potsdam, brandenburg, panama and london. On reabsorbing the partly absorbed potsdam,

serum with panama (factors Iv) no appreciable agglutination resulted when specific antigens of potsdam, brandenburg, panama and london were used. Amersfoort, therefore, removed only the agglutinins of factors en from the potsdam serum, leaving the agglutinins of factors Iv to be absorbed by panama.

The fact that amersfoort almost completely exhausted muenchen serum for the homologous specific antigen suggests that the second (d-) factor, α -phase, is similar to the specific phase (factor d) of muenchen; the small residues of agglutinins left in stanley and typhi sera for their homologous specific antigens after absorption with amersfoort cannot be explained at present.

SUMMARY AND CONCLUSIONS.

A new type of pathogenic Salmonella for the fowl has been described. Its somatic "O" antigen corresponds with factors VI, VII of potsdam. It occurs only in the specific phase, but its flagellar "H" antigen contains at least two distinct and separate antigenic complexes, which commonly occur in organisms that are not even remotely related. The one complex (the first, en-, antigen, β -phase of Kauffmann and Mitsui) contains factors enx, which represent also the factors of the specific phase of abortus-equi. The other complex (the second, d-, antigen, α -phase of Kauffmann and Mitsui) contains factor d, which comprises the type phase of stanley, muenchen and typhi.

Single cells containing factors enx, on multiplying, constantly yield variants containing factor d as well as offspring that retain antigenic complex enx. In the same way single cells containing apparently only specific factor d will bring forth new cells, most of which retain the parental antigenic structure, but a small proportion of the progeny will acquire specific factors enx instead of d.

When a broth culture of amersfoort in either the

enx or d phase and in an apparently pure form, is used for the preparation of sera, agglutinins of approximately the same titre for both variants are produced in the sera. The purity of the phase culture must be judged by the agglutination test, using heterologous sera which contain agglutinins either against factors enx or d.

As a result of the information given above the following antigenic structure is proposed for Salmonella amersfoort:

Somatic "O" antigen - VI, VII.

Flagellar "H" antigen -

(1) α - phase of Kauffmann and Mitsui - d-

(2) β - phase of Kauffmann and Mitsui - enx

11. During the course of 1937 an outbreak of a fatal disease occurred among a group of adult fowls on a farm near Onderstepoort. The symptoms and lesions presented were indistinguishable from those of an ordinary virulent outbreak of fowl typhoid. The disease was investigated by my colleague, Mr. J.D.W.A. Coles, who made spleen cultures on agar from three birds; the cultures were handed to me for identification and I spread seed material from each culture onto MacConkey's bile-salt agar in Mason tubes. After 24 hours incubation both small and large non-lactose fermenting colonies appeared in two of the Mason tubes. Some of these colonies were picked and mixed separately with drops of a gallinarum serum dilution on glass slides. All the small colonies tested were readily agglutinated by the gallinarum serum, but all the large colonies failed to react with this serum. A pure culture of the small colonies was obtained and labelled culture 360. Several of the large colonies were now tested against various "O", type and group sera. No agglutination, whatsoever occurred with any group serum, but some of the colonies flocculated when mixed with typhi and stanley type sera, while others were agglutinated by type sera containing factors enx,

enlv, enlw and eh (abortus-equi, potsdam, dar-es-salaam, onderstepoort). All the colonies tested were agglutinated by "O" sera containing factors VI, VII (cholerae-suis, potsdam). Moreover, all the colonies tested were flocculated by amersfoort "O" and "H" mixed sera (factors VI, VII and d - enx). It appeared, therefore, from these preliminary tests that the organisms from the large colonies, labelled culture 359, were related to amersfoort, and a rabbit was immunised for the production of antiserum.

When culture 359 was plated so as to give several well separated single colonies, like amersfoort, some of these were found to agglutinate only with a type serum containing factors enx, enlv, enlw or eh (abortus-equi, potsdam, dar-es-salaam or reading), while others were flocculated only by type sera containing factor d (typhi, stanley and muenchen). In order to make sure that the culture used was unquestionably pure it was single-celled. It was found that the single-cell obtained from the colony that was agglutinated by type sera containing factors enx etc. produced daughter organisms which, on sub-cultivation, gave rise to colonies some of which agglutinated with enx sera, while others (about 12 per cent) were agglutinated by type sera containing factor d. Moreover, the single-cell procured from the colony that was flocculated by type sera containing factor d yielded bacilli, which on sub-cultivation produced colonies occurring in both the phases (d and enx). Some of the colonies (about 90 per cent) were agglutinated only by sera containing factor d, while a smaller number were agglutinated by type sera containing factors enx, enlv, enlw or eh. The bacilli of culture 359, therefore, also occurred in two specific phases, the α - and β -phases of Kauffmann and Mitsui (1930), the organisms which occurred in the one phase constantly dissociating into bacilli which were present in both phases. As the organism occurred only in the specific

phase the dissociation was confined to that phase; non-specific variants were not encountered at any time.

In order to settle the identity of culture 359, agglutination and absorption tests were carried out as shown in Table 12. After absorption tests had been performed with amersfoort the identity of the strain was determined, and no further tests were performed.

TABLE 12.

Antigen	Amersfoort 336 serum absorbed by Amersfoort.	Amersfoort 336 serum absorbed by 359.	Amersfoort 336 serum unabsorbed.	359 serum absorbed by Amersfoort 336.	359 serum absorbed by 359.	359 serum unabsorbed.
Amersfoort "O"	0	0	800	0	0	1600
Amersfoort "H" <u>d</u> (α)	0	0	12800	0	0	12800
Amersfoort "H" <u>en</u> (β)	0	0	6400	0	0	12800
359 - "O"	0	0	800	0	0	1600
- "H" (α)	0	0	12800	0	0	12800
- <u>en</u> (β)	0	0	6400	0	0	12800

0 = less than 1:100

The results of Table 12 show that amersfoort absorbed all the agglutinins ("O", "H" d- type and "H" enx type) from the homologous serum as well as from 359 serum; on the other hand, 359 completely exhausted both its own serum and amersfoort serum. Amersfoort (culture 336) and culture 359 should, therefore, be regarded as identical. But the original amersfoort (culture 336), was obtained in pure culture from dead chickens during a virulent outbreak of a septicaemic disease in very young chickens at Amersfoort, while the present strain, culture 359, was isolated in conjunction with gallinarum (vide infra) from adult fowls suffering from a fowl typhoid-like disease. In the epizootic discussed above (1) and in my previous paper (Henning, 1937), amersfoort 336 was apparently the sole cause of the mortality in the chicks; but in the present outbreak it is not quite clear whether amersfoort 359 or gallinarum 360 was the primary cause of the disease. The probability is that gallinarum 360 was the more important etiological agent, and that amersfoort 359 gained admission into the body after its resistance had been lowered by fowl typhoid.

Whereas the organisms comprising amersfoort culture 359 were motile, those of culture 360, obtained from the small colonies, were non-motile. Culture 360 was tested, therefore, both serologically and by means of fermentation reactions. The latter are given below (Table 25) and are typical for gallinarum. Agglutination and absorption tests were carried out with culture 360 and gallinarum 43, obtained from the National Collection of Type Cultures, and the serum of the latter. An antiserum for culture 360 was not prepared, but a one-sided absorption was carried out and it was found that, like gallinarum 43, culture 360, completely removed all the agglutinins from the serum of gallinarum 43. Moreover, the latter serum agglutinated the "O" antigen of culture 360 up

to full titre (1:1600). Both serologically and by means of fermentation reactions (*vide infra*), therefore, culture 360 resembled gallinarum 43, and it should be regarded as a strain of gallinarum.

The fermentation reactions of amersfoort culture 359 are also given below (Table 25).

For purposes of comparison Table 20 is included in order to show the antigenic structure of different organisms that occur in the α and β phases of Kauffmann and Mitsui.

TABLE 20.

Organisms known to show α - β phase variation in their specific phases (partly after Kauffmann & Tesdal, 1937).

	"O" Antigen.	H. specific Antigen.		Author.	Origin.
		α -phase.	β -phase.		
S. Abortus-bovis	I IV	<u>b</u>	<u>enx</u>	Bernard (1935)	Aborting cows.
S. Schleissheim	IV	<u>b</u>	<u>Z₅</u>	Kauffmann & Tesdal (1937)	bovine.
S. hvittingfoss	XVI	<u>b</u>	<u>enx</u>	Tesdal (1936)	gastro-enteritis in man.
S. chester	IV V (XII)	<u>eh</u>	<u>enx</u>	Kauffmann & Tesdal (1937)	gastro-enteritis in man.
S. brandenburg	IV (XII)	<u>lv</u>	<u>en</u>	Kauffmann & Mitsui (1930)	gastro-enteritis in man.
S. bispebjerg	IV (XII)	<u>a</u>	<u>enx</u>	Kauffmann (1936a)	gastro-enteritis in man.
S. abortus-equi	IV (XII)*	?	<u>enx</u>		aborting mares.
S. potsdam	VI VII (XII)	<u>lv</u>	<u>en</u>	Kauffmann & Mitsui (1930)	gastro-enteritis in man.
S. oslo	VI VII (XII)	<u>a</u>	<u>enx</u>	Tesdal (1937)	gastro-enteritis in man.
S. amersfoort	VI VII (XII)	<u>d</u>	<u>enx</u>	Henning (1937)	Septicaemia disease in fowls.
S. typhi	IX (XII)	<u>d</u>	<u>j</u>	Kauffmann (1936b)	
S. dar-es-salaam	I IX (XII)	<u>lv</u>	<u>en</u>	Kauffmann & Mitsui (1930)	typhoid

* Kauffmann & Tesdal (1937) consider that S. abortus-equi will probably also be found to show α - β variation at times.

111. During spring of 1936 a very virulent epizootic occurred in few day old chicks at the School of Agriculture near Potchefstroom. Some of these chickens were forwarded to Onderstepoort for investigation and were examined by Mr. Coles, Chief of the section of Poultry Diseases. Heart-blood and spleen cultures made by him yielded a pure growth of a gram-negative bacterium that was handed to me for identification. The organism was found to be very actively motile, and it did not ferment lactose. When it was tested against various "O", type and group, sera by means of slide agglutination, it was readily agglutinated by "O" sera containing factors IV and V, by typhi-murium type serum and by group sera. The organism was also found to be di-phasic, and the culture was labelled 357. A rabbit was immunised with a killed saline suspension of a fresh agar culture, and a good serum was obtained. As the preliminary test indicated that culture 357 is probably related to typhi-murium, cross-agglutination and absorption tests were first performed with this organism (table 13).

TABLE 13.

Antigen.	Typhi-murium serum (Glasgow) absorbed by typhi-murium.	Typhi-murium (Glasgow) serum absorbed by 357.	Typhi-murium (Glasgow) serum unabsorbed.	357 serum absorbed by typhi-murium.	357 serum absorbed by 357.	357 serum unabsorbed.
typhi-murium "O"	0	0	800	0	0	800
typhi-murium type	100	100	100,000	0	0	25600
typhi-murium group	100	100	50,000	0	0	6400
357- "O"	0	0	800	0	0	800
357- type	100	100	100,000	0	0	25600
357- group	100	100	50,000	0	0	6400

0 = <1:100. On account of the high titre of the typhi-murium type and group serum a small residue (1:100) of unabsorbed agglutinins were left after the absorption.

The results of Table 13 clearly show that the antigenic structures of culture 357 and typhi-murium (Glasgow) are identical; culture 357 removed all the agglutinins ("O", type and group) from typhi-murium (Glasgow) serum, as well as from the homologous serum, while typhi-murium (Glasgow) completely exhausted both its own serum and 357 serum.

The agglutination (titre 1:100) which is recorded in the absorbed sera in columns 2 and 3 of Table 13 is attributed to the high titre of the unabsorbed serum; as stated above, sera of very high agglutination titres are very unwieldy for absorption tests, because it is extremely difficult to remove the last trace of agglutinin, even when the homologous antigen is used for the absorption.

After several strains of pure culture of typhi-murium (357) were obtained from a number of the chickens, this organism was considered to be the etiological agent of the epizootic, and an attempt was made to determine the source of the infection. As the first deaths took place only a few days after hatching, it was thought that the infection was probably obtained from the breeding hens through the eggs. Two successive slide agglutination tests were performed with the blood of the breeding stock; but both tests were negative and no carriers could be found among the hens. In the case of ducks Warrack and Dalling (1933) observed that infected eggs were laid only when the titre of the affected birds was high and that the agglutination^{titre} of the sera obtained from reactors dropped considerably during the course of the laying season. Whether the same condition holds for fowls cannot be stated at present, and it is not certain whether the existence of carriers escaped notice on account of the lateness of the tests - serological tests were performed only some weeks after typhi-murium had been proved to be the cause of the epizootic. None of the eggs were examined for typhi-murium

infection, and Salmonellas could not be detected in the ovaries of any of the hens examined. The source of the infection, therefore, still remains obscure.

IV. During the course of an investigation of another fowl typhoid-like epizootic among adult birds, Mr. Coles again made agar cultures from the heart-blood and spleen of the affected birds, and handed these to me for further study. The cultures (three in number) did not appear ^{to be} pure, and some seed material from each one was thinly spread on MacConkey's bile-salt agar in Mason tubes. One of the cultures yielded only lactose-fermenting colonies and was discarded; but from both the others several large and small non-lactose fermenting colonies were obtained, suggesting the existence of a mixed infection. The small colonies were readily agglutinated by gallinarum serum; a few of these were picked, cultured and labelled culture 415. The large colonies were tested against various "O", type and group serum dilutions on glass slides. A distinct fine granular agglutination was obtained with the "O" sera of senftenberg, and anatum; coarse floccules were produced by group sera (e.g. cholerae-suis var Kunzendorf serum) and by newport, reading, onderstepoort and ^{another} type sera. Some of the large colonies were sub-cultured and labelled culture 414, and a rabbit was immunised with it. The preliminary tests showed that culture 414 was related partly to senftenberg and anatum on account of its "O" antigen and partly to newport, reading, onderstepoort and anatum on account of its "H" specific antigen, and that it was di-phasic. Cross-agglutination and absorption tests were, therefore, performed, first with culture 414, anatum and senftenberg (Table 14).

TABLE 14.

Antigen.	Anatum S. ab. by Anatum.	Anatum S. ab. by 414	Anatum S. un- absorb- ed.	414S. a.b. Anatum	414S a.b. 414	414 S. unab- sorbed	L ₂ S. a.b. 414	L ₂ S unab- sorbed	Kott- bus speci- fic S. a.b. 414.	Kottbus speci- fic un- absorb- ed.	414 S.ab. L ₂	414 S.ab. Kott- bus.	414 S.ab. Anatum V. Muen- ster.	414 S. ab. Senf- ten- berg.	Senf- ten- berg 414	Senf- ten- berg S. unab- sorbed.
Anatum "O"	0	0	400	0	0	800										
Anatum type	0	0	3200	0	0	12800										
Anatum group	0	0	6400	0	0	6400										
414 - "O"	0	0	400	0	0	800	0	800	0	0	0	800	0	400	0	400
414 - type	0	0	3200	0	0	12800	-	0	0	3200	12800	0	0			
414 - group	0	0	6400	0	0	6400	-	1600	0	800	200	800	1600			
L ₂ - O						800	0	800			0	0				
L ₂ - type						0		3200			0					
L ₂ - group						6400	0	6400			0					
Kottbus "O"						0			1600	1600		0				
Kottbus type						3200			0	3200		0				
Kottbus group						1600			800	3200		0				
Anatum V. Muenster "O"						800							0			
Anatum " type						6400							0			
Anatum " group						1600							0			
Senftenberg "O"						200								400	800	

0 = less than 1:100, S = Serum, a.b. = absorbed by.

The results of Table 14 show that culture 414 has the same antigenic structure as anatum. Culture 414 completely absorbed all the agglutinins ("O", type and group) from anatum serum, as well as from the homologous serum, while anatum completely exhausted the sera of culture 414 and of itself. Anatum var. muenster completely exhausted both the "O" and specific agglutinins from 414 serum, but it merely reduced the non-specific titre from 6400 to 1600. Culture 414 should, therefore, be regarded as a strain of anatum.

Culture 415, obtained from the small colonies, was tested against gallinarum 43 serum and was agglutinated by it to full titre (1:1600); it also completely absorbed gallinarum 43 serum, showing that it contained the same antigenic components as gallinarum. The fermentation tests given below (Table 25) are also typical for gallinarum. Culture 415 should, therefore, be regarded as a strain of gallinarum. Antiserum for culture 415 was not prepared and the absorption test performed was one-sided.

In this outbreak also it is not certain whether anatum culture 414 or gallinarum 415 was the primary cause of the disease. Salmonellas were found in two cultures only - three were made - and both contained anatum as well as gallinarum. On account of the frequency of gallinarum infection, however, and on account of the predominance of gallinarum colonies in the first subcultures made, it seems probable that the organism of fowl typhoid was the main etiological agent in this outbreak.

In addition to these outbreaks of Salmonella infection in poultry, 149 epizootics of fowl typhoid and 45 outbreaks of disease in young chicks were also investigated. In most of the cases heart-blood and spleen cultures were made by Mr. J.D.W.A. Coles and submitted to me for identification. Some of the cultures were made by me personally, and culture 206 was obtained by Dr. Martinaglia from one of a number of

chicks thought to be affected with bacillary white diarrhoea. On plating culture 206 I noticed that both large and small colonies appeared on the agar within 24 hours. As large and small colonies have frequently been observed in cultures of pullorum that were unquestionably pure, especially after allowing the cultures to stand a day or two at room temperature, the existence of a mixed infection was not suspected; nevertheless, a few of the small, and some of the large colonies were picked and cultured separately. The large colonies yielded a dense growth of actively motile organisms, while the small colonies gave rise to a much poorer growth of non-motile bacteria. In order to determine the purity of the two cultures, my colleague, Dr. J.H. Mason, kindly undertook to single-cell them; the growths obtained from the single-cells were labelled culture 207 and culture 208 for the non-motile and motile bacteria respectively.

The two cultures were now tested against various "O", type and group sera. Culture 207 was agglutinated only by "O" sera containing factor IX, while culture 208, which proved to be monophasic, was flocculated by "H" sera containing factor d, as well as by "O" sera with factor IX.

With a view to carrying out complete absorption tests, antisera were prepared against both culture 207 and 208. But as culture 208 was agglutinated with sera containing "O" factor IX and "H"-specific factor d, it was evident that the organisms comprising the culture were closely related to S. typhi, which contains both these components. Absorption tests were, therefore, performed with S. typhi, as shown in Table 21.

TABLE 21.

ANTIGEN.	typhi-serum absorbed by typhi.	typhi serum absorbed by 208	typhi serum unabsorbed.	208 serum absorbed by typhi.	208 serum absorbed by 208	208 serum unabsorbed.
typhi "O"	0	0	3200	0	0	1600
typhi "H"	0	0	25,600	0	0	12800
208 - "O"	0	0	3200	0	0	1600
208 - "H"	0	0	25,600.	0	0	25,600

0 = less than 1:100.

The results of these tests clearly show that culture 208 absorbs all agglutinins ("O" and "H") from S. typhi serum, as well as from its own serum, while S. typhi completely exhausts both the homologous serum and 208 serum. Culture 208, therefore, has the same antigenic structure as S. typhi, and should be regarded as a strain of this organism. S. typhi is not regarded as a pathogen for poultry, and it is not known to be carried by fowls. It is true that Emmel (1936) claims to have isolated S. typhi, S. paratyphi-A, S. paratyphi-B and other salmonellas from the intestinal contents of fowls suffering from enteritis due to coccidiosis or verminosis, but he gives no information on what basis the organisms were recognised, and his conclusions require confirmation before they can be accepted. *For comparison see biochemical tests Table 25.*

As culture 207 was readily agglutinated by "O" sera containing factor IX, and as pullorum infection was suspected, absorption tests were performed with S. pullorum, which like culture 207 is non-motile. The results, which are given in Table 22, show that culture 207 absorbs all the "O" agglutinins from pullorum serum and that pullorum completely exhausts the serum of culture 207. But pullorum and gallinarum have the same somatic antigenic components, so that it is not possible to determine by means of a serological test alone to which of these two types culture 207 belongs; a final differentiation can be made only by means of fermentation tests (Table 25). According to these tests culture 207 corresponds to Salmonella pullorum.

TABLE 22.

Antigen.	Pullorum serum absorbed by Pullorum.	Pullorum serum absorbed by 207.	Pullorum serum unabsorbed.	207 serum absorbed by Pullorum.	207 serum absorbed by 207.	207 serum unabsorbed.
Pullorum "O"	0	0	3200	0	0	3200
207 - "O"	0	0	3200	0	0	3200

0 = less than 1:50.

Pullorum = Pullorum ~~B~~ 26 of the N.C. of type cultures.

Of the 139 outbreaks of fowl typhoid 137 cultures were obtained from fowls and two from turkeys. Against four of these, antisera were prepared for the purpose of performing absorption tests with strains of gallinarum and pullorum obtained from the National Collection of Type Cultures of the Lister Institute. In Table 23 the results are given which were obtained with culture 29 - the results obtained with the other three cultures (249, 314 and 340) are similar, but are not given. Table 23 shows that culture 29 absorbs all the "O" agglutinins from gallinarum serum, as well as from the homologous serum, while gallinarum exhausts both its own serum and 29 serum. Similar results were obtained when pullorum serum was substituted for gallinarum serum, and pullorum cultures used for the absorption tests instead of gallinarum. Cultures 29, 249, 314 and 340, therefore, resemble both gallinarum and pullorum serologically, but their fermentation reactions (Table 25) corresponded to those of gallinarum, so that they should be regarded as strains of gallinarum. The other 135 cultures were used for unilateral absorption tests of gallinarum serum, and were found to remove all the "O" agglutinins from the serum; the fermentation reactions of all these cultures also resembled those of gallinarum.

TABLE 23.

Antigen.	Gallinarum S.serum a. b. gallina- rum.	Gallina- rum s.a.b. 29.	Galli- natum s.unab- sorbed.	29 S. a.b. galli- narum.	29 S. a.b. 29.	29 S. unab- sorbed.
Gallinarum "O"	0	0	1600	0	0	1600
29 - "O"	0	0	1600	0	0	1600

Gallinarum = gallinarum 416 of N.C. of type cultures.
 0 = less than 1 in 50.
 a.b. = absorbed by.
 S = serum.

A study was also made of 55 cultures obtained from ^{a number of} few day old chicks suffering from an acute disease, and from the ovaries of hens that gave a positive agglutination test for pullorum. Against three of these, cultures 317, 322, and 436, antisera were prepared for absorption tests. The results, which are given in Table 24, show that culture 317 removes all the "O" agglutinins from pullorum serum and from the homologous serum, while pullorum also completely exhausts both these sera. Similar results were also obtained with cultures 322 and 436 and pullorum. When gallinarum was substituted for pullorum identical results were obtained, so that identification of the cultures could not be made entirely on the basis of the serological test - fermentation tests were necessary for the complete differentiation between gallinarum and pullorum; these are given in Table 25. Unilateral absorption tests were performed with the other 52 cultures and pullorum or gallinarum serum, resulting in the complete absorption of the sera. But when fermentation tests were carried out, it was found that the reactions of 42 of the cultures resembled those of pullorum, while the other 10 corresponded to gallinarum.

TABLE 24.

ANTIGEN.	Pullorum Serum absorbed by Pullorum,	Pullorum Serum absorbed by 317.	Pullorum Serum unabsorbed.	317 Serum absorbed by Pullorum.	317 Serum absorbed by 317.	317 Serum unabsorbed.
Pullorum "O"	0	0	3200	0	0	1600
317 - "O"	0	0	3200	0	0	1600

Pullorum = Pullorum B.G.26
 0 = less than 1 in 50.

According to the fermentation reactions, therefore, 45 of the cultures from few day old chicks and infected ovaries of adult hens should be regarded as pullorum, while the other 10 cultures fall under gallinarum. The clinical symptoms and lesions presented by the chicks from which pullorum cultures were isolated did not differ materially from those which yielded cultures of gallinarum. A diagnosis of infection with either gallinarum or pullorum in very young chicks should, therefore, not be made, unless fermentation tests have been carried out, as well as serological tests.

TABLE 27.

Summary of the results obtained with the antigenic analysis of 318 strains of Salmonellas isolated from domestic animals and birds in South Africa.

Type of Salmonella.	No. of strains studied.	Origin of organisms	"O"-antigen	"H" - Antigen		
				Type (specific)		Group (non-specific)
				α -phase	β -phase	
<u>Enteritidis</u> var. <u>dublin</u>	97 ^x	96 calves and 1 human	1X, (X11)	<u>gp</u>	-	-
<u>Enteritidis</u>	3	Calves	1X, (X11)	<u>gom</u>	-	-
<u>Typhi</u>	1	Chick	1X, (X11)	<u>d</u>	(j?)	-
<u>Gallinarum</u>	149	139 Adult fowls 10 few day old chicks	1X, (X11)	-	-	-
<u>Pullorum</u>	48	42 chicks 3 ovaries of hens	1X, (X11)	-	-	-
<u>Abortus-equi</u>	1 ^{xx}	adult horse (tendo-vaginitis)	1V, (X11)	-	(<u>enx</u>).	-
<u>Typhi-murium</u>	10	2 calves 2 canaries 1 finches 1 canary-food 1 pig 1 chickens 1 sheep 1 rabbits	1V, V (X11)	<u>i</u>	-	1,2,3.
<u>Typhi-murium</u> var. <u>Copenhagen</u> (Storrs)	1	foal (purulent arthritis).	1V, (X11)	<u>i</u>	-	1,2,3.
<u>Cholerae-suis</u> (Kunzendorf).	6	pigs	VI, VII (X11)	-	-	1,3,4,5.
<u>Bovis-morbificans</u>	1	pork	VI, VIII	r	-	1,3,4,5.
<u>Anatum</u>	1	fowls	X, 111	<u>eh</u>	-	1,4,6.
<u>Onderstepoort</u>	1	sheep	XIV	<u>eh</u>	-	1,4,5.
<u>Amersfoort</u>	2	1 chickens 1 adult fowls	VI, VII, (X11)	<u>d</u>	<u>enx</u>	-

x Two of these strains were ^{now} motile and were, therefore, devoid of H-specific factors gp.

xx The one strain studied had lost its motility and, therefore, did not contain an H-antigen, factors enx.

O-factor (X11) has been recently added by Kauffmann (1935b) and is supposed to occur in a number of different species that are not related.

Since the completion of this paper 10 more strains of dublin have been isolated from calves and 17 more strains of gallinarum have been obtained from outbreaks of fowl typhoid. In their antigenic structure and biochemical reactions the dublin strains corresponded to those dublin strains described above and the reactions of the gallinarum strains resembled those of the gallinarum cultures given above. The total number of dublin strains, therefore, should be 107 and of gallinarum 166, and the total number of Salmonellas 345.

TABLE 25.

FERMENTATION REACTIONS OF THE DIFFERENT TYPES OF
SALMONELLA STUDIED.

Type of Organism.	No. of cultures tested.	Bitter	Stern	d. tartarate Jordan & Harmon)	No. of cultures tested.	Glucose	Lactose	Dulcitate	Saccharose	Mannite	Maltose	Avabinose	Rhamnose	Inulin	Inosite	Salicin	Galactose	Sorbite	Laevulose	H ₂ S Production.	Indol	Litmus Milk	Motility	Species of animal or source from which culture was obtained.
enteritidis var. dublin.	50	50-	50-	36 x 14xxx	97	x	-	x	-	x	x	21x 76-	79x 18-	-	-	-	x	x	x	x	-	Alk	94x	Calves 96 Human
dublin 154	1	-	-	x	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 170	1	-	-	xx	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 175	1	-	-	xx	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 283	1	-	-	x	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 295	1	-	-	xx	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	+	Calf
dublin 303	1	-	-	x	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 329	1	-	-	x	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 342	1	-	-	x	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 441	1	-	-	x	1	x	-	x	-	x	x	-	-	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin 445	1	-	-	x	1	x	-	x	-	x	x	-	-	-	-	-	x	x	x	x	-	Alk	x	Calf
dublin Pesch. 256	1	-	xx	xx	1																			
" Knox (2206A)	1	-	-	xx	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	
" Cambridge I	1	-	xx	xx	1	x	-	x	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	
" Topley	1	-	xx	x																				
Paracoli savage 255	1	-	-	xx																				
enteritidis 216	1	x	-	xx	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Calf
" 290	1	x	xxxx	xxxx	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Calf
" 418	1	x	xxxx	x	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Calf
" M.7	1	x	xxxx	xx	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	
" D.5	1	x	xxxx	x																				
" Weybridge	1	x	xxxx	x																				
" Jena	1	x	xxxx	xx																				
" var. Rostock 3747	1	-	-	x																				
" var. Moscow	1	x	-	x																				
" var. Blegdam	1	x	-	xx																				

Cholerae-suis 365 (Kunzendorf)	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	Fig (swine fever).
" 381	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	"
" 382	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	"
" 383	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	"
" 384	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	"
" S.F.8.	1	x	-	x	1	x	-	-	-	x	x	-	x	-	-	-	x	x	x	x	-	Alk	x	"
" (Kunzendorf)	1	x	-	x																				
Cholerae-suis (America)	1	x	-	xxxx																				
Bovis-morbificans 391	1	x	xxxx	x	1	x	-	x	-	x	x	x	x	*	x	-	x	x	x	x	-	Alk	x	Pork
Bovis-morbificans (Basematl)	1	x	xxxx	xxxx																				
Bovis-morbificans (Bladden-Scott)	1	-	xxxx	xxxx																				
Amersfoort 336	1	x	xxxx	x	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Chickens
" 359	1	x	xxxx	xxxx	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Fowl
Onderstepoort 282	1	x	xxxx	xxxx	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Sheep
Anatum 414	1	x	xxxx	x	1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	Fowl
" 3701	1	x	xxxx		1	x	-	x	-	x	x	x	x	-	-	-	x	x	x	x	-	Alk	x	
" 3702	1	x	xxxx																					
Abortus-equi 219	1	x	-	x	1	1	-	1d	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	Horse
" W.H.2	1	x	-	x																				
Gallarum	40	0	0	xx	149	1	-	1	-	1	1	119 ¹ 20-	182 ¹ 17xd	-	-	-	1	1d	1	x	-	Alk	-	Fowls 137 Turkeys 2 Chickens 10
Gallarum 29	1	-	-	xx	1	1	-	1	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	Fowl
" 360	1	-	-	xx	1	1	-	1	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	Fowl
" 415	1	-	-	xx	1	1	-	1	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	Fowl
" 249	1	-	-	xx	1	1	-	1	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	5 day old chickens.
" 324	1	-	-	xx	1	1	-	1	-	1	1	1	1	-	-	-	1	1	1	x	-	Alk	-	hen

Gallinarium	340	1	-	-	xx	1	+	-	+	-	+	+	+	+	-	-	-	+	+	+	x	-	Alk	-	Fowls
Pullorum		15	-	-	-	45	+	-	-	-	+	-	37x 8-	35x 10xd	-	-	-	+	+	+	x	-	Neut.	-	Chickens and ovaries of hens
"	207	1	-	-	-	1	+	-	-	-	+	-	+	+	-	-	-	+	+	+	x	-	Neut.	-	Chicken
"	317	1	-	-	-	1	+	-	-	-	+	-	-	+	-	-	-	+	+	+	x	-	Neut.	-	Chickens and ovaries of Hen.
"	322	1	-	-	-	1	+	-	-	-	+	-	-	xd	-	-	-	+	+	+	x	-	Neut.	-	Chicken (7 day)
"	337	1	-	-	-	1	+	-	-	-	+	-	+	xd	-	-	-	+	+	+	x	-	Neut.	-	Chick.
"	436	1	-	-	-	1	+	-	-	-	+	-	+	+	-	-	-	+	+	+	x	-	Neut.	-	Chicks.

Stern: x, xx and xxxx indicate different degrees of discoloration, xxxx being the most deeply dark lilac colour.

d-tartrate: x, xx and xxxx indicate that $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ respectively of the medium in titre has been changed yellow.

x = Positive; + = acid, but no gas is formed; xd = delayed fermentation.

1^x = acid, but no gas formed 4 years ago, negative now.

The fermentation reactions given in Table 25 include the rhamnose test of Bitter, Weigmann and Habs (1926), the glycerin-fuchsin-broth test of Stern (1916) and the d-tartrate test of Jordan and Harmon (1928). By using solid agar media advised by Jordan and Harmon more clear-cut results were obtained than with the fluid media of Silberstein (1931); in positive reactions the colour of the indicator (phenol-red) was changed yellow by the acid formed. But the extent to which this discoloration of the agar occurred varied even with different strains of the same organism; stab cultures were made and the discoloration started from the inoculum, spreading from this point in all directions. In some cases barely a quarter of the medium was changed, while in others as much as a half or three-quarters had turned yellow.

Fifty of the strains labelled dublin gave negative tests with Bitter's rhamnose and Stern's glycerin-fuchsin-broth; with the d-tartrate test of Jordan and Harmon the indicator was changed yellow about half-way down the tubes (+ +) in 14 cultures, and about one quarter down the tube (+) in the remaining 36 cultures - 47 of the cultures were not ^{with} these media. Out of 97 cultures, 76 were arabinose negative and 21 were positive after 5 days incubation; 79 were rhamnose positive and 18 were negative after 48 hours incubation. But the rhamnose was generally fermented after 4 or 5 days in the incubator. The antigenic structure of all the 97 strains was identical with that of S. enteritidis var. dublin.

For comparison five stock strains of dublin were studied. Of these Pesch 256, Cambridge 1 and Topley were positive with Stern's glycerin-fuchsin-broth, while dublin (Knox) and Paracoli (Savage) 255 gave a negative reaction. All five reacted negatively with Bitter's rhamnose and positively with Jordan and Harmon's d-tartrate. On comparing the antigenic structure of Cambridge 1 and Topley with that of dublin (Knox) by means of agglutination and absorption tests the

three cultures were found to be identical; both Cambridge 1 and Topley completely exhausted dublin (Knox) serum.

Of the three strains which corresponded antigenically to S. enteritidis all gave a positive reaction to Bitter's rhamnose, two (cultures 290 and 418) reacted positively with Stern's fuchsin-broth and one (culture 216) failed to change it. All three cultures were positive with Jordan and Harmon's medium. The three stock strains, M.7. D.5, and Weybridge gave a positive reaction with Bitter's rhamnose, Stern's fuchsin-broth and Jordan and Harmon's d-tartrate.

Of the 13 strains which were antigenically identical with typhi-murium (Glasgow) all were Stern and d-tartrate positive, twelve were Bitter positive and one (culture 357) was Bitter negative. Eight of the cultures were inosite positive and two (strains 357 and 502) were negative; nine were positive and one (strain 357) was negative with rhamnose, while all the ten tested fermented arabinose. Culture 357 was both rhamnose and inosite negative. Moreover, a complete mirror absorption test performed with culture 357 (Table 13) showed without doubt that it is a strain of typhi-murium. Of the typhi-murium stock strains tested all five were Bitter and d-tartrate positive, three (Mutton 74, Glasgow and Weybridge) were stern positive, while two (Binns and Breslau) were Stern negative.

The one strain of typhi-murium var. ^{Copenhagen,} Storrs (culture 478) studied gave positive Bitter, Stern and d-tartrate reactions. It fermented both inosite and maltose, but not arabinose; while the stock strain Storrs 19500 fermented arabinose and inosite, but not maltose; it was also Bitter and d-tartrate positive, but Stern negative. The two strains of Copenhagen (659 and 1147) both fermented maltose and arabinose but not inosite; both were Bitter, Stern and d-tartrate positive. Antigenically, however, ^{Copenhagen} Storrs 478, Storrs 19500

and the two strains of Copenhagen were identical (Table 26).

For comparison 5 stock strains of paratyphi-B were included in the test. Of these three (D.C., Grey and Odense) were Stern positive and two (Schottmuller and Lowestoft) were negative; only one (Grey) was Bitter positive, the other four were negative. All five reacted negatively with Jordan and Harmon's d-tartrate.

The six strains which resembled cholerae-suis (Kunzendorf) antigenically were all Bitter and d-tartrate positive but Stern negative.

There was only one culture (strain 391) which had the same antigenic structure as bovis-morbificans. Like the original culture of Basenau it was Bitter, Stern and d-tartrate positive, but the strain of Sladden and Scott differed from it on account of its negative Bitter reaction.

The strain of anatum (culture 414) studied gave a positive reaction with Bitter, Stern and d-tartrate, resembling, therefore, the two stock strains (3701 and 3702).

Like abortus-equi WH.2, the one strain (culture 219) studied reacted positively to Bitter and d-tartrate, but negatively to Stern's glycerin-fuchsin-broth. It was found to be an aerogenic.

Both strains of amersfoort and the one of onderstepoort were positive with Stern, Bitter and d-tartrate.

All the forty gallinarum strains tested gave a negative reaction with Bitter and Stern, but a positive one with Jordan and Harmon's d-tartrate. Out of 139 strains 119 fermented arabinose, while 20 failed to do so after 4 days. All the cultures fermented rhamnose, but in the case of 17 of the strains the fermentation was delayed. All were dulcitate and maltose positive.

The fifteen strains of pullorum tested all failed to react on Bitter, Stern or d-tartrate. Thirty-seven of the

45 strains fermented arabinose, but 8 failed to do so. All the 45 strains fermented rhamnose, but in 10 the reaction was delayed. All were dulcitate and maltose negative, and all 45 strains were anaerogenic.

For the differentiation of S. gallinarum and S. pullorum the cysteine-gelatin medium of Hinshaw and Rettger (1936) was tried, but the organisms failed to grow in the medium so that no change could be detected in the tubes. The cysteine used was freshly prepared.

Only one strain of S. typhi was studied. The original culture, obtained from a chicken by Martinaglia, was found to be composed of a mixture of two organisms when I received it; the one non-motile and the other motile. After "single-celling", the non-motile culture was labelled "strain 207" and the motile one "strain 208". The former resembled pullorum and gallinarum antigenically (Table 22) and pullorum biochemically; while culture 208 was found to have the same antigenic structure as typhi (Table 21). The fermentation reactions of the latter, however, did not altogether correspond to those of the stock strains of typhi employed. Like typhi, culture 208 was Bitter and Stern negative, and d-tartrate positive. Four years ago culture 208 fermented dulcitate slowly, and maltose within 24 hours, but at present it fails to ferment dulcitate and the fermentation of maltose is delayed for about five days. All the stock strains of typhi used fermented maltose within 24 hours, but failed to ferment dulcitate. The fermentation reactions of culture 208 are, therefore, not quite typical of typhi, but its antigenic structure is identical with that of this bacterium. When it was found that a change had occurred in the fermentation reactions of culture 208 after four years, the serological tests were repeated. The results of these tests show that the antigenic structure of culture 208 has remained unaltered, and that it still resembles typhi.

When the biochemical reactions of the different

types of Salmonellas studied are compared with their serological reactions very marked differences may be exhibited by several strains belonging to the same serological type. For example, some striking variations in their fermentation reactions were manifested by the four strains of typhi-murium var. storrs (Copenhagen) studied; the one strain 478 described by me and the other 3 stock strains. All four gave positive Bitter and d-tartrate tests; three (Storrs 478 and Copenhagen 659 and 1147) were Stern positive and fermented maltose, while one (Storrs 19500) gave a negative reaction to these tests. Two (Storrs 478 and 19500) were inosite positive and two (Copenhagen 659 and 1147) were negative. All four were antigenically identical (Table 26).

Analogous variations have been observed in the case of typhi-murium, paratyphi-B, enteritidis, enteritidis var. dublin, bovis-morbificans and, to a lesser degree with typhi, pullorum and gallinarum.

SUMMARY.

Altogether 318 strains of Salmonella from different species of animals have been studied. The antigenic analysis obtained is summarised in Table 27, and the biochemical reactions are given in Table 26.

The results recorded in Tables 26 and 27, therefore, show that the biochemical reactions of Salmonellas, although very useful as complementary tests, cannot be solely relied upon for the identification of the type or species of organism. Many of the types which were indistinguishable on serological grounds were found to differ in their fermentation reactions, whereas others which differed markedly in their antigenic composition showed identical biochemical reactions. For the recognition of the type of Salmonella studied, reliance can be placed only on the antigenic analysis of the bacterium by means of carefully performed serological tests.

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