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Serological Variants of *Salmonella typhi-murium* Isolated from South African Animals.

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A NUMBER of epizootics in domestic animals caused by Loefflers *Bacterium typhi-murium*, containing both factors IV and V, have been described by Henning (1939). In the description given here we have confined ourselves mainly to the study of strains of *typhi-murium* lacking factor V. These were obtained from outbreaks of paratyphoid in pigeons, horses and cattle.

A. PIGEONS.

Three epizootics have been investigated. Since Moore's (1895) description of a septicaemic disease in pigeons due to a bacillus of the hog cholera group several outbreaks of salmonella infection have been recorded in these birds. In most of the epizootics the disease was a paratyphoid infection *per se*, but in the outbreaks described by Zingle (1914) and Cash and Doan (1931) the body was invaded by a salmonella and the infection occurred under certain adverse conditions like pigeon-pox or alleged myeloid hyperplasia of the bone-marrow. In Holland, Reitsma (1925) and Sahaya and Willems (1927) recorded the acute form of the disease in young birds and the chronic form in adults. In Germany, Beck and Meyer (1927) and Beck (1929) described outbreaks of *typhi-murium* in adult as well as young birds, while Berge (1929) regarded this organism as the most important cause of disease in pigeons. In America, Beaudette (1926), Emmel (1929), Jungherr and Wilcox (1934) and Edwards (1935, 1938) all investigated a septicaemic disease in pigeons in which *typhi-murium* was incriminated as the cause. All pigeon strains studied by Edwards were shown to be lacking in factor V. In most countries paratyphoid is generally regarded as one of the most serious diseases of pigeons.

Outbreaks of food poisoning in man have been traced to foodstuffs derived from pigeons affected with paratyphoid. Clarenburg and Dornickx (1932) described an epidemic involving 20 persons in a hospital at the Hague. The source of infection was proved to be

pudding containing as an ingredient pigeon eggs. The outstanding symptoms were fever, diarrhoea, vomiting and gastro-enteritis. *Typhi-murium* was obtained from the blood, faeces and urine of the patients as well as from the pudding. The eggs were obtained from pigeons infected with paratyphoid and *typhi-murium* was actually isolated from some of the suspected eggs.

Outbreak I.

History.

In August, 1938, one of us (D.H.) autopsied three valuable Oriental Frills brought to the laboratory by the owner, Dr. L. V. Pearson, from Durban for investigation. About two hundred birds were kept by him in well-constructed lofts with concrete floors, while the floors of the adjoining lofts were covered with drift sand. The pigeons were valued at about £300 and approximately as much had been spent on the housing.

The pigeons, composed of different breeds, were bought mostly from a local dealer; some of these were imported from the East and had previously given negative slide agglutination tests with *gallinarum;* others were imported from leading fanciers in England. The first deaths occurred in January, 1938, when 16 birds died within a few days. Death was usually very rapid—some pigeons that appeared normal the day before were found dead the following morning; others frequently developed symptoms of vomiting and diarrhoea which lasted for a few days before the birds died. Poisoning was suspected so that a complete change was made in the food, water and feeding utensils. After a few more deaths the mortality ceased. In March, however, some fancy pigeons were bought and placed in the loft; within three days one died suddenly and three developed diarrhoea. Two of the latter died and one recovered.

In May a number of pigeons were imported from England and mixed with some valuable birds bought locally. Within a few days four of these birds had contracted a severe diarrhoea, from which all died. Periodical outbreaks occurred until March, 1939, when this paper was written. In all about eighty pigeons have died from the disease and twenty carriers have been detected by means of blood testing. Some of the latter were birds imported from England and in one case a reactor was detected on its arrival in South Africa. This proves that the infection was picked up in England and that the disease also exists in England.

The disease was usually characterised by its sudden onset and rapid ending. Several pigeons developed a green diarrhoea which generally lasted for a few days before death occurred. A few lingered for two weeks or longer and became extremely emaciated. In some cases arthritis and swelling of the joints developed, leading to lameness and dropped wing; occasionally there were indications of serious nervous disturbances, like twisting of the head, twitching of the neck and incoordination of movements. Only two of the sick birds recovered: the one was destroyed before its serum could be tested, while the other one proved to be reactor.

The following interesting observations have been made during Dr. Pearson's outbreak.

(1) Last year Dr. Pearson imported from England an Oriental Frill hen (Ring No. 2650) which had to be destroyed shortly after its arrival owing to a broken leg and wing. Before it was killed, however, an egg was laid. A Homer hen (Ring No. 378) that was used as foster parent hatched the egg and reared the young pigeon derived from the egg. Later this young Oriental Frill (Ring. No. SAPA 135) was tested and found to be a reactor. Just previously the hen (No. 378) had hatched and reared eight of her own squabs which remained healthy and proved to be non-reactors on two successive tests.

The hen (Ring No. 378) proved to be a carrier after rearing 135. Later it was destroyed and found to suffer from oophoritis. *Typhi-murium* was isolated from the affected ovary.

(2) An imported Baldhead Tumbler (Ring No. 204) acted as foster parent to two Oriental Frill squabs. She hatched and reared them until they were about 3 weeks old, when she died from paratyphoid. One of the Squabs also died from paratyphoid when about 6 weeks old. The other young pigeon (Ring No. 130) was tested as soon as it was old enough to be bled and proved to be negative for one test. Its father (Ring No. 1045) was found to be a non-reactor during two tests, while its mother (Ring No. 2006), although giving a negative reaction at the first test, proved to be positive subsequently.

(3) A Mag-pie cock (Ring No. 3406) and hen (Ring No. 3420) were paired in 1938. The hen died from paratyphoid fever in February, 1939. The cock was tested twice; the first test, in February, was negative but a subsequent one in March was positive.

(4) A couple of imported Saddle-back Tumblers, of which only the cock (Ring No. 734) was a reactor, first produced two squabs (Ring Nos. SAPA 136 and SAPA 137) both of which reacted. The one, a male (No. 137), was slaughtered but cultures made from the internal organs and intestinal contents failed to yield *typhi-murium*, the titre of the second one (No. 136) was apparently going down and the bird was being kept under observation. As a result of the second mating two more eggs were laid and hatched; one of the squabs died in its shell and the other (Ring No. SAPA 125) proved to be a reactor (titre 1:100). As the dead squab was not available for study, cultures could not be made and the cause of death remained unproven.

In some cases Dr. Pearson had locked in their nest-boxes, with their non-reacting mates, birds that subsequently proved to be reactors without infecting the former. There was no evidence, however, that the birds were shedding the bacteria.

There is no doubt that the disease has been kept going on the premises by a number of apparently healthy pigeons that remained carriers. By means of serological tests performed by us at different occasions twenty reactors were detected among birds that appeared

quite normal and healthy; from the organs of some of these, *typhi-murium* has been isolated. It is thought that the infection was introduced by a pigeon imported from the East and bought from a local dealer in Durban by Dr. Pearson. At the time of its introduction this bird was suffering from a form of diarrhoea, but as it was not available for examination at the time of the investigation neither serological nor cultural tests could be performed.

Autopsy of affected birds revealed extensive intestinal catarrh, swelling of the spleen and liver, necrotic nodules in the liver and lungs, oophoritis and chronic (purulent) arthritis. A pure culture of a Gram-negative, non-lactose fermenting bacterium was isolated from the heart-blood and spleen of diseased birds, and studied by one of us (M. W. H.) for its antigenic structure. The organism (labelled culture 548) was found to be motile and diphasic; it was readily agglutinated by " O " sera of group B of the Kauffmann-White schema, by *typhi-murium* type serum and by group sera. Mirror absorption tests showed that *typhi-murium* completely exhausted the agglutinins from 548 serum, but that culture 548, although removing all the type and group agglutinins from *typhi-murium* serum, merely reduced its " O " titre from 800 to 400. Moreover, *typhi-murium* var. *Copenhagen* completely exhausted 548 serum and culture 548 removed all the agglutinins from *typhi-murium* var. *Copenhagen* serum. The antigenic structure of culture 548 can be regarded, therefore, as identical with that of *typhi-murium* var. *Copenhagen*, which lacks " O " factor V.

In order to determine the extent of the infection the owner was advised to have his flock tested for carriers. Thick blood smears were taken from 150 birds and tested by the rapid agglutination method, using as antigen a thick suspension of culture 548. Seven of the slides gave definite fine, granular flocculation, and about ten gave an indefinite reaction. The owner was advised to send the seven reactors to Onderstepoort for further testing. Soon after their arrival the birds were bled and their sera used for the agglutination test (Table I).

Subsequently all the remaining birds were tested twice by the tube agglutination method with the result that a further number (14) of reactors were detected; some of these are also recorded in Table I.

According to the information recorded in Table I it will be noticed that the sera of all ten birds contained exclusively " O " agglutinins. There was no trace of either type or group agglutinin in the serum of any of the " O " reactors at a dilution of 1:20.

Nine of the pigeons were slaughtered and autopsied, and cultures were made from the internal organs (heart blood, liver, peritoneum, spleen, intestine and ovary or testes) of each one. Four of the birds were males and five females. One of the former (No. 496) was affected with chronic arthritis of the left hock and two suffered from enlarged testes (Nos. 296 and 2062), but no other pathological changes could be detected in any of the male birds. Cultures made from the pus of the affected joint of one of the male birds (No. 496) and from the liver, spleen, testes and heart-blood

of all of them remained sterile. All five of the females were affected with oophoritis. In addition, two were suffering from a mild chronic peritonitis, and one from both peritonitis and pericarditis. All five showed a variable amount of swelling of the spleen, but no apparent abnormality could be detected in the liver. Heart-blood, spleen and liver cultures made from the females yielded no growth, but a gram-negative, non-lactose fermenting organism resembling culture 548 was obtained from the ovaries of all five. A similar bacterium was obtained from a culture made from the peritoneal fluid of one bird, but not from the peritoneal fluid of the other four, nor from the pericardial fluid of the one affected with pericarditis. Cultures made from the intestinal contents of all nine pigeons yielded only lactose-fermenting Gram-negative bacteria. In all cases the material was seeded on MacConkey's bile-salt agar in Mason tubes as well as in tetrathionate broth.

TABLE I

Agglutination Reactions of the Sera of Carrier Pigeons.

Serum of.	548 "O" Antigen.	548 "type" Antigen.	548 "group" Antigen.	Result of Organ Cultures.
*Female Pigeon No. 458.....	20	0	0	Ovary and heart blood pos.
Female Pigeon No. 463.....	100	0	0	Ovary, pos.
Female Pigeon No. 488.....	400	0	0	Ovary, pos.
Female Pigeon No. 492.....	200	0	0	Ovary, pos.
Male Pigeon No. 490.....	400	0	0	Negative.
Male Pigeon No. 490.....	100	0	0	Negative.
Female Pigeon No. 493.....	800	0	0	Ovary and peritoneal fluid, pos.
Male Pigeon No. 2062.....	80	0	0	Negative.
Male Pigeon No. 137.....	40	0	0	Negative.
Female Pigeon No. 378.....	80	0	0	Ovary, pos.
Rabbit immunised with 548...	800	12800	6400	—

0 = less than 1 : 20; pos. = positive culture from the organ stated, cultures from all other organs being negative; negative = no growth of *typhi-murium* obtained from any organs examined.

* At a subsequent test the titre of Pigeon 458 had dropped to 1 : 10.

Although Pigeon 548, the sixth female, also gave a positive reaction when a number of birds were tested in October, 1938 (Table I) the titre of its serum was so low (1 : 20) that it was considered advisable to keep it under observation for some time rather than kill it for the purpose of examining its organs.

In April, 1939, this bird was again bled and its serum tested. A definite "O" agglutination was obtained only in the first tube i.e. at a dilution of 1 : 10. There was no "H" agglutination at all and no distinct "O" agglutination at 1 : 20. Approximately a week after the test the pigeon was noticed sick, it appeared listless, and sat huddled up in a corner of its cage with its feathers ruffled and was suffering from diarrhoea. The evacuations were dirty-grey

and soiled the cloacal feathers. The bird refused to eat and died after an illness which lasted about a week. The carcase was autopsied, but apart from swelling of the liver and enteritis, no pathological changes could be seen. Cultures made from the heart-blood, liver and ovary yielded a poor growth of *typhi-murium*, but no *typhi-murium* could be found in the media seeded with intestinal contents. From October, 1938, until the time of its death in May, 1939, Pigeon 548 was kept alone in a cage and did not come in contact with any other bird.

The sera of a number of obviously sick birds were tested before death, but all failed to give " O " agglutinations at dilutions of 1:20 and over.

The six strains of non-lactose fermenting bacteria isolated from the female birds were found to be actively motile and diphasic; all six strains proved to be identical with culture 548. It is significant that all six female carrier birds harboured in their ovaries *typhi-murium* var. *Copenhagen* which yielded cultures of actively motile bacteria, whereas the agglutinins found in their sera were exclusively of the " O " variety. Only six female carriers were examined and *typhi-murium* were obtained from all six.* Rabbits injected with killed suspensions of these strains of *typhi-murium* produced in their sera type, group as well as " "O " agglutinins.

This observation is in agreement with the findings of Pijper and his co-workers on typhoid fever (Pijper, 1930; Pijper and Pullinger, 1928; Pijper and Crocker, 1937). They consider that a diagnosis of enteric fever " hinges on the demonstration of ' O ' agglutination ". Seven of the chronic human carriers studied all gave a marked and exclusive " O " agglutination in a serum dilution of 1:100 with a sensitive strain (Ty 901). Two urinary carriers that gave only " O " agglutination produced " H " agglutinins after a series of subcutaneous injections of typhoid vaccine. They confine their search for human carriers entirely to the complement-fixation and " O " agglutination tests, completely ignoring " H " agglutination. Weil (1921) attributed the close relationship between these two tests to the fact that complement-fixation is primarily provoked by " O " antigens. In practice Daubenton (1931) confirmed Pijper's work; by simply prohibiting all African Natives with positive " O " agglutination tests from working as cooks on a goldmine he succeeded in considerably reducing the incidence of typhoid fever.

Outbreak II.

History.

This outbreak (Rogers) occurred in a flock of about 50 Homer pigeons kept in wooden lofts with the floors covered with wood shavings. Attached to the lofts is an aviary of wild birds, and in another loft about twenty yards away some fancy pigeons are kept.

* Since going to press, another four females were autopsied and *typhi-murium* was isolated from the ovary of each one.

The Homers have been kept in the present lofts for about three years. Apart from a few losses due to so-called "canker" (probably trichomoniasis) no deaths have occurred until October, 1938, when five young pigeons suddenly developed nervous symptoms associated with twisting of the neck and falling over backwards. The duration of the disease was usually about five days. One of the sick birds brought to the laboratory (Allerton) was autopsied and it showed the following lesions:—swelling of the spleen, catarrh of the intestine, and caseous purulent material in the spaces between the cerebrum and cerebellum; a Gram-negative, non-lactose fermenting bacterium (Culture 576) was isolated from this purulent material. This organism was found to be motile and diphasic; it also fell into group B of the Kauffmann-White schema. Agglutination and absorption tests showed that it resembled culture 548 in every respect; like culture 548 it lacked factor V, and its type and group antigens were identical with those of *typhi-murium*.

Outbreak III.

A third outbreak of *typhi-murium* infection in pigeons (Pietersen) was also studied by us. The birds were bought at a dispersal sale and were transported for several hundred miles to their new quarters where the disease broke out. A pure growth of *typhi-murium* (culture 629) was obtained in heart-blood, spleen and liver cultures made from several pigeons. Serological tests performed in the same way as for culture 548 showed that the bacterium is a strain of *typhi-murium* which contains both factors IV and V, differing, therefore, from the organisms isolated in the two previous outbreaks.

In the three outbreaks studied the following symptoms and lesions have been observed:—

Symptoms.

(*a*) Acute and peracute symptoms are usually manifested by young birds and sometimes by adults when the disease suddenly makes its appearance in a loft that was previously free from infection. In peracute cases, a bird that was apparently healthy the night before is found dead the following morning without having shown any symptoms of disease. In acute cases the pigeon lives for a day or two and typical symptoms have had time to develop. The bird is noticed to be off its food, it stands listless and huddled up with its feathers ruffled and it may suffer from severe diarrhoea, vomiting, thirst, pneumonia and progressive weakness. Sometimes there are nervous symptoms leading to convulsions, and paralysis of the muscles of locomotion and flight. When there has been continuous scouring the bird becomes extremely exhausted after a day or two with its vent feathers badly soiled. The mortality is always very high and recoveries are rare, but when an affected bird recovers it generally remains a carrier and so serves as a continuous source of infection. Sometimes, as in the case of Dr. Pearson's outbreak, considerable losses are sustained from this acute form of the disease in birds of all ages.

(*b*) Subacute and chronic symptoms are generally shown by adult birds. The affected pigeons frequently suffer from a form of diarrhoea which may last for weeks or even months. They usually lose condition in spite of good food and become progressively weaker and weaker until they are finally extremely emaciated before the end arrives. Birds affected with pneumonia may also live for some days before they die. Nervous symptoms and arthritis are fairly common. In this latter case the affected joints are swollen and painful, there is drooping of the wings and lameness or paralysis of the affected limb. When the nervous system is affected the birds may suffer from convulsions, twisting of the neck, incoordination of movements and paralysis of the muscles of locomotion and flight. All chronic cases should be regarded as dangerous as they may serve as reservoirs of infection as long as they remain alive. All the female reactors autopsied by us were found to be suffering from oophoritis and *typhi-murium* was isolated from the affected ovaries.

Lesions.—In acute cases there are generally indications of a septicaemia giving rise to swelling of the spleen and liver, acute catarrhal enteritis, and pneumonia; sometimes small greyish necrotic nodules are scattered throughout the lung and liver tissue, and even the pectoral muscles.

In chronic cases the carcase is usually extremely emaciated; there is softening and atrophy of the pectoral and leg muscles, and sometimes numerous greyish nodules are found in the pectoral muscles; there is arthritis with a purulent or caseo-purulent material distending the joint capsule; there may be necrotic nodules on and ulceration of the mucous membrane of the intestine, necrotic nodules in the liver and lungs, pericarditis and peritonitis. Oophoritis is common in females and males sometimes suffer from orchitis. Meningitis may be a lesion in some outbreaks.

Some infected birds may live for months and so act as carriers without showing any obvious signs of illness. Such birds are particularly dangerous as they may set up a virulent epizootic at any time when they are brought in contact with healthy birds.

Beaudette (1926) has found *typhi-murium* in the unabsorbed yolk-sacs of young birds and isolated this organism invariably from the ovaries of female carriers.

As shown above all the six female carriers autopsied by us suffered from chronic oophoritis, and *typhi-murium* was isolated from the ovary of each one. Clarenburg and Dornickx (1932) obtained *typhi-murium* from the eggs of carrier birds, but so far we have not yet succeeded in isolating the bacterium from eggs.

There are several factors which predispose the birds to infection with paratyphoid organisms, e.g. overcrowding, chilling, exposure to unhygienic conditions, transportation for long distances, infection with an intercurrent disease like pigeon-pox, or exposure to any factor that is liable to reduce the vitality and resistance of the birds.

B. Equines.

Henning and Clark (1938) described an outbreak of purulent arthritis in the foals of a stud in the Orange Free State. *Typhimurium* var. *Copenhagen* was found to be the cause of the disease. A pure culture of this organism was obtained from the pus of the affected joints. Agglutination and absorption tests were performed in the same way as with culture 548 and identical results were obtained.

According to information obtained from the manager of the stud where the outbreak occurred there is no evidence that pigeons have ever been kept in or near the stables; but the place abounds with Rock Doves, *Dialiphila phaeonota*.

C. Bovines.

More recently one of us (M.W.H.) studied an outbreak of calf-pneumonia in a dairy herd in Johannesburg. Several calves were reported sick from time to time and the majority of these died. The affected calves were weak and emaciated, lay most of the time and developed a dry cough; the breathing was usually fast and there was a dirty purulent discharge from the nose; diarrhoea was sometimes present. Three sick calves were killed and autopsied, and the following pathological changes were observed: The carcase was extremely emaciated; hard casein clots (and in one case, wood shavings) were found in the abomasum. In the first calf the liver was swollen and light yellowish-brown in colour, and several small light necrotic-looking areas were noticed on the cut surface; the spleen was slightly enlarged. In the other two cases the liver and spleen appeared normal. In all three calves the most outstanding changes were found in the thorax—there were hydrothorax and hydropericardium, and the lungs were extensively affected; there were adhesions between the costal and pulmonary pleurae, and large portions of the lung tissue were hard, consolidated and dirty in colour. On section numerous abscesses, varying in size, were exposed, the largest ones being up to 3 c.m. in diameter; irregular light-grey necrotic foci were scattered in the tissue between the abscesses. The contents of the abscesses were generally semi-fluid and varied in colour from dirty-white to slatey-blue; some of the abscesses appeared vacuolated. Cultures were made from the heart-blood, liver, spleen and lungs of all three calves, and organ specimens were taken for histological study.

In the first calf a pure culture of a Gram-negative non-lactose fermenting bacterium was obtained from the liver, spleen and heart-blood; this organism was labelled culture 580 and is described below. The culture from the lungs yielded a mixed growth of a Gram-negative organism (probably the same as culture 580), a Gram-negative, bipolar staining organism and a pleomorphic Gram-positive cocco-bacillus.

Cultures made from the organs of the second and third calves did not yield any organisms resembling those of culture 580 obtained from the first calf, but the growth from the lungs of the second calf

was composed predominently of Gram-positive diphtheroids and a few colonies of a Pasteurella. The cultures made from the lungs of the third calf yielded an apparently pure growth of Gram-positive diphtheroids.

The cocco-bacilli obtained from the three calves appeared to be identical. They refused to grow on ordinary meat-infusion agar, but on serum agar or blood agar they gave rise to a faint confluent growth after 24 hours' incubation. The individual colonies were very fine and barely visible in less than 48 hours. When the cultures were incubated for two or three days the colonies became larger and more distinct. Morphologically and culturally the organism resembles *corynebacterium pyogenes*, but a more detailed study of the bacterium is being undertaken and will form the subject of another paper. As the lung lesions presented by the three calves resemble those usually associated with *corynebacterium* infection (Lovell and Hughes, 1935) the cause of the pneumonia is provisionally attributed to this organism. The presence of the *Salmonella* (culture 580) in one calf is ascribed to secondary causes—this organism probably gained admission into the tissues of the calf after its resistance had been lowered by pneumonia due to the *corynebacterium*. A vaccine produced from culture 580 and used in the affected herd did not lower the incidence of calf pneumonia.

The significance of *pasteurellas* in the lungs of the two calves cannot be appraised at present. It is well known that *pasteurellas* frequently invade the pulmonary tissues of cattle and sheep suffering from pneumonia (Henning and Brown, 1936) but their presence may be due either to primary or secondary causes. As these *pasteurellas* were found to be non-pathogenic for mice and guinea-pigs they can be regarded as secondary invaders.

Like strain 548, culture 580 was tested against various " O ", type and group sera of the Kauffmann-White schema and it was also found to be a strain of *typhi-murium*. Moreover, mirror absorption tests proved it to be a IV-variant of *typhi-murium* identical with culture 548 and, therefore, with *typhi-murium* var. *Copenhagen*. The owner of the dairy states that pigeons have never been kept on the premises and that the calves have never been off the premises. The only other record of the isolation of the IV-variant of *typhi-murium* from a bovine is that of Hohn and Harrmann (1937).

During his study of the paratyphoid B group Schutze (1920) found the two strains, Binns and Timson, serologically alike and called them the Binns type; he also showed that they were serologically related to *typhi-murium*. White (1926) failed to isolate a specific strain from *Binns* and concluded that it occurs permanently in the non-specific phase; he also noticed that it contained qualitatively a little less of the *aertrycke* " O " complex. As a result the following assignment was given to *Binns*:—IV, V: — : 1, 2, 3. After studying several strains of *Binns*, however, Edwards (1936) showed (1) that they all contained specific components characteristic of *typhi-murium* and (2) that they all lacked " O " factor V. He, therefore, amended the antigenic formula of *typhi-murium* var. *Binns* to read IV: i: 1, 2, 3. This is identical with the formula assigned by Kauffman (1934) to the organism

typhi-murium var. *Copenhagen* described by him and it is also identical with the formula given by Edwards (1935) to *typhi-murium* var. *Storrs*. But Landsteiner and Levine (1932) were the first to notice that certain strains of *typhi-murium* may be devoid of "O" factor V.

Recently several other workers have reported strains of IV-variants. Thus, Zahn (1935) found one out of 74 cultures of *typhi-murium* studied; Hoffman and Edwards (1937) and Höhn and Herrmann (1937) have isolated a number of strains of this variant from pigeons. Höhn and Herrmann have recorded one culture from a calf. Edwards (1938) studied 155 strains of *typhi-murium* of animal origin. All the IV-variants encountered were obtained either directly or indirectly from pigeons; one culture was isolated from rabbits which had been in contact with infected pigeons. All thirty cultures obtained from pigeons were IV-variants. Later Edwards studied several more IV-strains from pigeons, also one from a duck and one from a chicken.

Discussion of Biochemical Reactions.

In the biochemical tests the following points were observed:— The eight cultures from the Pearson outbreak were identical biochemically as well as serologically. Their antigenic structure resembled that of Kauffman's *S. typhi-murium* var. *Copenhagen*, but unlike the latter they all fermented inosite; their reaction to the Stern test was definitely positive while Kauffman's strain was only slightly positive. Moreover, the latter have a much stronger reaction with Jordan and Harmon's test than the Pearson strains. The Roger strain (culture 576 differed from the Pearson strains biochemically by failing to ferment inosite, but resembled them otherwise, both biochemically and serologically. The Pietersen strain (culture 629) which was a typical *typhi-murium*, containing both factors IV and V, gave a negative Stern reaction and a strongly positive Jordan and Harmon test, otherwise it resembled the Pearson strains biochemically.

The Calf strain (culture 580) resembled the Pearson strain serologically and also biochemically excepting for the fact that it gave a slightly positive Stern and a strongly positive Jordan and Harmon reaction. The Foal strain (culture 478) was a typical IV-variant serologically but biochemically it differed from the Pearson strains by giving a strong Jordan and Harmon reaction and by failing to ferment Arabinose.

The organism obtained from a chicken outbreak (culture 357), a typical *typhi-murium*, differed biochemically from two Stock strains of *typhi-murium* (Glasgow and Weybridge) by failing to react to the Bitter test and by not fermenting inosite. The two canary strains (cultures 581 and 626) resembled the two stock strains of *typhi-murium* both antigenically and biochemically.

These results show that the eight cultures of *typhi-murium* (IV-variants) isolated from the Pearson outbreak were all identical antigenically and biochemically, but that the four strains of organisms isolated from the four separate outbreaks (Pigeons 2,

TABLE 2.

Biochemical Tests of Various Strains and Variants of S. typhi-murium *and of one Strain of* Paratyphi-B.

Number and Nature of Culture.	Source.	Bitter Test.	Stern Test.	Jordan Test.	Glucose.	Lactose.	Dulcite.	Saccharose.	Mannite.	Maltose.	Arabinose.	Rhamnose.	Inulin.	Inosite.	Salicin.	Xylose.	Sorbite.	Laevulose.	Indol.	H_2S.
S. typhi-murium var. *Copenhagen* No. 548	Pigeon (Pearson).	±	+	±	+	−	+	−	+	+	±	+	−	±	±	+	+	+	−	+
S. typhi-murium v. *Copenhagen*—																				
No. 586	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 588	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 589	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 593	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 603	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 612	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 643	Pigeon (Pearson).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 576	Pigeon (Rogers).	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 580	Calf	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
S. typhi-murium—																				
No. 581	Canary	+	±	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 626	Canary	+	−	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 629	Pigeon (Pieterson)	+	−	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
No. 527	Chicken	−	±	+	+	−	+	−	+	+	+	+	−	±	−	+	+	+	−	±
(Glasgow)	Stock	+	±	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
(Waybridge)	Stock	+	−	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
V. Storrs (No. 19500)	Stock	+	−	+	+	−	+	−	+	−	+	+	−	+	−	+	+	+	−	+
S. typhi-murium V. *Copenhagen* (No. 1147)	Stock	+	±	+	+	−	+	−	+	+	+	+	−	−	−	+	+	+	−	+
Paratyphi-B. (Scott)	Stock	−	−	−	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−	+
S. typhi-murium V. *Copenhagen* No. 478	Foal	+	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+	+	−	+

+ = positive, − = negative, ± = slightly positive, +, ++ and +++ (in Jordan) degrees of positive reaction. *typhi-murium* var. *Copenhagen* and *typhi-murium* var. *Storrs* = *typhi-murium* var. *Binns*. Stern = Fuchsin broth according to Stern (1916). Bitter = Rhamnose medium of Bitter Weigmann and Habs (1926). Jordan = d−tartrate agar of Jordan and Harmon (1928).

Calves 1, Foals 1) were all different in their biochemical reactions, although they were all identical serologically. Moreover, not one of the four strains of *typhi-murium* (IV-variants) described by us gave the same biochemical reaction as either the Copenhagen or Storrs variety of the bacterium.

Summary.

Outbreaks of *typhi-murium* have been described in pigeons (3), foals (1) and calves (1). The antigenic structure of the different strains isolated was studied and it was shown that, with the exception of one strain from an epizootic in pigeons, all were lacking in factor V. The strain obtained from the *one* epizootic in pigeons contained both factors IV and V.

By means of agglutination tests several carriers were detected among the pigeons, but all the reactors contained exclusively "O" agglutinations—no trace of H. agglutination could be detected in any one of the carriers. All the female carriers suffered from oophoritis and actively motile strains of *typhi-murium* were obtained from all the affected ovaries.

It was not possible to isolate *typhi-murium* from the internal organs studied and intestinal contents of any one of the male carriers.

The diseases in the calves was associated with a cocco-bacillus infection, probably *Corynebacterium pyogenes*, which may be the primary cause of the pneumonia.

In foals the organism isolated from the pus of the affected joints is probably the real cause of the joint-ill.

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