PATHOGENESIS AND CONTROL OF EXPERIMENTAL SALMONELLA INFECTIONS

by

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This work is dedicated to the sweet memory of my beloved parents who still continue to be a great fountain of inspiration to me: to strive, to seek and to venture into pastures new.

PREFACE

In spite of the fact that typhoid fever was recognised as early as 1659 by Thomas Willis (Huckstep, 1962) and the causative organism 'B. typhosus' was discovered by Eberth in 1880, the enteric fevers and salmonellosis still continue to pose a challenge to the human ingenuity and intelligence even in this modern era of great technical and social advancement. In 1961, a report on typhoid fever by the World Health Organisation stated, "Although this disease is not a major public health problem in most European or other developed countries, it continues to be one in vast areas of the world." Typhoid and paratyphoid fevers occur from time to time in the economically developed countries, e.g. the Zermatt outbreak in Switzerland, 1962 and the Aberdeen outbreak in Scotland, 1964. Salmonellosis, especially salmonella food poisoning, is a greater problem in the more economically advanced countries.

The salmonellae often grow without visible signs in apparently wholesome food. The salmonellae are responsible for more outbreaks of food poisoning throughout the world than all other known bacterial and chemical poisons put together. In 1954, according to Leff (1957), out of a total of 8961 outbreaks in England and Wales, 5269 were due to salmonellae, and reports from most parts of the world confirm that Salmonella typhimurium is the commonest cause of salmonella food poisoning. Despite the dramatic curative effects of treatment with chloramphenicol, relapses

are more frequent than in the days prior to specific drug treatment, and complications and convalescent carriage still occur.

The present position as regards the treatment of enteric fevers, although vastly improved, is thus not completely satisfactory; and the same is true of the early diagnosis and prophylaxis of the fevers. There is indeed a great need for the development of improved methods of control by active immunisation with blander and more potent vaccines, and cure of patients and treatment of carriers with a drug that is effectively bactericidal and at the same time free from the toxicity of chloramphenicol. However, it is apparent that further progress in the understanding and the control of these diseases would be contingent on a better understanding of their pathogenesis and of the factors involved in acquired resistance to the infection. It is doubtful whether any evidence of significance regarding the human disease can be obtained from the study of experimental infections with S. typhi in small laboratory animals. S. typhi injected intraperitoneally in large doses into mice causes a disease of short duration terminated by a "toxic" type of death after 2-3 days or by recovery with little tendency to prolonged carriage (Ørskov and Kauffmann, 1936). The organisms multiply at the site of the injection and are not widely disseminated (Bacon, Burrows and Yates, 1951). Fortunately, however, the systemic infection, "mouse typhoid",

caused by S. typhimurium which can be readily reproduced experimentally in mice, resembles in considerable degree the natural typhoid and paratyphoid fevers of man. Also S. typhimurium is closely related antigenically to S. paratyphi B, which is the cause of the most commonly occuring paratyphoid fever in Britain. For these reasons S. typhimurium infections in mice were used as the model for the present studies on the pathogenesis and control of salmonella infection.

PLAN OF STUDY

The investigation consisted of three parts:-

(I) Studies of pathogenesis.

1. Investigation of the role of fimbriae and flagella in the pathogenesis of mouse typhoid:-

   (a) The virulence of pairs of S. typhimurium differing in the presence or absence of fimbriae or flagella was compared in mice infected intraperitoneally or orally. The strains in each pair were closely related, one having been derived from the other by mutation or phage-mediated transduction.

   (b) The above mentioned S. typhimurium strains and, in addition, fimbriate and non-fimbriate enteropathogenic Escherichia coli strains, were tested by De's (1953) technique with ligated rabbit gut loops.

2. Investigation of experimental salmonellosis in albino rats.

(a) Oral and intraperitoneal injection of albino rats with fimbriate and non-fimbriate S. typhimurium.

(b) Localisation of possible sites of penetration of the gastro-intestinal tract by S. typhimurium.

(c) Passage down the gastro-intestinal tract of orally administered S. typhimurium.

3. The effect of adhesion of fimbriate organisms (S. typhimurium and S. flexneri) on human leucocytes in in vitro experiments.

(II) Electron-microscope studies.

(a) Examination of shadowed (Au:Pd.) and negatively stained (1 per cent. phosphotungstic acid) S. typhimurium organisms to demonstrate fimbriae and flagella.

(b) Examination of shadowed and unshadowed specimens to demonstrate adhesion of fimbriate S. typhimurium to human leucocytes, red blood cells and platelets.

(c) Preparation and examination of ultrathin araldite sections of S. typhimurium and its fimbriae and flagella.

(d) Examination of ultrathin araldite sections of the duodenum of albino rats to study the mode of penetration of the epithelial mucosa of the gastro-intestinal tract by S. typhimurium.

(III) Studies of control of infection.

Active Immunisation.

Comparison of the protective potencies in mice of (a) live attenuated vaccines and (b) killed vaccines; including heat-killed, phenol preserved vaccines and acetone-killed vaccines (used with and without an adjuvant)prepared from non-fimbriate/non-flagellate S. typhimurium and its phage-transductant fimbriate/flagellate derivative.

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C O N T E N T S

REVIEW OF LITERATURE

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THE GENUS SALMONELLA

The generic name "Salmonella" was adopted in 1934 by the Sub-Committee of the International Association of Microbiologists. The generic definition adopted by the Salmonella Sub-Committee in 1934 was modified by the Committee in 1949 (Edwards and Ewing, 1962) and it now stands as follows:-

"A large genus of serologically related, gram-negative and non-sporing bacilli; 0.4 to 0.6 by 1 to 3 microns in the usual dimensions but occasionally forming short filaments; showing, with certain exceptions, a motile peritrichous phase in which they normally occur; in fact adhering to the pattern of S. typhi in staining properties and morphology. Rarely fermenting lactose or sucrose, liquefying gelatin or producing indol. They regularly attack glucose with, but occasionally without, gas production. All the known species are pathogenic for man, animals or both."

The type species is Salmonella cholerae-suis (Breed et al. 1959).

Long before the development of modern bacteriology and the isolation of the typhoid bacillus, William Budd's (1856) accurate epidemiological study of typhoid fever outbreaks indicated that the disease was contagious and that the infective agent was excreted in the faeces of the patients (Morgan, 1952). William Budd also rightly

believed that the contamination of milk and water probably played a role in the spread of the disease. S. typhi was the first member of the genus to be isolated and described. It was described by Eberth in 1880 in the tissues of patients and it was isolated in 1884 by Gaffky. Following this, other members of the genus were isolated from cases of typhoid-like fevers. S. cholerae-suis was isolated and reported by Salmon and Smith in 1885; S. enteritidis was isolated by Gaertner in 1888 from a patient who died following the consumption of contaminated meat. Durham and de Noeble (1898) described S. typhi-murium which they had isolated independently from patients suffering from gastro-enteritis following the ingestion of infected meat. The rapid isolation of closely related members of the genus from a number of diverse sources soon led to inevitable confusion. However, the pioneer work of White (1925, 1926) who recognised the importance and the necessity of considering recent discoveries concerning bacterial variation in the characterisation of the bacilli; placed the classification of salmonellae on a sound basis. This work was confirmed by Kauffmann (1941) who modified, systematised and greatly extended it to form the present classification of the genus. This has made possible the rapid and accurate recognition of the serologic types, or "serotypes" as they are now called. The Kauffmann-White scheme in 1955 listed 343 serotypes (Edwards and Ewing, 1955), but in 1962 the list had increased to 758 (Edwards

and Ewing, 1962) and this is by no means the end of the growing list. The Kauffmann-White scheme is based on antigenic patterns. The serotypes have been arranged in groups, A, B, C - - - - etc., according to the O, or somatic antigens, they contain. The possession of one or more common antigens is regarded as essential for inclusion in the same group.

Antigens of Salmonellae

Most members of the group are flagellate and hence they possess the flagellar or H antigens in addition to the O or somatic antigens. Some members, for example S. typhi, have a third antigen (Vi) which is a somatic antigen. The flagellar antigen in many members of the genus is diphasic. The phases are called phase 1 and phase 2. The phase 1 flagellar antigenic components are labelled a, b, c, - - - - etc. and phase 2 flagellar antigens are labelled 1, 2, 3, - - - - etc. and e, n, x, z, - - - - etc. The flagellar phase 1 antigens are the more specific and the less widely shared. The somatic antigens are monophasic and are also accorded arabic numerals, i.e. 1, 2, 3, - - - 56, 57, 58. The O antigens associated with rough strains are different from those associated with smooth strains. There are also heat-labile and heat-stable antigens which are associated with the sheath or capsule.

According to Furth and Landsteiner (1928, 1929), the somatic antigens are made of several chemical components.

The studies of White (1929, a, b; 1931) make it clear that the somatic antigens are polysaccharide or have a polysaccharide component. Similar observations have been recorded by subsequent observers (Casper 1928, 1929; Combiesco et al., 1930; Basilewsky and Remgild, 1935). Studies by Freeman, Challinor and Wilson (1940) and Morgan and Partridge (1942) on S. typhi suggested that the O antigen is a polymolecular complex formed of a specific polysaccharide, a protein and a phospholipin. The protein component is said to be chemically and immunologically very similar to the conjugated protein prepared by Morgan and Partridge (1940) from the specific somatic antigen of Shigella dysenteriae. According to Freeman (1943) the somatic antigen of S. typhimurium chemically resembles that of S. typhi. The Vi antigen appears likewise to be of a glyco-lipid nature. It differs chemically in that on hydrolysis with acid it yields only half as much reducing sugar as the O antigen and it is precipitated in aqueous solutions by aluminium, uranyl, and lanthanum salts (Boivin and Mesrobeanu, 1938; Boivin, Izard and Sarciron, 1939 a, b). It contains no sulphur or phosphorus. The flagellar antigens appear to be of fibrous proteins of the myosin-keratin type.

Variations affecting the antigens of salmonellae

The successful classification of salmonellae is dependent upon the understanding and recognition of certain variations to which members of the genus are subjected.

O-H variation. This variation was studied by Smith

and Reagh (1903) and Beyer and Reagh (1904) and they worked with S. cholerae-suis. It was also studied and reported by Joos (1903) who worked with S. typhi. Motile organisms possess two types of antigens differing in physical characteristics, each of which stimulates in vivo its own particular antibody with which it reacts exclusively. One of these is associated with the flagella and is not found in non-flagellated organisms. It is heat labile and is progressively inactivated at temperatures above 60°C. After exposure to 100°C. for an hour its agglutinogenic property is lost. It is inactivated by acids and alcohol. The H antigens in the presence of homologous agglutinins are flocculated rapidly in large fluffy clumps which are easily dispersed. The other antigens called the O or somatic antigens are present in both motile and non-motile bacilli and are resistant to prolonged heating at 100°C. and to treatment with alcohol and dilute acids. The O agglutinins react much more slowly with their respective antigens than do the H agglutinins, irrespective of the motility or non-motility of the strain. In contrast to the loose floccular clumps produced by the H agglutinins, the O agglutinins produce finely granular clumps which are dispersed with difficulty. It should be emphasised that the term 'O antigen' does not include all the antigens that may be present in the non-motile strains but only those heat stable antigens present in the body of smooth organisms.

S-R Variation. Arkwright (1921) noted that certain bacterial cultures underwent changes in colonial morphology associated with changes in the character of growth in broth and stability of bacterial suspensions in saline. These degenerative changes were referred to as "roughness", since the colonies of the changed strains possessed roughened surfaces and irregular borders. The original condition of the culture was referred to as being in the smooth or "S" state and the changed culture as being in the rough or "R" state. R cultures tend to produce a granular growth in broth, and saline suspensions prepared with R cultures are much less stable than those prepared from S cultures. The serological properties of the O antigen of the rough strains are not the same as those of the smooth cultures from which they are derived. The rough cultures have a tendency to cross agglutinate regardless of whether their smooth parents were related or not.

V-W Variation. Some recently isolated strains of the typhoid bacillus are non-agglutinable by homologous O antiserum. Felix and Pitt (1934, 1934a) demonstrated that the O inagglutinable cultures had a special antigen which they called a virulence or Vi antigen. Kauffmann (1935) called colonies which contained Vi antigen, "V colonies" and colonies which lacked Vi antigen "W colonies". V colonies were agglutinated by Vi antiserum but not by pure O antiserum, whilst W colonies were agglutinated by O antiserum but not by pure Vi antiserum. Kauffmann also

found that on continual transfer each form gave rise to the other and he called this the V-W variation. The V-W variation is a widely distributed phenomenon. Intermediate VW forms are agglutinated by both Vi and O antisera.

M-N Variation. This term was introduced by Kauffmann (1936) to denote the variation between the mucoid (M) and the non-mucoid (N) forms of salmonellae. Kauffmann (1935) (1936) demonstrated a special M antigen in mucoid cultures. Mucoid forms are not agglutinated by O antiserum but if living mucoid cultures are injected into rabbits, agglutinins for the M form are produced. The M antigen of all salmonella organisms in which it has been examined is apparently the same, although the organisms which produce it belong to a variety of serotypes in different groups. Definite capsules can be demonstrated in some but not in all mucoid strains. Mucoid forms are inagglutinable by O antiserum, but this inagglutinability is annulled by heating at 100°C. for 1 to 2 hours.

Phase variation affecting the H antigens.

The specific groupphase variation. This was described by Andrewes(1922, 1925). The flagella may occur in two alternative antigenic forms. In the specific phase (phase 1) the H antigenic components are either peculiar to the particular species or are shared by only a few other species, whilst in the group phase (phase 2) the flagella contain antigenic components shared by many other species.

α-β phase variation. This variation was described by

Kauffmann and Mitsui (1930). It does not differ in principle from the specific-to-group phase variation. In this instance, the phase 2 contains the antigenic components e, n, x, etc. instead of 1, 2, 3, etc.

A special type of α-β phase variation. This was described by Edwards and Bruner (1938) where the phase 2 in S. worthington instead of containing the 1, 2, 3, etc. or e, n, x, etc. H antigens contained antigenic components of the a, b, c, series which had previously been met with only in phase 1.

Artificial phase variation. This is more of academic interest than of practical importance. Kauffmann (1936c) observed that if a certain strain S. typhi was cultured in broth containing anti-d serum, the d antigen was lost and replaced by a new antigen j.

Antigenic changes induced by bacteriophages. It has been known for a long time that bacteriophages bring about changes in the form of growth and antigenic behaviour of enteric organisms. The phages affect the somatic, envelope or sheath, and flagellar antigens and are thus able to transmit many physiological properties. Bruner and Edwards (1948) showed that O antigens 3, 10 could be changed to antigens 3, 15 and vice versa by the cultivation of S. anatum and S. meleagridis in semi-solid media containing respectively absorbed 10 and 15 antisera. This was shown to be the result of phage action by Iseki and Sakai (1953a, 1953b). Kauffmann (1953) noted that in

transductions involving H antigens, the O antigens of S. paratyphi A var durazzo (2, 12) was changed to 1, 2, 12. These findings were confirmed and extended by Stocker (1958, 1958a) who further reported that the form variations involving antigen 1 occurred regularly in cultures that were lysogenised with a type-A phage. Changes in the O antigens of Salmonella group C_2 brought about by phage action were described by Baron, Formal and Washington (1957) and Iseki and Matsumoto (1959), who found that salmonella organisms possessing the somatic antigens 8, and 20 carried a phage that was active on cultures having somatic antigen 8, or the complex 6, 8. When the cultures of salmonella possessing somatic antigens 6 and 8 were made lysogenic with such phages, forms of salmonellae having somatic antigens 6, 8, and 20 were obtained. Not only are the somatic antigens of salmonellae changed by phages, the sheath or envelope (K) antigens are also affected. The Vi phages act upon the Vi strains of S. typhi and the resultant lysogenic forms are resistant to the phages and are inagglutinable by Vi antisera. Phages also have marked effects upon flagellation of salmonellae. Sertic and Boulgakov (1936a) described a phage active on S. typhi that was specifically adsorbed to the H antigen and Rakieton and Bornstein (1941) described phages active on S. typhi and S. poona which produced lysogenic cultures that were non-motile and in which no H antigens could be demonstrated. Phages have also been used to mobilise

non-motile cultures through the induction of genetic material by transduction. This phenomenon was studied extensively by Stocker, Zinder and Lederberg (1953) who identified six homologous genetic factors - the mutation of any of which may cause lack of motility. Further it is possible to transduce antigens in a variety of salmonella types by the action of temperate phages (Zinder and Lederberg, 1952; Lederberg and Edwards, 1953; and Edwards, Cherry and Davies, 1955).

Fimbriate - non-fimbriate variation. Duguid et al., (1955) and Duguid and Gillies (1957) showed that fimbriate cultures underwent reversible variation between fimbriate and non-fimbriate forms and that this variation could generally be controlled by conditions under which the bacteria were cultivated. They found that the fimbriate form became dominant when the cultures that were transferred at 48 hour intervals under the following cultural conditions:-

(i) aerobically in unagitated tubes of broth;

(ii) aerobically in unagitated shallow layers of broth;

(iii) aerobically on agar slants with excess water of condensation;

and(iv) anaerobically on agar slants.

The non-fimbriate form became dominant in cultures grown serially under the following cultural conditions:-

(i) aerobically on agar plates or dry agar slants;

(ii) aerobically in shallow broth layers rotated continuously;

(iii) anaerobically in tubes of broth;

and (iv) aerobically in tubes of glucose broth.

Conversion from one form to the other usually requires several subcultures under the determining conditions. Fimbriation is often associated with the formation of a thin surface pellicle, and increased turbidity in broth.

Form variation. Certain minor somatic antigens of the genus overlap, that is they occur in more than one of the salmonella groups in the Kauffmann-White Scheme. Among those overlapping somatic antigens are 1, 6 and 12. While this form variation is known to affect only the so-called minor antigens of the bacteria, it may exert a pronounced effect upon their behaviour in agglutination tests. Not only does form variation affect the common or overlapping antigens that occur in more than one O antigen group, but it also occurs in antigens which distinguish different subgroups within a single O group.

It should be emphasised that S-R, V-W, M-N and form variations affect only the somatic antigens of the bacilli and their reactions in the corresponding sera. They have no effect on H antigens except as roughness affects the stability of bacterial suspensions and therefore their agglutination. In contrast, phase variation influences only the H antigens and is without effect upon the O antigens.

Flagella of Salmonellae

Flagella are long thread-like processes and their

arrangement on the bacterial cell may take one of four forms:- (a) monotrichate - a single flagellum at one pole; (b) amphitrichate - a single flagellum at each pole; (c) lopotrichate - a bunch of flagella at one pole and (d) peritrichate - numerous flagella arranged indiscrimately over the bacterial cell. A large number of bacteria, including a few cocci, many bacilli and most known spirilla and vibrios, are more or less actively motile by means of flagella. The flagella were first effectively demonstrated by Loeffler (1890). The central portion of the flagellar thread passes through the cell wall and is in direct connection with the cytoplasm. This was observed by Trenkmann (1890), Ellis (1902-03), Fuhrmann (1910) and Meyer (1912) and was confirmed electron-microscopically by Johnson et al. (1943) and Conn and Elrod (1947).

With the exception of a few well recognised serotypes such as S. gallinarum and S. pullorum, the salmonella species are generally motile, and flagellate. All the salmonellae appear to have the flagella arranged peritrichously. According to Leifson (1960) by far the most common shape of the flagella is a regular helix with a wavelength varying between 2.4 and 2.7 microns. Curly variants, organisms with flagella having a shorter wavelength, are encountered occasionally and these appear to be genetic mutants of the normal forms. In some serotypes, such as S. wichita, the curly variant appears to be

very stable, whilst in other serotypes such as S. typhimurium it appears to be much less so. According to Leifson the change of antigenic phase in the diphasic types of salmonella does not appear to be associated with any significant change of flagellar wavelength.

Extensive studies have been made, both on stained preparations (Leifson, 1960) and on living bacteria (Pijper, 1957) but the resolution obtained with the light microscope is insufficient to show any structure within the flagellum. Examination of bacterial flagella in the electron microscope has shown that they do not conform to the pattern found in the flagella of higher organisms but have a much simpler structure. Structural differences between flagella of different species have been demonstrated. For example the flagella of Bacillus brevis (de Roberts and Franchi, 1951, 1952) and Vibrio metchnikovi (van Iterson, 1953) are sheathed whereas most others are not. Helical structures have been found in a number of non-sheathed flagella by Starr and Williams (1952) for a motile diphtheroid bacillus; by Labaw and Mosley (1954) for an unidentified bacterium; by Braun (1956) for an E. coli; and Williams and Chapman (1961) for Spirillum lunatum. Kerridge et al. (1962) investigated the molecular structure of the flagella of S. typhimurium by electron microscopy and they reported that apart from surface irregularities it was not possible to show any fine structure in the untreated flagellum. In sections the

flagellum appeared hollow, possessing a five-fold symmetry. After partial degradation of the flagella by ultrasonic vibration, by sodium-dodecyl-sulphate, or heat treatment, spherical subunits were visible in the negatively stained preparations. These subunits correspond to the flagellin molecule; they have an estimated diameter of 45 Å and are arranged with hexagonal packing.

Since the finding by Gard (1944) that it was possible to prepare bacterial flagella in high yield by differential high speed centrifugation, studies on the chemical composition of flagella of Proteus vulgaris and Bacillus subtilis have been carried out by Weibull and Koffler and their associates (Weibull and Tiselius, 1945; Weibull, 1948; 1949a, b; 1950a, b, c; 1956; Kobayashi, Rinker and Koffler, 1959) and on the flagella of S. typhimurium by Ambler and Rees (1959). The flagella of these organisms consist of at least 98 per cent. protein and they disintegrate at acid pH values to give a single protein component. X-ray diffraction analysis of purified preparations of the flagella from Proteus vulgaris and Bacillus subtilis give diffraction patterns characteristic of the "keratin-myosin-epidermin-fibrinogen" group of proteins; the flagellar protein was called flagellin by Astbury, Beighton and Weibull (1955). Beighton, Porter and Stocker (1958) found minor differences in the X-ray diffraction patterns obtained from flagella of Proteus vulgaris and S. typhimurium.

Although there is as yet no rigorous disproof (Wilson and Miles, 1960) of the passive role of flagella in bacterial movement, the evidence that has accumulated in answer to Pijper's contentions (Pijper, 1946, 1947, 1948, 1949a, b) is overwhelmingly in favour of flagella as specialised organs of motility.

Fimbriae

In addition to flagella which are under some conditions demonstrable by the light microscope, bacteria may produce another smaller kind of filamentous appendage demonstrable only by the electron microscope. The presence of these appendages was reported by Anderson (1949), Houwink (1949), and Houwink and van Iterson (1950). Duguid and his associates (Duguid, Smith, Dempster and Edmunds, 1955; Duguid and Gillies, 1956, 1957, 1958; Gillies and Duguid 1958; Duguid 1959; and Duguid and Wright, 1959) carried out systematic studies of the appendages and suggested the name "fimbriae". Fimbriae are much shorter and smaller than flagella and lack the typical wavy spiral seen in stained preparations of flagella. They usually measure 0.005 - 0.01 micron in width and 0.3 to 1.0 micron in length. They are often numerous (100 to 250 per cell) and may be seen running over the surface of the bacterium and its cell wall and extending from it. In the Enterobacteriacease they have been observed in the following genera: Escherichia, Shigella, Salmonella, Klebsiella, Serratia and Proteus. The

fimbriate cultures undergo reversible variation between fimbriate and non-fimbriate forms. Fimbriation is associated with the formation of a thin surface pellicle and increased turbidity in broth cultures. One of the important characteristics of the fimbriate organisms is their capacity to agglutinate erythrocytes of the guinea-pig and also to a lesser degree those of other animals. Ox erythrocytes are scarcely agglutinated. This adhesive or haemagglutinating property is very closely associated with fimbriation and is a simple, practical way of indirectly demonstrating the presence of fimbriae. Non-fimbriate strains do not produce haemagglutination of guinea-pig erythrocytes. Haemagglutination by the fimbriae is inhibited in the presence of 0.5 per cent. of D-mannose. If mannose is added after red cells have been agglutinated by mixture with fimbriate organisms the reaction is reversed. This inhibition and reversal of haemagglutination of guinea-pig red cells is also effected by α-methyl-mannoside solution in the same low concentration as mannose (Duguid and Gillies, 1955). These workers observed that fimbriation interfered with O agglutination. They also demonstrated that fimbriae were antigenic and gave rise to antisera having high titres. Anti-fimbrial sera can be prepared by the injection of living suspensions of fimbriate cells. The anti-fimbrial sera are prepared by absorption of crude serum with non-fimbriate suspensions of the homologous bacterial culture.

Heating to 60°C. does not alter their fimbriate state and bacteria so treated are agglutinable by anti-fimbrial sera or by adsorbed anti-fimbrial sera; they also agglutinate guinea-pig red cells. Duguid and Gillies also found that heating to 100° for 1 hour or 120° for 30 minutes resulted in the loss of fimbriae and such treated organisms were no longer agglutinated by anti-fimbrial sera. The haemagglutinating property is thermolabile at 90°C.

Agglutination of fimbriate bacilli by fimbrial antiserum is relatively rapid and loosely floccular in appearance; the clumps are intermediate between those resulting in O and H types of agglutination.

Chemical analysis by Old (1963) showed that the fimbriae were protein structures which were unrelated to the cell wall, the capsule and the flagella.

Functions of fimbriae

The natural function of fimbriae is still unknown. Houwink and van Iterson (1950) suggested that they act as organs of attachment, holding bacteria to solid nutrient substrates or mineral particles on which nutrient solutes concentrate from dilute media such as in natural waters. The adhesive action of fimbriae is proved by their role in haemagglutination (Duguid et al., 1955). Maccacaro and Angelotti (1955) suggested that the fimbriae greatly increased the surface area of the bacteria and that their function was nutritional. The occurrence of haemagglutinating activity in many pathogenic bacteria and the

adhesion of fimbriate strains to the epithelial cells of the guinea-pig and human colon (Duguid and Gillies, 1957) led to the suggestion that the pertinent adhesive properties may have their natural function in fixing the bacteria to the surface of host cells.

Fimbriae of salmonellae

The presence of fimbriae has been demonstrated in species of the genus Salmonella (Duguid and Gillies, 1958a; Duguid and Wright, 1959). The latter workers demonstrated that salmonella types otherwise unrelated antigenically contained closely related fimbrial antigens. This work was extended by Campbell (1961) whose work may be briefly summarised as follows:- Approximately 90 per cent. of the salmonella strains examined (other than S. gallinarum and S. pullorum) were found to produce typical haemagglutinating fimbriae. The remaining 10 per cent. of strains were found to be permanently non-fimbriate. S. gallinarum and S. pullorum produced non-haemagglutinating fimbriae, morphologically identical with those of the other salmonellae. The study of antigenic structure of the fimbriae of salmonellae by the preparation of antisera against the fimbriae of 25 strains belonging to 22 different serotypes using cross-adsorption techniques, revealed five different fimbrial antigens which were designated F1, F2, F3, F4 and F5. The F1 antigen was present in all of 97 fimbriate strains belonging to 60 different serotypes (including S. gallinarum and S. pullorum). The

F2 antigen occurred only in 26 serotypes, while the fimbrial antigens F3, F4 and F5 were found to be shared by only 4, 3 and 5 serotypes respectively. A provisional table of fimbrial antigenic formulae of the salmonella group was also drawn up by Campbell. All fimbriate strains of the same serotype were found to be identical in their fimbrial antigenic structure.

Bacteriophage types of Salmonellae

Salmonella typhi phages

The Vi antigen of S. typhi which was discovered by Felix and Pitt (1934, 1934a) is present in practically all freshly isolated cultures of typhoid bacilli. Shortly after the description of Vi antigen, bacteriophages specific for the Vi (or V) form of S. typhi were isolated by Craigie (1936), Craigie and Brandon (1936, 1936a), Sertic and Boulgakov (1936) and Scholtens (1936). Certain of these phages attacked only the V form of S. typhi and exhibited a limited activity toward the V form of S. paratyphi C. They were totally inactive against W cultures of S. typhi and other salmonella types. Through neutralisation tests with anti-phage sera, Craigie and Yen (1937) established four serologic types of Vi phages. These phages differed not only in serum neutralisation tests, but also in plaque size and thermal death point and they were designated Vi phage types I, II, III and IV. Later Desranleau and Martin (1950) described two additional serologic types of Vi phage designated V and VI, and

Brandis (1955) described a Vi phage known as type VII. The type I phage lysed Vi cultures of practically all strains of S. typhi and phage types III and IV lysed the majority. Phage types V and VI were less active and many V form S. typhi cultures were not lysed by them. The behaviour of type II was most unusual. It lysed only a few cultures but displayed a high degree of adaptability and developed a marked selectivity for the type of S. typhi on which it was propagated. By adaptation, Craigie and Yen (1938, 1938a) were able to obtain preparations of phage II which attacked almost all cultures of S. typhi in their possession. The changes occurring in phage II were shown to be of two different kinds:- (a) phenotypic or host induced modifications that are non-mutational in origin, and (b) host-range mutants selected by growth on a particular host and involving genotypic changes. The phenotypic modifications readily assumed their former character when grown on their original host, the genotypic modification did not. The literature on this subject has been extensively reviewed by Anderson and Williams (1956). Thus S. typhi strains were classified into a number of sub-types, each of which was lysed in high dilution by a different preparation (or preparations) of type II phage. These have come to be known as "phage types" and the procedure by which they are distinguished as "phage typing". While many races of Vi bacteriophages of serologic type II have been isolated and found to be adaptable to various

phage types of S. typhi, only one of these (phage II of Craigie and Yen) has been used to produce internationally accepted standard preparations for phage typing. Craigie and Yen found that the cultures of S. typhi were always lysed by the same adapted phage, and that the "phage type" of a culture did not change when it was kept as a stock culture, passed through experimental animals, or when it was recovered repeatedly over long periods from the faeces of a carrier. Not only are the phage types constant, but there is a remarkable agreement in the behaviour of cultures recovered from a single epidemic toward the phages used for typing. Craigie and Yen thus uncovered a valuable aid in the study and control of typhoid fever.

According to Bernstein and Wilson (1963) the typing scheme in current international use includes 72 phage types of S. typhi. The technique originally described by Craigie and Yen and modified by Craigie (1941) has been changed only slightly. The status of bacteriophage typing was summarised by Craigie and Felix (1947) and Anderson and Williams (1956) who made certain suggestions for the standardisation of the method. The adapted races of phage type II are designated A, B, C, etc. Some of the letters are further subdivided to denote related but non-identical types, as B1, B2 and B3. Recently as it became apparent that the alphabet would soon be exhausted, the phages have been given numerical designations (at present 25 - 46 inclusive).

Bacteriophages of other Salmonella serotypes.

It is possible to apply phage typing to a variety of salmonella serotypes. The systems of typing devised for these salmonellae are based on the isolation of a number of symbiotic phages from cultures of each of the serotypes and cross-matching the cultures of the type against the phages isolated from that type. Among such systems could be mentioned those devised by Lilleengen (1952) for S. pullorum and S. gallinarum; by Smith (1951a, 1951b) for S. thompson, and by Atkinson and her associates (1952, 1955, 1956, 1956a) for S. adelaide, S. waycross, S. bovis-morbificans, and S. potsdam. Aside from S. typhi the two organisms most often subjected to phage typing are S. paratyphi B (Felix and Callow, 1943, 1951; Scholtens, 1955, 1959; and Anderson and Williams, 1956) and S. typhi-murium (Lilleengen, 1948; Felix and Callow, 1951; Felix, 1956; Anderson and Williams, 1956; Callow, 1959). At present, according to Edwards and Ewing (1962), ten phage types of S. paratyphi B and thirteen phage types of S. typhimurium are recognised by the International Centre for Enteric Phage Typing, London.

A second system of typing S. paratyphi B and S. typhi-murium is dependent upon recognition of the symbiotic phages that are carried by the individual strains of the two species. The methods were discussed by Scholtens (1959) and Boyd and Bidwell (1957).

Toxins of Salmonellae

Exotoxins are not produced by members of the Salmonella group. The endotoxins, which are the toxic material, are closely related to the bacterial body and are released on autolysis. They are heat stable.

Habitat of salmonellae

The salmonellae are most commonly found in the intestine of mammals, birds and man. The main reservoirs of infection are fowl, duck and pigs. Rodents, ruminants and carnivores are not uncommonly infected. Rats (Buchbinder et al., 1935; Cohrs et al., 1958) and mice suffer naturally from infection with S. typhimurium and S. enteritidis. The organisms may also be found in the blood, the lymphatic nodes, the ovary, the eggs of fowl and ducks, and also in water and sewage. They have also been isolated from vermin, marsupials and reptilia (Mackerras, 1954).

Distribution and range of pathogenicity

On the basis of their distribution and relationship to human disease the salmonellae may be divided into three groups (Dubos, 1952).

Group I

This group contains those species that are primarily pathogenic for man. They are: S. typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C.

Group II

This group consists of salmonella species which are primarily pathogenic for animals and birds but are known to cause disease in man. The relative incidence of this

disease varies in different geographical areas. Examples of members of this group are: S. typhimurium, S. choleraesuis, S. anatum, S. enteritidis, S. newport, S. weltevreden.

Group III

Members of this group, so far as is known, are pathogenic only for animals and birds.

Clinical classification of salmonella infection in man

1. Typhoid and paratyphoid fevers caused by S. typhi and S. paratyphi A and S. paratyphi B are commonly endemic in most underdeveloped countries of the world. The disease is characterised by a prolonged incubation period and predominance of septicaemic over intestinal symptoms.

2. Salmonellosis. All known salmonella species other than those (S. paratyphi B may be associated with food poisoning syndrome) mentioned in Group I are capable or potentially capable of producing the disease. The severity of the disease varies with the virulence potential of the species of the infecting salmonellae, age and general health of the patient. According to the three recognisable stages of pathogenicity, Newell (1959) proposed a further subdivision of this group into:-

(a) Symptomless excreter

The organisms are excreted for a variable period of time with no gastro-intestinal or other symptoms. It should be borne in mind that a healthy carrier, who may not even recall having had an acute attack of typhoid, and who is frequently only an intermittent excreter of the

germ (Nelson and Pijper, 1951) is usually the unwitting cause of epidemics and of localised endemic typhoid.
(b) An illness of a gastro-intestinal type with possibly some transient general disturbance, such as headache, mild pyrexia lasting for less than 48 hours. This subdivision includes food poisoning, which is, rather paradoxically not an uncommon occurrence in the more developed countries.
(c) A systemic illness of the enteric fever type, alone or following an illness of types (a) and (b). Although the proportion of individuals who after ingesting organisms of any salmonella type will enter clinical stages (a), (b) or (c) depends partly upon individual susceptibility and partly upon the number of organisms ingested, the type of organism is remarkably constant in its effects. (McCullough and Eisole, 1951)

Host specificity

Some members of the genus have a strong host specificity such as S. typhi and S. paratyphi B for man; S. abortus-equi for horses; S. abortus-suis for pigs and S. gallinarum and S. pullorum for poultry. Other salmonellae have a wide host range. S. typhimurium with its world-wide distribution is the most conspicuous example. Though certain types are far commoner in man than others, practically every known species of salmonella is potentially capable of infecting man. Even S. gallinarum (Topley Wilson 1960, p. 878), which has been regarded as restricted to fowls, has been isolated from a human patient.

Pathogenesis

General. Typhoid, paratyphoid fevers, salmonellosis and enteric-like fevers are a serious public health problem not only in the under developed countries of the world but also (especially salmonella food poisoning), in the more advanced and developed countries such as Britain, United States of America and Europe. The term bacterial food poisoning denotes an acute gastro-enteritis caused by bacterial contamination of food or drink. The infective type of food poisoning is commonly caused by a member of the salmonella group. S. typhimurium and S. enteritidis are perhaps the commonest causes. The food has usually stood for a time in warm weather, thus allowing multiplication of the organisms, but it does not smell nor does it look bad. The infection may come from animals or poultry whose flesh is used for food, or the food may be contaminated from the faeces of infected mice, rats or man (patients and carriers). The food poisoning outbreak is likely to involve all the members of a family. Larger outbreaks will follow social gatherings, where large numbers of persons partake of food from a common source of supply which had been imperfectly cooked. Complete recovery is usual in the course of a week, but there is a mortality of about one per cent.

The first salmonella species isolated from food poisoning was S. enteritidis. It was isolated by Gaertner in 1888 (Wilson and Miles, 1960) from the meat of an

emergency-slaughtered cow at Frankenhausen in Germany. At autopsy the same organism was isolated from the cadaver as that isolated from the meat. In 1898, Durham in England and de Noble in Belgium described another organism which they isolated simultaneously from patients suffering from food poisoning and the meat that they consumed. This organism was called S. aertryke - after the village in Belgium where the outbreak took place. This organism is now called S. typhimurium. This name was given by Loeffler in 1892 (Wilson and Miles, 1960) to an organism which he isolated from a mouse epizootic, and which was subsequently found to be identical with S. aertryke. Intensive interest and study of the salmonella group during recent years by workers from all over the world have brought to light many other species of the genera which are concerned in the production of food poisoning. The frequency of the salmonella species involved in causing Salmonellosis differs in different countries, as will be apparent from what follows.

(i) In Great Britain (Wilson and Miles, 1964) the common species of salmonella involved in food poisoning are:-

S. typhimurium, S. enteritidis, S. thompson, S. newport, S. cholerae-suis, S. bovis-morbificans, S. dublin, S. seftenberg, S. derby, S. eastbourne, S. stanley and S. aberdeen.

(ii) In the United States of America (Edwards and Bruner, 1943 and Felsenfield and Young, 1949), salmonellae commonly

associated with gastro-enteritis are:-

S. typhimurium, S. newport, S. panama, S. oranienberg, S. diego, S. bareilly, S. montevideo, S. bredeney, and S. thompson.

(iii) In Denmark (Wilson and Miles, 1960) the types most commonly responsible for outbreaks are:

S. typhimurium, S. enteritidis and S. dublin.

It is apparent from the above that in all three countries S. typhimurium tops the list and in Britain it is responsible for three-fourths of all salmonella food poisoning outbreaks. This is also true in the Federation of Malaya (Singh, 1955), Australia and New Zealand (Josland, 1952, Atkinson, 1956).

Further progress in the understanding and control of typhoid fever, paratyphoid fevers and salmonellosis would be contingent on a better understanding of their pathogenesis and factors involved in acquired resistance to infection. Few infectious diseases have lent themselves to experimental study and reproduction in laboratory animals with the classic simplicity and clarity of the pioneer experiments of Pasteur and Koch in their studies on anthrax and rabies, and tuberculosis respectively. Laboratory investigations into the pathogenesis and control of typhoid have been hampered by the fact that S. typhi and the paratyphoid bacilli only cause disease in human beings and are not natural pathogens for the common laboratory animals. Experimental infections of small

laboratory animals with those organisms bear little relationship to the natural disease in man. The problems that arise in the control of the typhoid fevers are concerned with the particular host parasite relationship and the aim of laboratory methods is to simulate this relationship as closely as possible in small animals. The pioneer work of Ørskov, Jansen and Kabayashi (1928) has shown that mouse-typhoid caused by S. typhimurium runs a very similar course to typhoid fever in man. S. typhimurium is also a natural pathogen of the mouse and has a somatic antigenic structure identical with that of S. paratyphi B (Felix, 1954). Experimental mouse-typhoid can be accepted as a satisfactory model of a natural systematic salmonella disease. Recently, Edsall et al., (1960) reproduced experimental disease which resembled mild typhoid fever in man by feeding live cultures of typhoid bacillus to young chimpanzees and in so doing confirmed the earlier classical reports of Grünbaum (1904) and Metchinkoff and Besredkha (1911). Experiments with chimpanzees are hampered by the high costs and the technical difficulties involved in handling and housing the animals.

Salmonella species employed in experimental salmonellosis

Experimental studies on the pathogenicity of salmonellae for laboratory animals have been confined only to a few species, namely, S. typhi, S. typhimurium, S. enteritidis and S. paratyphi A and B.

Animals used in experimental salmonellosis studies

(i) Small laboratory animals: rabbits, guinea-pigs, rats and mice. The mouse is the animal of choice (Hobson, 1959).

(ii) Large laboratory animals: chimpanzees (Metchinkoff and Besredkha, 1911 and Edsall et al., 1960).

(iii) Birds: one-day-old chickens (Clemmer et al., 1960 and Campbell, 1961).

(iv) Human volunteers: Hormaeche, Peluffo and Aleppo (1936), working with S. typhimurium showed that large doses of the organisms were required to produce food poisoning in man.

Route of infection

Though the general consensus of opinion today is that salmonella infection most commonly occurs via the alimentary tract, there is an increasing volume of literature from various workers (Varela and Olaita, 1942; Netter, 1950; Varela and Ocha, 1953; and Datta and Pridie, 1960) suggesting that salmonella infection in man is possible through the agency of infected airborne particles. These workers have isolated salmonellae and other faecal organisms from the nose, pharynx and sputum of man. This raises the question of the role of the respiratory tract as a possible portal of entry of salmonella organisms. Trillet and Kaneko (1921) succeeded in infecting mice by aerosols of S. typhimurium and showed that the dose required to initiate infection was very much smaller by the

respiratory route than the per-cutaneous, conjunctival and oral routes. Clemmer et al. (1960) reported that chicks could be infected with salmonellae by the respiratory route. These workers used ten different salmonella strains and showed that the organisms proliferated in the lungs. Darlow, Bale and Carter (1961) infected mice by the inhalation of a mono-dispersed aerosol of S. typhimurium suspended in phosphate buffer (pH 7.6). The animals were exposed to the spray in a modified Henderson apparatus (Henderson, 1952). The resulting disease was characterised by specific primary pneumonia. They reported that the lethal dose was very much smaller than that by the oral route, and that the lethal dose was approximately the same as that by the intra-peritoneal route. Moore (1957) showed that guinea-pigs whose eyes were protected by goggles were not infected when exposed in an environment that was heavily contaminated with S. enteritidis. Wilson (1956 and 1958) reported that one-day-old chicks were very heavily infected by a fine spray of S. pullorum. Darlow and Bale (1959) showed that the flushing of a lavatory-pan could produce an aerosol of particles sufficiently small to be inhaled into the alveoli of the lungs. They suggested that there was the possibility of infection with faecal pathogens occurring as a result of flushing lavatory-pans.

The site or sites of infection and mode of penetration of the mucous membrane of the gastro-intestinal tract.

This is still a point of issue and presents a big gap in our knowledge of pathogenesis. It was believed that the bacilli passed through the stomach, proliferated in the intestine and thence invaded the blood stream. This view has been generally discarded since it seems that the heavy infection that occurs during the second and third weeks of an attack of typhoid is due, in large part, to the passage of bacilli into the intestine from the gall-bladder and perhaps from the Peyer's patches and lymphoid follicles. It has been suggested that the bacilli usually gain access to the tissues via the tonsils and lymphoid tissue of the pharynx rather than through the intestinal wall (Brion and Kayser, 1902 and von Origalski, 1904). More recently, Darlow et al. (1961) working with S. typhimurium described successful infection of mice by inhalation of mono-dispersed aerosol. However, for the present, whether or not infection occurs via the tonsils or the respiratory tract in some cases, there are apparently no justifiable grounds for discarding the traditional view that the more usual and the commonest portal of entry of the organisms is the intestinal mucosa and thereafter they are carried via the lymphatics to the bloodstream and the tissues. As to the possible mode of penetration of the intestinal mucosa, little or nothing is known.

Fimbriae and their possible role in penetration of the intestinal mucose and pathogenesis

Duguid and Gillies (1959) showed that fimbriate

strains of dysentery bacilli adhered to epithelial cells which had been scraped from portions of normal colon freshly excised from a human patient and from freshly killed guinea-pigs. The adhesiveness for the intestinal epithelium was invariably associated with fimbriation and haemagglutinating activity. The adhesion was not affected by the abundant mucin present. Campbell (1961), in experiments designed to investigate the effect of fimbriation on the virulence or infectivity of S. typhimurium, S. enteritidis and S. pullorum for mice or chickens, came to the conclusion that the fimbriate phase strains were more virulent than the non-fimbriate phase strains by the oral route of infection, while by the intraperitoneal route the strain in either phase was equally virulent. His observations further suggested that the fimbriae played a role in establishing a transient pathogen in the intestinal tract in competition with the resident commensals. Campbell further observed, in studies of the course of infection in starved mice, that the fimbriate phase S. typhimurium differed from its non-fimbriate phase in more readily breaching the defence mechanisms of the intestinal mucosa to colonise the underlying tissue.

Pathogenicity of Salmonella species for laboratory animals

Experimental studies with S. typhi. Although many experimental patterns involving a variety of experimental animals have been employed, few investigators have attempted to reproduce the essential features of the infection

with the organisms as are observed in man. Frankel and Simmonds (1886) and later Remlinger (1897) were apparently the first to claim success in the production of oral infection in laboratory animals with S. typhi. In both rabbits and mice, the ingestion of food contaminated with typhoid bacilli followed by starving the animals for 2 or 3 days resulted in enteritis, severe and frequently fatal septicaemia, and engorgement or ulceration of the Peyer's patches. Grünbaum (1904) infected four chimpanzees by feeding typhoid cultures or infected stools and was able to produce a disease which was characterised by malaise, diarrhoea, fever, a rising titre or positive Widal reaction and the Peyer's patches showed characteristic changes, but he did not describe his procedures or results in detail. A few years later Metchnikoff and Besredka (1911) undertook more extensive studies. They experimented with chimpanzees, gibbon, guinea-pigs, young and adult rabbits and monkeys belonging to four different species. They experimented with fourteen chimpanzees, and three of these were fed pure cultures of S. typhi, while the other eleven were given mixtures of contaminated faeces and cultures or simply faecal material. The resulting disease was characterised by fever, enteritis, enlarged Peyer's patches, positive blood cultures and rising agglutinin titres were observed in most of their test animals. However they did not check these findings regularly in each and every test animal. They also produced a disease essentially similar

to that in the chimpanzees, in a gibbon. In contrast, oral infection experiments in ten guinea-pigs, eighteen young rabbits, six adult rabbits and fifty-one monkeys belonging to four different species (only two cynomolgus) failed to show any signs of infection. Grünbaum had also obtained negative results in two experiments with Macacus rhesus monkeys. In a subsequent study Metchnikoff and Besredkha (1911) challenged ten additional chimpanzees, seven of which were subjected to procedures calculated to provide some degree of immunity. It was observed that some 6-8 days after the challenge, six of the ten experimental animals developed fever, typhoid bacteriaemia and also showed most of the features which have been mentioned earlier. Except for the reported successes in producing oral S. typhi infection of mice by Wassermann and Sommesfeld (1915) and Bulow (1950) the research work in this particular field has been stagnant since the experimental studies of Metchnikoff and Besredkha, who very rightly concluded that pathogenesis and immunity in typhoid could only be studied effectively in experiments wherein the route of infection, the general clinical course of the disease, and the induced pathological alterations resembled those seen in typhoid in man. Nevertheless, experimental infection with S. typhi has during the 50 years or so been virtually confined to intraperitoneal inoculation of guinea-pigs and in more recent years the mouse. There is no doubt that the many small animal studies for example that

of Edsall (1946) involving the ingenious variations of intra-cerebral inoculations and that of Reilly et al. (1935) of inoculation into the mesenteric glands, have contributed greatly to the characterisation of the typhoid bacillus, its antigenic composition, the relationship between antigenic components and invasiveness following parenteral inoculation and the immune response in animals and man, but it is evident that during that period the investigation emphasis was not on the problems of the portal of entry, the distribution of the organisms in the host, the nature of the basic immune response, and other fundamental problems of experimental pathology of oral S. typhi infection. Instead the emphasis was on relatively isolated problems, amenable to conventional microbiological, immunological and histopathological techniques. Edsall et al. (1960) confirmed the observations of Grünbaum (1904) and Metchnikoff and Besredkha (1911) that a disease bearing a resemblance to human typhoid fever could be produced in chimpanzees. Although the findings varied somewhat from animal to animal, in the opinion of these workers several generalisations were possible with regard to the response of the chimpanzees to oral infection with S. typhi. In their series of experiments, a majority of the animals exhibited typhoid bacteriaemia after 4 to 10 days of oral infection; most had fever of 1 to 9 days duration - generally coincident with the bacteriaemia although there was a tendency for the bacteriaemia to

appear first; about half excreted typhoid bacilli in the faeces and this excretion was in every instance preceded by an "eclipse" period following the usual transient appearance of the infecting organisms the day after the challenge; anti-typhoid O and H antibodies were present a few days after the onset of demonstrable infection in every animal; gross and microscopic changes characteristic of human typhoid fever were seen in the intestinal and the mesenteric lymphatic tissues, spleen, liver and gall-bladder; the carrier state was observed to develop in a small proportion of the infected animals and the biliary tract was shown to be a source of persisting infection in one such animal. The principal apparent differences between the typhoid disease as seen in chimpanzees and man were:-

(i) the incubation period was relatively shorter in chimpanzees; (ii) the clinical course of the disease in chimpanzees was also relatively mild and brief. Only a few animals in the whole experimental series appeared seriously ill during the course of the infectious process. The hypertoxicity, typhoid facies, stupor, extreme lethargy, etc. which are so generally associated with the disease in man were not discernible in the infected chimpanzees. (iii) the pathological changes, although typical of mild typhoid in man did not include in any of the animals examined any evidence of ulceration of the Peyer's patches or any of the other complications of typhoid fever seen in man. However,

it should be borne in mind that not all human typhoid fever is severe or "typical" and this is particularly so in young children, in whom the disease is usually mild or even abortive. Since most of their experimental animals were in a stage of maturation comparable to children under 10 years of age, it seemed reasonable in the opinion of these workers to assume that their response to typhoid infection could be compared both clinically and pathologically to the pediatric rather than to the adolescent or the adult typhoid fever in man. Consequently, the relatively mild course of the disease in chimpanzees, and the lack of ulceration of the Peyer's patches do not necessarily set apart the chimpanzee syndrome from the disease as seen in man. Moreover, it has long been recognised that a significant number of swollen Peyer's patches even in adults suffering from typhoid never come to ulceration, but resolve as convalescence proceeds. One of the features of the disease in chimpanzees which is also true for the disease in man was the striking lack of uniformity from animal to animal. The disease ranged from severe systemic and localised illness to a wholly inapparent infection detectable only by serologic means. Thus the experimental chimpanzee data supported the widespread impression that in typhoid fever as in many other infectious diseases, the exact replication of the clinical, laboratory and the pathological picture from animal to animal or from man to man, is the exception rather than the rule. The possible

causes of this difficulty of exact replication of disease in individual members of the species, man or chimpanzees, may be numerous but in the opinion of Edsall and his associates, in the chimpanzees the two prominent causes were:-

(i) The variety in degree and in kind of the intestinal parasitism observed in the experimental chimpanzees, and (ii) the variation in the inhibitory properties of chimpanzee stools towards the S. typhi organisms.

Experimental studies with S. typhimurium

The effects produced by the administration of living cultures of S. typhimurium to small rodents are entirely different from those produced in them by laboratory infection with S. typhi. S. typhimurium is a natural pathogen of these animals and it gives rise to a characteristic disease known as mouse-typhoid. The disease is produced when a living culture of S. typhimurium is administered by the oral or subcutaneous or intraperitoneal routes, though the time to death is longer by oral infection. The organism has a very definite invasive power for the tissues of the mouse and other laboratory rodents. A virulent strain will kill 50% or more of the mice infected intraperitoneally with a dose of 100 bacilli as compared with 50-100 million S. typhi organisms (Wilson and Miles, 1960). Mice dying within 2 to 3 days after infection with a moderate dose of a virulent strain of S. typhimurium will succumb with an acute septicaemia and with few obvious

lesions, while mice dying after the more usual period of 5 to 10 days often show characteristic lesions - varying degrees of splenic enlargement often associated with small necrotic foci in the spleen, somewhat larger necrotic lesions in the liver and sometimes scattered pneumonic patches in the lungs, with a minimal pleural effusion. These lesions have been described by many workers (e.g. Seiffert, Jahncke and Arnold, 1928).

The spread of the organisms from the intestinal lumen

The spread of infection from the intestine and the subsequent involvement of the various tissues was studied and described by Müller (1912) and Ørskov et al. (Ørskov, Jensen and Kobayashi, 1928; Ørskov and Moltke, 1928; Ørskov and Lassen 1930). Most workers agreed that the intestine is the chief site of entrance of the organisms to the tissues, but diverse opinions exist with regard to their mode of dissemination from the intestine to the various organs. Müller (1912), using several strains of salmonella which he administered orally, concluded that the passage from the intestine to the various organs of the experimental animal depended on the strain and the infecting dose employed. He found that with an extremely virulent strain and a large infecting dose the bacteria passed from the intestinal lumen into the blood stream to cause a primary bacteriaemia. On the other hand with an organism of low virulence and a small infecting dose the infection spread by way of the lymphatics. This then

proceeded to the next stage of secondary bacteriaemia. Ørskov and his associates also observed differences in the mode of dissemination of the virulent and non-virulent strains of salmonella, but differed in their conclusion from that of Müller, in that according to these workers both the virulent and non-virulent strains of salmonella passed from the intestine into the lymphatic system, but the virulent strain (S. typhimurium) spread from the regional lymph nodes via the thoracic duct to the blood stream and from thence to the internal organs; whereas the non-virulent strain (S. paratyphi B) caused a localised infection in the mesenteric nodes where the bacteria persisted for several weeks. Similar findings were reported by Krough-Lund (1928) who experimented with S. typhi. The organisms remained in the mesenteric lymph nodes and did not spread any further. Seiffert (1918) on the other hand found S. typhimurium in the blood, lymph nodes and the internal organs several hours after infection. His findings were similar to those of Müller. Kliger and Oltizki (1930, 1931) who investigated the course of oral infection with S. enteritidis (using a strain of medium virulence) in 6 white mice. These workers concluded that the infection did not induce a primary bacteriaemia but tended to localise in the first stage in the mesenteric lymph nodes from whence at a later stage the bacteria entered the blood stream and the internal organs. They considered the mesenteric lymph nodes to be the main region

of infection and that the mesenteric lymph nodes were actually not only the first site of infection but also the last to be cleared of the infecting strain in cases of recovery.

Fimbriae and dissemination of salmonella organisms through the intestinal mucosa

Campbell (1961) showed that the fimbriate phase of S. typhimurium 7471 was not only orally more virulent for mice and one-day chicks than the non-fimbriate phase of the strain, but that it also tended to disseminate more rapidly into the tissues of the mice and chicks. He reported that 30 seconds after ingestion of the organisms the heart blood from 17 of 20 mice infected orally with the fimbriate strain yielded the organisms on culture. Likewise, cultures of liver and spleen samples taken within 1-5 min. were positive only when the infecting strain was in the fimbriate phase.

Fate of salmonella organisms after reaching the intestinal lumen

There is also much disagreement amongst workers about the fate of salmonella organisms in the intestinal lumen. Müller (1912) found the bacteria in the intestine during all stages of infection. Ørskov et al. (1928) on the other hand claimed that the bacteria disappeared entirely from the lumen of the intestine shortly after the oral infection and that only a small number of the organisms succeeded in passing through the intestinal wall. The mode

of penetration was not known. Seiffert (1928) noted the presence of the infecting organisms in the intestine during all the stages of infection and he claimed that only non-virulent strains disappeared rapidly from the intestine. Kligler and Olitzki (1930, 1931) did extensive work to investigate this point and they showed the organisms tended to remain in the intestine during all the stages of infection and that this was not in any way related to the virulence of the infecting strains. Working with S. enteritidis, they showed that the state of infection in the intestinal tract varied with the stage of infection. During the first stage of infection (the incubation period) the organisms remained in the lower part of the intestinal tract; while during the second stage, that is at the peak of the infection, the infecting organisms were occasionally found in the upper part of the tract, and finally during the third stage of illness, i.e. the carrier stage, the organisms were localised solely in the upper part of the intestine.

Quantitative infection studies

Hobson (1956 and 1957a) showed that the intraperitoneal injection of 5000 organisms of a virulent strain of S. typhimurium (S2/446) which produced a fatal infection for 96 per cent. of the inoculated mice, resulted in progressive bacterial proliferation in the first few days of infection. The peak of mortality was at the 7th day of infection and bacterial populations of the order of 10^7 organisms were found in most mice killed at that time.

Reduction of the infecting dose to 10 organisms appeared to affect the speed and probability of attaining a critical final bacterial population. Only 50 per cent. of the infected mice died, but many of the survivors continued to carry the potentially virulent bacteria. As in the experimental infections with a large dose, the moribund mice always contained 10^8 to 10^9 viable organisms. Quantitative experiments showed that a progressive bacterial multiplication occurred in all mice in the first 7 to 14 days after infection with 10 organisms. Thereafter in mice which were destined to survive, overt bacterial multiplication gradually ceased and bacterial counts of the viscera gradually fell during the subsequent 2-3 weeks. The course of events in mice infected with less virulent strains of S. typhimurium has received less attention. Hobson (1957) investigated this point using a strain of S. typhimurium of reduced virulence - a single step streptomycin-resistant mutant (S2/R) derived from a virulent strain S2/446. The streptomycin-resistant mutant was found to be less virulent for mice than the parent strain. The difference in virulence was only observed when the mice were infected with small numbers of the test organisms. Although the streptomycin-resistant strain caused fewer deaths than the parent strain it remained capable of establishing persistent infection in the majority of the animals. The significant difference in the behaviour of the two strains in vivo was that after clearance from the

blood, the multiplication of the infecting organisms in the tissues began more rapidly with the virulent strain and was more progressive, whereas the multiplication of the mutant was delayed, was less in degree and was apparently suppressed within a few days of infection. The mutant strain was apparently identical antigenically with the parent strain and was equally toxic. The mice dying of infection with this strain did so when the bacterial population had reached a level similar to that obtained in the terminal stage of infection with the virulent strain. The essential difference between the two strains was the speed and probability of attaining the critical final bacterial population of 10^8 to 10^9 organisms. The outcome of mouse-typhoid infection could thus be considered to depend on an equilibrium between the rate of bacterial growth and the rate of the development of the host's resistance. The balance could be tipped in favour of the organisms by increasing the size of the inoculum so that a large bacterial population was achieved early before the host defence mechanisms could be mobilised. In an earlier publication Hobson (1956) had reported that the host survival from S2/446 infection could be increased significantly by maintaining bacteriostasis throughout the first few days of infection by the administration of furazolidine. Although all the animals remained carriers at the end of the treatment, four relapsed and in them the subsequent course was closely similar to that seen in experiments with

the strain S2/R.

Experimental salmonellosis with Salmonellae paratyphi A and B and other types

S. paratyphi B is of interest in experimental salmonellosis because it occupies a position in some ways intermediate between that of S. typhi and S. typhimurium. It is a natural pathogen of man but not of rodents. It is antigenically very closely related to the mouse typhoid bacillus. When injected into mice it kills them much more quickly than does S. typhi, but it is less virulent for mice than S. typhimurium. When administered orally as shown by Ørskov et al. (1928) it has a limited ability to invade the tissues and to multiply in them, and it rarely gives rise to fatal infections.

Gerichter (1960), in a thorough and critical review on the subject of experimental infection of laboratory animals with salmonellae, reported that an oral infecting dose of 5×10^9 S. typhi and S. paratyphi B to white mice caused infection of the spleen, liver, lungs, gall-bladder, mesenteric lymph nodes, jejunum and the blood. The percentage of infected spleens was higher than that of the other organs. The bacteria appeared in the various organs in about 2 minutes, and in the blood as early as 20 seconds. According to Gerichter the speed and the intensity of the invasion by the organisms from the gastro-intestinal tract to the blood stream depends neither upon the volume of the dose nor upon the contents of the mouse's stomach. He

found the minimum oral infecting dose was 5×10^5 organisms. The bacterial counts on blood samples collected by cardiac puncture 2 to 3 minutes after oral infection were 5×10^2 organisms per ml. of blood. The bacterial count in the blood increased and reached the peak (about 3×10^3) after 20 minutes. After that he discovered that the numbers of organisms decreased thus:- 1.6×10^3 after 1 hour; 8×10^2 after 2 hours; and 1.7×10^2 after 3 hours. In spite of this considerable decrease the blood did not become sterile until after several days. In the infected spleen a different process was observed. Initially only a small number (5 x 10 per 100 mg. of spleen) of organisms was found but after 24 hours these had increased to 1.2×10^2 and to 5×10^2 after 4 days.

S. cholerae-suis is highly virulent for rabbits. Milner and Schaffer (1952) showed that one-day-old chicks can be readily infected by mouth with different types of salmonellae.

Bacterial carriage in survivors of mouse typhoid

Mice that recover from experimental infection with S. typhimurium commonly continue to harbour the causative organism. In most early published works, the infected animals were herded together in communal cages and under these conditions over 80 per cent. of the survivors were found to be persistently infected (Topley, Greenwood, Wilson and Newbold, 1927-28). Virulent strains of S.typhimurium have, however, a great capacity to spread from mouse

to mouse (Topley, 1920-21, 1921; Amoss, 1922); thus it is impossible to determine from herd experiments the full significance of the carrier state. Carriage may represent a common result of a single infection with a known bacterial inoculum, or it may be the outcome of heavy and repetitive re-infection of mice which were capable of eradicating the primary experimental infection. Topley and Ayrton (1923-24) found a high incidence of carriage in survivors of a group of mice that had been segregated immediately after infection. The organisms were, however, frequently absent from the excreta of carriers or were present only intermittently. The extent of the latent disease was not determined, nor did their results or the later work of Ørskov and his associates (Ørskov, Jensen and Kobayashi, 1928; Ørskov and Lassen, 1930) show the mode of development of the carrier state. Survival with persistent infection may be the outcome of two possibilities; either all the mice pass through a generalised infection during which some acquire the ability to reverse the course of the disease, or some mice are innately resistant and are able from the onset of infection to suppress bacterial multiplication.

Hobson (1957) carried out infection experiments in which the mice were observed under strict segregation after infection with a small number of virulent S. typhimurium organisms. He followed the course of the disease quantitatively to show the relationship of the carrier state to

the acute disease. The results indicated a chronic bacterial carriage in the majority of the survivors resulting from a single exposure to infection. The quantitative studies suggested that the initial bacterial inoculum established a progressive infection in all the animals. The in vivo bacterial proliferation appeared to reach a peak at the 14th day of infection, and thereafter successive groups of survivors showed progressively diminishing bacterial counts. There was also evidence of the establishment of an increasing stable equilibrium between the host and the parasite with the increase in duration of infection. Many of the 28-day survivors harboured more than the original bacterial inoculum. Hobson suggested that the chronic carrier was an animal that had acquired during the course of infection the ability to reverse the trend of the disease before the bacterial counts attained a lethal level which was of the order of 100 million organisms. It is interesting that chronic carriers are able to live in apparently perfect equilibrium with numbers of bacteria sufficient to establish a progressive disease sometimes terminating in death in other mice. There are four possible explanations for this namely:-

(i) infection results in acquired immunity;

(ii) there is an impressed variation to avirulence, rendering the organisms incapable of renewed invasion of the host tissues;

(iii) the harboured organisms may be harmless to their

carrier host merely because the host has passed out of the age-group which is susceptible to the disease before the bacterial population could attain a critical level.

or (iv) as suggested by Dubos (1954) and Davies (1954), the failure of the organisms to cause relapse in chronic diseases may be due to changes in the biochemical environment of the infective lesion, such that the potentially virulent organisms are rendered incapable of progressive multiplication.

Fimbriae and persistence of infection in the gastro-intestinal tract

Duguid and Gillies (1957) suggested that the invariable fimbriation of many Bact. coli strains could play some part in their maintenance as commensals in the gastro-intestinal tract. Campbell (1961) reported that after oral infection of mice the percentage of infected animals was higher in the group that had been infected with the fimbriate S. typhimurium strain, and that although the degree of intestinal infection shortly after inoculation was equal with fimbriate and non-fimbriate cultures, the non-fimbriate culture was gradually eliminated from the gastro-intestinal tract by the second to the fourth day following the administration of the bacterial inoculum and only a small percentage of mice in the group remained infected. The recurrence of the infection amongst the mice in the non-fimbriate group about the 12th day of infection coincided

with the re-invasion of the intestine, during the septicaemic phase of mouse-typhoid. On the other hand a higher percentage in the group infected with the fimbriate organisms showed persistent intestinal infection during the first two weeks.

Experimental salmonellosis using De and Chatterje's technique of ligated loops of rabbit gut

The problem of human diarrhoea and its aetiology has been investigated by many people in a wide variety of ways and it is now known that a number of well-defined bacteria are able to cause this disease in man. Unfortunately, many of the organisms which affect man fail to cause diarrhoea in animals, and those organisms which affect both man and animals do not always give reproducible results when tested on animals under experimental conditions. Although some organisms cause diarrhoea in animals under field conditions, it may be impossible to cause diarrhoea in experimental animals kept in the laboratory. Because of these difficulties, we know little about the properties of an organism which enable it to cause diarrhoea. We only know that groups of organisms such as Vibrio cholerae, Salmonellae, Shigellae and others, possess this ability. A new approach to this problem became possible as the result of the work of De and Chatterje (1953). They described a technique whereby loops of rabbit small intestine were ligated at each end and strains of V. cholerae were then injected into the ligated loops; virulent strains

caused dilatation of the rabbit gut, whereas non-virulent strains were without effect. De et al.(1956) reported results of testing E. coli strains, these showed that strains known to have caused infantile diarrhoea gave a positive reaction, whereas with strains of doubtful aetiological significance, some were positive and some negative. De et al. (1960) continued their work on V. cholerae and were able to demonstrate that after growing V. cholerae in 10 per cent. peptone water, the sterile filtrate produced a positive reaction on the ligated rabbit gut. The work suggests that the ability of V. cholerae to cause diarrhoea may be due to some soluble product of growth, possibly an exotoxin. Taylor et al. (1958 and 1961) reported similar results with E. coli strains. Similar studies were also carried out by Jenkin and Rowley (1959) and McNaught and Roberts (1958) who showed that the accepted enteropathogenic serotypes isolated from patients with diarrhoea gave a positive reaction in the rabbit gut, whereas identical serotypes from healthy babies, water, or other sources, were negative. Taylor and her associates have also shown that strains of E. coli causing urinary infection gave negative reaction, which suggests that a positive rabbit gut test may be related to the ability of the organism to cause diarrhoea. These workers failed to produce this reaction with sterile extracts of E. coli or with their growth products. Taylor and Wilkins (1961) reported that some strains of Salmonella and Shigella irrespective of

their source caused dilatation of the ligated loops. Taylor and her associates (1958) had published a picture of a section of a positive loop showing many gram-negative bacteria adhering in a layer closely applied to the surface of the villus but the possible role of fimbriae or flagella in causing a positive loop reaction had not been investigated in the past by these or any other workers.

In vitro studies with mouse macrophages

In order that invading organisms may survive and produce disease or establish parasitism in the host, the invaders have not only to overcome the host's defences, but must also multiply in the tissues of the host. Experimental infection studies with various organisms have shown that injected bacteria are rapidly cleared from the circulation and come to be lodged mainly in the liver and spleen which are the principal organs of the reticulo-endothelial system. The multitude of technical difficulties involved in studying the fate of bacteria within fixed cells of the liver and spleen have led workers (Suter, 1952; Roger and Tompsett, 1952; Tompsett, 1954; Furness, 1958; Lyang, 1960; Rowley and Whitby, 1959; Whitby and Rowley, 1959; Mackeness, 1960; Jenkin and Banacerraf, 1960; and Mitsuhashi, Ichiei and Tanaka, 1961) to investigate the behaviour of bacteria within peritoneal macrophages or other animal cells that can be grown in tissue culture. The investigations on the fate of bacteria in the liver and spleen suggest that whereas the

population of a virulent strain of salmonella increases, that of an avirulent strain slowly declines. Since mouse serum lacks a bactericidal mechanism it could be assumed that the destruction of bacteria by the mouse is dependent on its cellular defences (Marcus, Esplin and Donaldson, 1956; Muschel and Muto, 1956). Thus it has been suggested that the virulence of strains of salmonella for the mouse reflects the ability of the strain to multiply within the phagocytic cells - presumably those of the recticulo-endothelial system. Furness (1958) found that the mouse peritoneal macrophages phagocytosed virulent and avirulent strains of salmonella to the same extent in the presence of 10 per cent. heated calf serum. After an initial bactericidal period, the virulent strain multiplied within the mononuclear cells whilst the avirulent strain maintained itself only in small numbers. These results are contrary to the findings of Rowley and Whitby (1959) who studied the fate of the same strains of S. typhimurium within mouse macrophages. These latter workers observed that the virulent strain required a greater amount of serum opsonins to be phagocytosed than the avirulent strain, but once phagocytosed both strains were killed at the same rate. It should be noted that, in all their experiments, specific antiserum was used as a source of opsonic factors. The interaction between the host and the bacterial parasite is complex and the survival of one or the other is favoured by such factors as: (a) the titre

of specific and non-specific opsonins, (b) the ability of the phagocytic cells to ingest and kill the bacteria and (c) the capacity of the bacteria to multiply within the host environment.

Jenkin and Rowley (1960), working with strains of salmonella and E. coli, reported that unless gram-negative bacteria were opsonised they were not phagocytosed by mouse peritoneal macrophages. Jenkin and Benacerraf (1960) showed that virulent strains of salmonellae opsonised with normal mouse plasma were not phagocytosed as well as avirulent strains and also that a much larger percentage of the virulent organisms survived and multiplied within the macrophages. The differences in the observations of Whitby and Rowley, and Jenkin and Benacerraf can be explained by the fact that the former workers used specific antiserum as a source of opsonic factors. Under these conditions the virulent strains of salmonella were treated by the mouse peritoneal macrophages as if they were avirulent. Jenkin and Benacerraf (1960) also showed that virulent bacteria treated with plasma from BCG-infected mice were better phagocytosed and killed by peritoneal macrophages, than were those treated with plasma from normal non-immune mice. Mitsuhashi, Ichiei and Tanaka (1960) reported that mononuclear phagocytes derived from the abdominal cavity of normal mice allowed intracellular multiplication of a virulent strain of S. enteritidis (116-54). They observed that bacterial multiplication

was rapid and the phagocytes infected in incubated macrophage tissue cultures were destroyed within 3 days.

Enhanced susceptibility to oral salmonella infection of streptomycin-treated mice

Bohnhoff and his associates (Bohnhoff et al. 1954; Miller, 1959; and Bohnhoff and Miller, 1962) have described the effect of certain antibiotics on the susceptibility of mice to salmonella infection. A single oral dose of streptomycin increased susceptibility to such a degree that fewer than 10 S. enteritidis organisms (streptomycin-resistant) by mouth were sufficient to initiate infection in more than half the mice, while approximately 10^6 were required to infect half the non-streptomycin-treated controls. Meynell (1955), using the method of Bohnhoff and associates, found that a lethal infection was initiated by very small numbers (LD 50 <5 organisms) of S. typhimurium in mice treated on the preceding day with 50 mg. of streptomycin orally whereas in the untreated control group of mice the LD 50 exceeded 10^6 organisms. Ushiba et al. (1955) treated small numbers of mice with 2 mg. of streptomycin per day orally for 3 days before oral inoculation and found increased incidence of infection with a strain of S. enteritidis. Freter (1955) used streptomycin to establish V. cholerae in the intestinal tract of guinea-pigs but the method also involved starvation for 4 days before and 1.5 days after inoculation which was preceded by the administration of calcium

carbonate, accompanied by sodium bicarbonate and streptomycin, and followed by morphine. In later experiments he used streptomycin, erythromycin and nystatin in repeated doses orally to render the intestines of mice and guinea pigs susceptible to infection with S. flexneri or V. cholerae (Freter, 1956). According to Bohnhoff and Miller (1962) even penicillin in sufficient dosage per os increased susceptibility to infection with S. enteritidis almost as effectively as streptomycin. The enhanced susceptibility of the mouse's intestinal tract is believed to be due to the change in the enteric micro flora (Miller and Bohnhoff, 1963), resulting from the antimicrobial action of streptomycin (or penicillin) within the lumen of the gut. They found that all gram-negative bacilli were initially eliminated but most of them returned to their pre-treatment numbers and incidence within a few days, E. coli rather more slowly than the others. According to them, the elimination of Bacteroides from the enteric microflora seemed most directly correlated with enhanced susceptibility to Salmonella infection following oral administration. They found that certain strains of Bacteroides inhibited multiplication of salmonella in vitro and the establishment of such strains in the intestinal tract by oral inoculation partially restored the lost resistance of streptomycin-treated mice.

Studies with organisms grown in vivo to reveal the bases of microbial pathogenicity

This subject has been extensively reviewed in the Symposium on "Microbial behaviour *in vivo* and *in vitro*" by experts actively engaged in this field (Smith and Taylor, 1964). The bases of microbial pathogenicity are the metabolic processes and products of pathogenic organisms that, in contrast to those of non-pathogens, enable them to produce disease in a susceptible host. Our knowledge of these bases of pathogenicity in most instances is vague especially for those organisms that grow to large numbers in the host before producing symptoms of the disease.

The compounds responsible for the aggressive, toxic, and immunogenic properties of pathogenic organisms are usually proteins, carbohydrates, and lipids, either existing free or present as mixed complexes, for example, lipoproteins and mucopolysaccharides. Similar compounds are present in non-pathogenic species. The general metabolic processes whereby bacteria derive energy and synthesize the main proteins, carbohydrates and other compounds are probably similar for all organisms (pathogenic and non-pathogenic) and are not significantly altered by a change in conditions of bacterial growth. The concern here, however, is with the small peculiarities in the molecular structure of an end product that make it toxic, aggressive or immunogenic. These peculiarities are certainly controlled by the microbial genome but their production is phenotypically determined especially by the nutritional conditions. Hence although minimal media are useful in

exploring such general processes as growth, reproduction, and protein synthesis, optimal media are of paramount importance when interest centres on these specific end products having biological significance in determining virulence. When a pathogenic organism grows in the tissues of a host and produces disease, we know that all the compounds and processes required to create pathogenicity are being produced. Hence the particular nutritional conditions of the host tissue during the infection and disease are fully adequate for the pathogen as regards the formation of virulence factors. It must be emphasized here that the decisive nutritional conditions in the host tissues undergoing bacterial attack may be different from normal physiological conditions and are continually changing (Dubos, 1954). The pathogen may react in some way to these semipathological conditions and to the mobilisation of the host defence mechanisms; in fact, some virulence factors may well be produced as a direct reaction to the defences of the host. It is at present impossible to produce <u>in vitro</u> even the physiological conditions operative in the normal host, not to mention the conditions occurring during inflammation and tissue breakdown by bacterial products. The peculiar nutritional and other environmental requirements of various pathogens that lead to their preferential attack on specific tissues in disease (e.g. <u>Brucella abortus</u> in bovine cotyledon which is rich in the <u>abortus</u> growth promoting factor (erythritol) Williams

Karpie and Smith (1963)), C. diphtheriae in the throat and tonsils and N. gonorrhoeae in the urinogenital tract) are at present not understood and certainly not reproducible in vitro. In view of the aforesaid considerations, it would be surprising if the complex metabolic activity of the pathogens in vivo were not different from that in vitro. Also every worker is aware of the successive loss of virulence and change in morphology during the artificial cultivation of recently isolated pathogens, and this would indicate the magnitude of the probable difference between organisms grown in vivo and in vitro.

The success of tissue culture in producing certain immunogenic viral preparations makes the use of such techniques for investigation of problems of microbial pathogenicity tempting, but it must be emphasised that the growth of a pathogen in tissue culture is still a long way from growth in vivo and that some aspects of pathogenicity mechanisms would be missed in such studies. Thus, most cells growing in tissue culture are quite different from their parent cells in vivo and this difference is often accompanied by a change in their susceptibility to certain viruses (Ross and Syverton, 1957; and Swim, 1959). The pathogenicity of viruses changes in tissue culture (Enders, 1952; and Edney, 1957). Furthermore, in studies of intracellular bacteria maintained in tissue culture the choice of cells may well be determined by technical considerations rather than by their relationship to the

infection *in vivo*. Monocytes are often used in such studies since they are relatively easily maintained for long periods whereas the polymorphonuclear cells that play an important role in most infections are rarely used because, at the moment, they are difficult to maintain in culture.

It seems, therefore, when seeking substances responsible for pathogenicity, that there is much to be said for studying organisms and their products obtained from infected animals. Many virulence factors have indeed been recognised *in vitro*, for example, the capsular polysaccharides of *Streptococcus pneumoniae* (Felton and Bailey, 1926), just as many virulence factors can be formed by avirulent organisms. However, in the diseased animal a virulent organism is more likely to produce its whole armoury of factors, whereas under conditions *in vitro* there is a distinct possibility of one or more being absent. Nor is it beyond the realm of possibility that *in vitro* compounds are formed that appear to be virulence factors but are never produced *in vivo*.

Comparative studies of organisms grown in vivo and in vitro

Olitzki and Godinger (1963) demonstrated that *S. typhi* Ty 2 grown *in vivo* was more virulent for mice than its corresponding culture grown *in vitro*. These authors also showed that extracts of infected organs acted as infection-promoting substances. *In vitro* experiments performed by Olitzki and Kaplan (1963), proved that the majority of

organ extracts of mice infected with strain Ty 2 markedly inhibited the bactericidal action of serum on S. typhi.

The growth of enteric pathogens such as Salmonella, Shigella and V. cholerae is suppressed by the normal gut flora, and if the latter are removed by streptomycin, subsequent infections with pathogenic organisms flourish (Watkins, 1960; Lankford, 1960; Lev, 1963; Meynell and Subbaiah, 1963; Meynell, 1963). The mechanisms of this phenomenon are becoming clearer from results of experiments in vivo and in vitro. Formal et al.(1961), working with germ-free animals, showed that growth of S. flexneri was suppressed by E. coli, which in vitro did not produce a colicine or any other recognised inhibitory factor. Meynell and Subbaiah (1963), using a new method for estimating bacterial division in vivo, showed that the contents of the colon and caecum of a normal mouse had a bacteriostatic and weakly bactericidal action towards orally administered S. typhimurium. Meynell (1963) showed that this activity was dependant on an Eh of - 0.2 V and the production of much volatile fatty acid by the normal flora, which are mainly obligate anaerobes. A similar environment in vitro had an identical inhibitory and weakly bactericidal action on S. typhimurium. Removal of the normal flora with streptomycin raised the Eh to + 0.2 V and reduced the concentration of volatile fatty acid, thereby producing conditions favourable for the multiplication of salmonellae.

The other examples of the use of pathogens grown *in vivo* for studies on pathogenicity are - *Bacillus anthracis* which was the first *in vivo* grown pathogen to be examined by Smith and his associates (Smith, 1958 and 1960) and *Pasteurella pestis* (Burrows and Bacon, 1954; 1956a, b). Application of these techniques has led to the recognition of a growth stimulant - erythritol - as the cause of the predilection of *Brucella abortus* for various bovine foetal tissues (Smith *et al.*, 1962b; Keppie, 1964).

Control of salmonella infection

General measures to control typhoid and paratyphoid infection

The main conclusion which emerges quite clearly from the mass of evidence on record is that the single important source of enteric infection consists in the typhoid or paratyphoid bacilli that are living or proliferating within the bodies of infected persons, and that the whole problem of prevention (neglecting for a moment the possibility of active immunisation) consists in stopping the various routes by which the bacilli may pass from the intestine of one individual to the mouth of the other. The ultimate source of enteric infection would in all cases be the excreta of an infected human being and this source is seldom remote. Hence effective control demands the eradication of infection at the source by successful treatment of the patients and prevention or cure of the carrier state but unfortunately this problem in practice is not so simple

or straightforward. In addition to prophylactic immunisation, control measures involve improvements in general living standards, personal hygiene and public health, and environmental sanitation; provision of safe water supplies and sewage disposal and supervisory control of preparation and handling of food and drink.

General measures to control salmonellosis

Newell (1959) logically suggested that the control measures for salmonellosis must vary with the state of economic development of the country concerned.

Control measures recommended for economically developed countries

In these countries the immediate aim should be to prevent the entry of any pathogenic salmonellae into the kitchens, canteens, restaurants and food processing plants. Their presence even in small numbers is an unjustifiable risk. Efforts should also be directed to the complete severance of the animal cycle, or if this is not possible of the animal-vehicle-human chain, coupled with measures to control the less frequent direct animal-human link.

Control measures recommended for the under-developed countries

A different method of approach is necessary in these countries because:

(i) there is a greater possibility of human-human, the direct animal-human, and human-animal-human modes of transmission;

(ii) most of the under-developed countries are situated in tropical or sub-tropical zones and lack efficient food storage facilities, and this allows active and rapid multiplication of the pathogens in contaminated foods;

(iii) there is a great variation in the customs and habits in the distribution and preparation of food.

Some of these factors are closely linked with the lack of safe water supplies, methods of disposal of excreta, generally poor environmental sanitation, personal hygiene, and education. These environmental hazards cannot be controlled without an enormous expenditure of the rather limited sources of the country concerned, for example on public health engineering which under the prevailing conditions is unlikely to be available for a considerable time to come. However, it would be unwise to assume that the most effective step for the control of the problem is the control or limitation of the human-human spread. It may well be simpler, cheaper, and more effective to start with measures designed to stop the human-animal spread or to interrupt the animal-animal cycle. Indeed a pilot study on these lines in such regions together with such general measures as: the improvement of living standards, public health and sanitation through education; the provision of fixed abodes and thereby fixed addresses; properly conducted population census, surveys of incidence of diseases and proper maintainence

of records of carriers of diseases, births and deaths, would be well worth while. I have no doubt that such measures will be most rewarding because the result would be a happy, healthy population which would strive and forge ahead to prosperity and its achievements would soon justify and repay manifold all manner of expenditure incurred in the execution of such projects.

Prophylactic immunisation against enteric fevers

Following the discovery of the typhoid bacillus in 1880 by Eberth, human beings were probably first immunised against typhoid fever by Wright in 1897 (Wilson and Miles, 1960). The first large-scale active immunisation was carried out in the British Army in South Africa during the Boer War, with controversial results (Cockburn, 1955). The British troops in India were vaccinated against typhoid in 1899 (Kalra, 1959). The Antityphoid Committee, Great Britain (1913) produced figures (referring to the incidence of typhoid fever among the inoculated and uninoculated troops stationed abroad) which were compatible with the view that active immunisation significantly reduced the incidence of enteric infection in environments in which such infection was particularly liable to occur. As a consequence of the fairly successful experiences of the British, antityphoid immunisation was introduced in 1908 (Russel, 1912) in the United States Army. The extensive British experience in India provided Greenwood and Yule (1915) an opportunity to compare typhoid rates in the

unvaccinated and vaccinated volunteers. These workers concluded that the efficacy of typhoid immunisation had been clearly established. Subsequent investigators have generally agreed that antityphoid vaccination was effective in lowering the incidence and case fatality rates of typhoid in the military organisations. These studies are open to criticism on the grounds that few if any were adequately controlled and some epidemiologists like Cockburn (1955), while not denying the apparent protection afforded by the typhoid immunisation, have questioned the validity of the conclusions. This doubt has been further aggravated by the number of reports during the last two decades, on the occurrence of an unexpected number of cases of typhoid in the vaccinated groups, for example typhoid in soldiers during the World War II in the Mediterranean region (Tribby et al., 1948), in the Pacific area (Syverton et al., 1946), and elsewhere (Jordan and Jones, 1953). Indeed the report by Marmion et al. (1953) has resulted in some degree of uncertainty regarding the degree of immunity induced by the disease itself. Besides, although immunisation against typhoid fever has been carried out for over half a century, there is little conclusive evidence as to the degree of protection it affords or the best methods of preparation and assay of the vaccines. Typhoid fevers cannot be studied adequately in the usual laboratory animals except perhaps in the chimpanzees as originally suggested by Metchinkoff and Besredka

(1911) and more recently by Edsall et al. (1960) and Gaines et al. (1961). The reason for this is that the infection in the small laboratory animals bears no resemblance whatsoever to the infection in man. Moreover, the laboratory potency tests of the vaccines in mice and rabbits are impossible to interpret conclusively because the results obtained seem to depend more on the tests and the methods employed than on the quality of the vaccines. Hence the only way to solve the problems presented by the controversial laboratory results and opinions of different laboratory workers, field workers, health authorities and others regarding the value of the various typhoid vaccines, is a properly controlled field trial. The general principles of carrying out such controlled field trials of vaccines have been clearly put forward by a number of authors, for example Bell (1948), Luykx (1949) and Bradford Hill (1951, 1952). However, it should be borne in mind that there is no ready-made plan that can be strictly followed in all circumstances. The planning of a field trial should be based on general principles but the final programme should be based upon the experience gained from a carefully planned pilot study in the region concerned. It is interesting that the World Health Organization has now been associated for several years with extensive collaborative studies on typhoid vaccination. From 1954 to 1956 an admirable investigation was carried out in Yugoslavia (Yugoslav Typhoid Commission, 1962) showing

that for four years a heat-killed phenolised typhoid vaccine gave substantial protection and that an alcoholised vaccine gave no significant protection. In 1958 a controlled field trial was carried out in the U.S.S.R. (Khasanov, Kheifets and Salmin, 1962) with an enteric polyvaccine containing complete antigens extracted from broth cultures of typhoid, paratyphoid and dysentery organisms and adsorbed on to calcium phosphate by the method of Raistrick and Topley (1934) as modified by Kossova and Nechaeva (1956). It was found that a single injection of the polyvaccine gave sufficient protection against typhoid during a period of 10 months. More recent studies (Cvjetanovic and Outschoorn, 1963) were developed in continuation of the earlier ones and controlled field trials were planned and carried out in 1960 in British Guinea and Yugoslavia and in 1961 in Poland and the U.S.S.R. These were based on testing in the field two highly stable monovalent typhoid vaccines - one acetone-killed and dried and the other heat-phenol-killed and dried in British Guiana and Yugoslavia and in parallel with the controlled field trials, collaborative laboratory studies were carried out on these vaccines employing a variety of methods with a view to selecting a laboratory assay method that would predict the protective value of the typhoid vaccine in man. The two sterile dried vaccines mentioned above were included in the hope of being able to establish an international reference preparation of

a typhoid vaccine of proved protective value in man.

The results (Cvjetanovic and Outschoorn, 1963) of the field trials in British Guiana and Yugoslavia showed that both the acetone vaccine and the heat-phenol vaccine were highly effective in protecting man against typhoid, and that there was protection even two years after inoculation with two doses of the vaccines. The heat-phenol vaccine, though effective to some extent (as noted in the earlier field trial in Yugoslavia) was not as effective as the acetone-killed vaccine. The preliminary results of the field trials in Poland and the U.S.S.R. are in agreement with the results obtained in British Guiana and Yugoslavia. In the field trial in the U.S.S.R. a heat-phenol vaccine and two chemical vaccines proved effective while an alcohol vaccine proved less effective, though not significantly so, than the heat-phenol vaccine. In the laboratory studies the vaccines were assayed by active and passive mouse protection tests, and by titration of agglutinins in serum of immunised rabbits. None of the methods examined permitted the protective power of typhoid vaccines to be determined accurately, but in the active mouse protection tests, under certain circumstances, there was some reflection of the protective power of the vaccines in man. It is evident that more work is essential to develop laboratory tests, the results of which would correlate with results obtained in the field. The identification of the essential protective antigen or antigens would be an enormous advance.

Experimental studies on immunisation in the laboratory

One of the most persistent problems in the control of enteric fevers and salmonellosis is the induction of an adequate degree of immunity by artificial means. The protective value of immunisation by the use of currently developed vaccines and methods of immunisation is still not clear and it seems probable that recovery from the disease confers the highest degree of protective immunity in man (Felix, 1951).

Mice, chimpanzees and more recently mouse macrophage cultures have been used in experimental studies on immunisation against salmonella infections. The subject of experimental immunisation has been adequately reviewed in recent publications by Jenkin and Rowley (1963); Jenkin, Rowley and Auzins (1964); Turner, Jenkin and Rowley (1964) and Rowley, Turner and Jenkin (1964).

Studies using mice

In experimental studies with small laboratory animals, the closest parallel to human typhoid fever is the mouse disease caused by S. typhimurium (Ørskov, Jensen and Kobayashi, 1928). It has been shown that survivors from actual infection were more resistant to a challenge infection than vaccinated animals (Topley, Wilson and Lewis, 1925; Topley, Greenwood and Wilson, 1931). In most of these studies, the survivors represented only a small proportion of the original herd which had been infected with virulent S. typhimurium. These findings

have been thought to indicate that heat-killed organisms lacked some essential antigen which is needed to stimulate immunity in the host. Several attempts have been made to improve the quality of the vaccine for example by using alcohol-killed organisms (Schutze, 1941) or purified somatic polysaccharide (Casper, 1929; Raistrick and Topley, 1934). In general the results were little better than those with the heat-killed vaccines. The bearing of such findings on the nature of immunity in mouse typhoid and inference of practical importance in the design of improved vaccines against typhoid fever must depend on the validity of the comparison between the vaccinated animals and survivors of infection. The conventional method of vaccination, for example that employed by Greenwood, Topley and Wilson (1931), involves the administration of two doses equivalent to 100 to 1000 million organisms on well separated occasions. Vaccination usually causes few deaths, hence this group of animals at challenge represents virtually all the mice which were immunised. On the other hand, survivors from a previous infection have undergone a radically different experience before challenge. Quantitative studies by Hobson (1956, 1957a) have shown that even a small number of organisms of a virulent S. typhimurium establish a progressive infection in most animals with gross bacterial proliferation followed by a slow reduction of bacterial counts in survivors, and many of these remain carriers. Thus the total amount of bacterial

antigen liberated in the tissues would be much greater than that administered to the vaccinated group in the form of vaccine. That survivors from infection are more resistant to challenge infection than the vaccinated animals may be due to the fact that this group of animals had been exposed to more antigen, but not necessarily to better antigens (Hobson, 1957a). Furthermore, infection with as few as 10 organisms of an avirulent S. typhimurium can result in the death of 50 per cent. of the infected mouse population within 28 days of infection (Hobson, 1959a). Thus the survivors could represent either animals that had been successfully immunised during the course of infection or an innately resistant fraction of the mouse population selected by infection. Webster (1924 and 1932) and Gowan (1952) demonstrated that large differences in genetic susceptibility to infection exists among the mice. Thus the different responses to challenge of the vaccinated animals and the survivors from infection could merely be a reflection of the differences in the genetic make-up of the two groups. Hobson (1957) compared the resistance of vaccinated mice to challenge infection with that of survivors of infection with a strain of S. typhimurium of reduced virulence, in which there was no gross overt bacterial multiplication, and in which mortality and natural selection was minimal. He found that the survivors following infection developed a progressive resistance to reinfection with virulent strains.

The degree of resistance was greater than that of the vaccinated mice, although the primary infection had not caused any significant degree of natural selection or higher serum titre of O antibody.

Studies using chimpanzees

Laboratory testing of typhoid vaccines has been customarily performed in mice. Although it is possible to distinguish between differenct types and batches of vaccines by mouse protection tests, there is no direct correlation between the mouse protective potency of a vaccine and its ability to protect man. Gaines and his associates (1961) felt that more pertinent information on typhoid prophylaxis might be obtained with a species more closely related to man. Accordingly they selected the chimpanzee. Edsall et al. (1960) defined the conditions under which typhoid infection could be established consistently in the chimpanzee. Gaines et al. (1960) reported that recovery from infection conferred immunity to a second challenge, and they explored the following issues in the course of their study:-

(i) whether typhoid vaccine conferred protection against experimental disease;

(ii) comparison of the protective effectiveness in chimpanzee of typhoid vaccines assayed in mice, and

(iii) the capacity of purified Vi and O antigens, either singly or in combination, to induce immunity to infection.

The following immunising products were employed in the study; monovalent acetone-killed and dried vaccine, prepared by Landy's (1953) method; heat-killed and phenol preserved vaccine; Vi antigen isolated from a Vi strain of E. coli (Webster, Landz and Freeman, 1952); and purified O antigen (phosphorylated lipopolysaccharide) derived from S. typhi O 901 (Webster, Sagin Landy and Johnson, 1955).

The results of this study were summarised thus:-

(i) The two vaccines (the acetone-killed and the heat-killed) afforded a significant protection to the immunised chimpanzees against a challenge which produced disease in the non-immunised animals. Both the vaccines provided excellent protection against infection by homologous or heterologous strains.

(ii) Considerable emphasis has been given to selecting Vi-containing typhoid bacilli for vaccine strains and to the employment of methods to prepare vaccine which would result in the maximal retention of this antigen (Felix, 1941, 1951 and Landy 1953). Gaines and his associates found that the resistance of chimpanzees to infection with typhoid bacilli was significantly increased by the administration of Vi-containing whole vaccines, regardless of their manner of preparation. The protection provided by the heat-killed phenol-preserved vaccine was essentially the same as that from the acetone-killed, although the latter type of vaccine presumably contains a greater

quantity of the Vi antigen (Landy, 1953). Whether similar vaccines prepared from non-Vi variants of these strains would protect under the same conditions remains for future work to determine. It is noteworthy however that in mice such non-Vi vaccines are relatively ineffective in protecting against virulent challenge. This finding that vaccines of markedly different immunogenic potency for mice produce apparently equal immunity in chimpanzees is of particular interest. It would appear that the mouse tests for vaccine potency do not accurately reflect the efficacy of vaccine in primates. The relationship between vaccine potency for mice and primates however remains to be established.

(iii) With respect to the individual antigenic components the protection provided to chimpanzees by the isolated antigens was less than that induced by the whole vaccines. However, in assessing the magnitude of the protection induced by the purified antigen, it should be borne in mind that the whole vaccines and the isolated antigens were not compared in the same experiment. Since the responses of the chimpanzees varied appreciably from one experiment to another, and the challenges undoubtedly fluctuated from one test to another despite uniform handling and standardisation, it would be premature to draw final conclusions regarding the performance of the whole vaccines versus that of the purified antigens. Clearly the findings with isolated Vi antigen justify further

study to evaluate more accurately the effectiveness of the antigen in immunisation of man against typhoid fever.

Role of H antigen in protection against typhoid fever

Laboratory evaluations (Ikic, 1956; Edsall et al., 1959 and Standfast, 1960) of the vaccines used in the Yugoslav field trials in 1953 indicated little difference in the two vaccines as judged by active or passive protection tests in mice. Variations in relative potency depended on the type of test performed. However, one consistent finding was that of greater stimulation of H antibody in rabbits by the heat-phenol vaccine, although when the two preparations were tested in humans H antibody levels were not markedly different (Yugoslav Typhoid Commission, 1957). These results led to the suggestion that H antigen had been given insufficient attention as a possible factor in protection against typhoid fever. Edsall (1956); Edsall et al.(1959) and Tully et al. (1963), during the course of their studies on experimental typhoid fever in chimpanzees, investigated the response of the chimpanzees to challenge with virulent typhoid bacilli subsequent either to immunisation or challenge with a strain of S. typhi containing only H antigen. These studies showed no evidence that the H antigen was important for protection against typhoid fever in the chimpanzee.

Immunising potency of vaccines prepared from bacteria grown in vivo and in vitro

Another demonstration of the differences between

bacterial products found *in vivo* and *in vitro* is the efficacy of living attenuated vaccines in cases where killed vaccines are relatively ineffective, e.g. the protection afforded by B.C.G. vaccine against tuberculosis in man and S.19 vaccine against contagious abortion in cattle. These attenuated vaccines appear to grow to some extent in the tissues of the host and persist for relatively long periods (Pierce, Dubos and Schaffer, 1956; Wilson, Taylor and MacDirmid, 1959) and in this way they may have an opportunity to produce essential antigens in optimal amounts at the most effective site or sites. The killed vaccines may be ineffective because antigens originally present in the cultures are destroyed by the sterilisation process. However, the methods used for killing are usually relatively mild. An alternative explanation is that the protective antigens are not present to any significant extent in the *in vitro* culture. Studies by Burrows and his associates (Burrows and Bacon, 1954, 1956a, b) with *Pasteurella pestis* grown *in vivo* have thrown light on some of the problems of pathogenesis and immunity in plague. These workers showed that virulent and avirulent strains of *P. pestis* that were indistinguishable by any *in vitro* test could easily be recognised by their behaviour in the peritoneum of a mouse. The avirulent strain was phagocytosed progressively until all the bacteria disappeared. The virulent strain was also phagocytosed initially, but within half an hour some

organisms had become phagocytosis-resistant and these multiplied and killed the host. Fukui et al. (1957) have shown that P. pestis behaves in guinea-pig lungs exactly as in Burrow's experiments. After an initial lag period, virulent (but not avirulent) cells become resistant to clearance by the lung macrophages. Cavanaugh and Randall (1959) found that P. pestis became resistant to phagocytosis by polymorphonuclear leucocytes after a period within monocytes. Fukui et al. (1960) showed that the resistance was associated with two new antigens V and W. Hence resistance to phagocytosis by polymorphonuclear leucocytes appears to be a major aspect of the aggressive action of P. pestis. Smith and his associates (Smith et al., 1960; Cocking et al., 1960; Keppie et al., 1960) showed that P. pestis grown in infected guinea-pigs possessed components responsible for toxicity and immunogenicity in the species. They questioned the validity of the claim that Fraction I, a non-toxic component of the envelope obtained from saline extracts of acetone-dried P. pestis by Baker et al.(1952), is per se the basis of immunity in mice. With regard to the aggressive action of P. pestis, both the extract and the residue enhanced the virulence of P. pestis for guinea-pigs and interfered with the phagocytosis of P. pestis by mouse and guinea-pig phagocytes. As a result of this work on P. pestis grown in vivo, similar toxic and immunogenic products have been obtained from P. pestis grown in vitro. Olitzki and

Godinger (1963) in the course of their studies on S. typhi grown in vivo and in vitro, found that S. typhi strain Ty 2, grown in vivo and employed as acetone-dried vaccine, possessed a higher immunising potency than an acetone-dried vaccine prepared from descendants of the same parent strain grown in vitro. Immunogenic substances were isolated from infected organs of mice and guinea-pigs and an immunogenic substance from the peritoneal fluid of the guinea-pigs was concentrated by precipitation with ethanol.

Adjuvants and antibody production under their influence

Adjuvants are substances which when mixed with antigens improve antibody production. Many simple proteins are poor antigens when injected alone. The use of alum-precipitated antigens has been universally adopted in the preparation of toxoids for human use, and has been applied to other immunising agents such as pertussis vaccine. Usually the alum-precipitated antigens are injected subcutaneously and under these conditions give rise to higher antibody levels which persist for a long time. Mixtures of mineral oil and aqueous solutions or suspensions of antigens were introduced by Freund and his colleagues and have been extensively used especially in animals (Freund et al., 1940 and 1948; Freund and McDermett, 1942; Freund and Bonanto, 1942, 1944, 1946). The antigen in such preparations is contained within tiny droplets, which compose the watery phase of the water-in-oil emulsion (Freund's incomplete adjuvant). An injection of such a

preparation leads to increased antibody production, as compared with that which follows injection of a saline solution of the protein. Antibody production is still further increased when various tubercle bacilli and Nocardia, or their cell walls, or the chloroform soluble wax which is extracted from human types of Myco. tuberculosis, is added to the oil phase of the mixture (Freund's complete adjuvant). The use of complete adjuvant is impossible in man on account of the attendant severe local reactions and the induction of hypersensitivity to tuberculoprotein. Two or three weeks following the subcutaneous injection of antigens in the incomplete adjuvant, a soft ill-defined granuloma (Humphrey and White, 1963) develops at the site of infection. The injected antigen can be detected in the area of such a granulomata for many weeks. It seems clear that the locking away of the antigen in the watery vesicles of the mineral-oil-emulsion serves to delay its absorption and elimination from the locality of injection. Antibody-containing plasma cells are also present in the granulomata. Presumably, like alum adjuvants, the incomplete Freund's mixture derives its main effects from a prolongation of the antigenic stimulation of regional lymph nodes, as well as from such contribution by the local granuloma itself.

Specific chemotherapeutic treatment of enteric fevers and salmonellosis

Until 1947 there was no specific drug for the

treatment of typhoid; with the best nursing and general treatment, the mortality was often between 10 and 30 per cent. The original report on the use of chloromycetin in the treatment of typhoid was published by Woodward *et al.* in 1948. This discovery of the first effective drug against typhoid fever was a major advance. Woodward *et al.* (1950) found that the action of the drug was bacteriostatic and not bactericidal, however much the dosage was increased. No drug has yet been found to equal chloramphenicol but several problems still remain to be solved. Relapses are more common than in untreated cases, carriers are unaffected and complications still occur, and side effects of the drug continue to be reported (Marmion, 1955). Since 1948 tetracycline preparations such as aureomycin, terramycin and achromycin have been tried but with only limited success. In 1951 Woodward *et al.* and Smadel *et al.* published reports of trials with cortisone alone and cortisone plus chloramphenicol in typhoid. They reported that cortisone and chloramphenicol administered together had much speedier action than chloramphenicol alone, but the complications and relapses still occurred and there were dangers associated with the combined therapy. Thus the present position as regards the treatment, although vastly improved, is not completely satisfactory.

Treatment of the carrier state

The treatment of the chronic carrier of typhoid is

notoriously difficult and the problem is made worse because of the difficulties in detecting carriers. Chloramphenicol is often ineffective either alone (Woodward, Smadel and Ley, 1950) or in combination with other antibiotics (Vaichulis, Littman, Ivy, Zubowicz and Kaplan, 1950). There is some evidence that large doses of penicillin (Jersild, Neukirch, Rann, Riwats-Ericksen and Tulinius, 1952; and Michaux, 1963) or penicillin in combination with other agents (Vaichluis, Littman, Ivy, Zubowicz and Kaplan, 1950; and Bigger and Daly, 1949) are at times effective and more recently success has been reported with ampicillin (Troy, 1964 and Christie, 1964). For the present this antibiotic alone or combined with cholecystectomy when there is evidence of disease of the gall-bladder (Tynes and Utz, 1962) may be the treatment of choice.

Hence it will be evident that because the presently available drugs are unreliable cures of salmonella infections of man (especially in cases of enteric fever), the emphasis is on prevention rather than cure. The present work was designed to investigate possible mechanisms of invasion and pathogenesis, and to evaluate immunising procedures, using S. typhimurium infections in mice as a model which may elucidate some of the mechanisms involved in enteric infections in man.

MATERIALS AND METHODS

MATERIALS AND METHODS

The organisms used in the experimental studies were as listed below and in tables I and V. Each was examined serologically to confirm its identity.

(1) Twenty-one strains of *Salmonella typhimurium* were obtained from Professor J.P. Duguid (see tables I and IV).

(2) Thirty-seven *Escherichia coli* were tested; 31 were obtained from Professor J.P. Duguid and 6 were from Dr. J. Taylor (see table V).

(3) Two *Shigella flexneri* strains were obtained from Professor J.P. Duguid.

(4) One strain of *Vibrio cholerae*, Ogawa type, was obtained from Dr. H.K. Ghosh of this Department.

The following abbreviations are used to denote the characteristics of the strains of *S. typhimurium* used:

N =	non-fimbriate	F =	fimbriate
nfl =	non-flagellate	FT =	phage-transduced fimbriate
fl =	flagellate	MF =	mutagen-induced fimbriate
flt =	phage-transduced flagellate		
SR =	streptomycin-resistant		

Rhamnose-negative = not fermenting rhamnose.

Mice

These were LAC grey mice bred locally and white Swiss mice bred at the Clinical Endocrinology M.R.C. Research Unit, University of Edinburgh. In each experiment an equal number of male and female animals were used but all

mice in any one cage were of the same sex. No mouse under five weeks of age was used in the experiments. The mice were fed on a pellet diet that was free from salmonellae and water was given ad libitum. The stock mice were kept in a separate room which did not house infected animals, and they were brought into the room for infected animals just before administration of the bacterial inoculum. The temperature in this room was maintained at about 22°C.

Experimental Salmonellosis I

The virulence of fimbriate and non-fimbriate strains of S. typhimurium in mice inoculated orally or intraperitoneally was compared in two series of experiments.

(a) The first series compared the virulence of non-fimbriate/flagellate (N/fl) strains of S. typhimurium with that of fimbriate/flagellate (FT/fl) variants obtained from these by phage-mediated transduction.

(b) The second series compared the virulence of non-fimbriate/non-flagellate (N/nfl) strains with that of fimbriate/non-flagellate (FT/nfl), non-fimbriate/flagellate (N/flt) and fimbriate/flagellate (FT/flt) transductants.

Series (a)

The parent strains compared in this series were non-fimbriate/flagellate (N/fl). The virulence of six different strains (each belonging to a different phage type) was compared with that of their fimbriate transductants in 4 groups of paired experiments as follows:-

Group I. Rhamnose-negative phage-transduced fimbriate (FT)

strains and non-fimbriate (N) parent strains.

Pair no. 1 S. typhimurium 1289FT/f1

S. typhimurium 1289N/f1 (parent strain phage-type 1 var 2/U41)

Pair no. 2 S. typhimurium 1294 FT/f1

S. typhimurium 1294N/f1 (parent strain phage-type 2b/14a)

Pair no. 3 S. typhimurium 1287FT/f1

S. typhimurium 1287N/f1 (parent strain phage-type 2a/13)

Group II. Streptomycin-resistant (SR) rhamnose-negative phage-transduced fimbriate strains and non-fimbriate parent strains.

Pair no. 1 S. typhimurium 1289FT/f1

S. typhimurium 1289N/f1 (parent strain phage-type 1 var 2/U41)

Pair no. 2 S. typhimurium 1294FT/f1

S. typhimurium 1294N/f1 (parent strain phage-type 2b/14a)

Group III. A rhamnose-positive fimbriate(F) strain and a non-fimbriate(N) strain considered to be a mutant of it.

S. typhimurium 1566 F/f1 phage-type 1a/U57

S. typhimurium 1566 N/f1 phage-type 1a/U57

Both lines were derived by Professor Duguid from a single culture of S. typhimurium that had been isolated from a pig in Indiana and supplied by Dr. P.R. Edwards. Serologically they are of the Copenhagen variety, lacking the

somatic antigen V.

Group IV. Rhamnose-positive, non-fimbriate (N) strains and their fimbriate mutants (MF) obtained by the use of $MnCl_2$ as a mutagenic agent by Dr. D.C. Old (1963).

Pair no. 1 S. typhimurium 629MF/fl

S. typhimurium 629N/fl (parent strain phage type 2c/14)

Pair no. 2 S. typhimurium 1436MF/fl

S. typhimurium 1436N/fl (parent strain phage type 4/U38)

Series (b)

The parent strains in this series were non-fimbriate/non-flagellate (N/nfl). The virulence of two different parent strains (both of phage type 2a/13 and rhamnose-negative) was compared with that of their variants in 3 experiments as follows:-

Expt. no. 1 S. typhimurium 6351N/nfl (parent strain)

S. typhimurium 6354FT/flt

Expt. no. 2 S. typhimurium 6351N/nfl (parent strain)

S. typhimurium 6352FT/nfl } phage transductants

S. typhimurium 6353N/flt }

Expt. no. 3 S. typhimurium SW573N/nfl (parent strain) (Friewer and Leifsen, 1952).

S. typhimurium SW576N/fl (flagellate mutant)

S. typhimurium SW576FT/flt (phage transductant variant of SW573)

Enhancement of virulence of test strains for mice

Two mice were injected intraperitoneally with about 10^6 organisms of the test strain from a culture grown in broth overnight at 37°C. When the mouse was moribund or dead, the spleen was removed with aseptic precautions and cultured in broth and on blood agar and MacConkey plates. A single colony was picked to broth culture and the passage was repeated through another mouse. Then a single colony was picked from the culture derived from the spleen of the second passage mouse and was cultured overnight in broth. After confirmation of the identity and status of this culture, freeze-dried stock cultures were prepared and these were later used to produce the inocula for the experiments.

Method of transduction of fimbriate to permanently non-fimbriate strains of S. typhimurium

This was mainly carried out by Professor J.P. Duguid and the method employed was that described by Campbell (1961).

A permanently non-fimbriate strain does not normally produce fimbriae even after repeated subcultures in broth. However fimbriae are readily produced by these permanently non-fimbriate strains following transduction by a phage from a fimbriate strain.

Preparation of phage lysate

A fimbriate culture of the avirulent S. typhimurium (F) strain SL497 was obtained by serial aerobic broth subcultures. Dilutions of PLT22 phage (Stocker et al., 1953)

of 10^4, 10^5 and 10^6 per ml. in 5 ml. of melted semi-solid agar at 50°C. were mixed with about 4 x 10^7 organisms (0.1 ml. of the fimbriate 18-hour broth culture). Then the 5 ml. seeded melted agar was spread over an agar plate (containing 20 ml. of agar) and it was allowed to set. After four hours' incubation faint growth was discernible; plates showing phage plaques were selected. The semi-solid agar from these plates was harvested into 5 ml. broth and the material was centrifuged to remove the organisms and agar. The clear supernatant fluid which contained the phage (F) was then pipetted off and pasteurised at 58°C. for 1 hour. Another phage lysate was prepared in the same way by growing it on an avirulent, non-fimbriate S. typhimurium (NF) strain SW576.

Titration of the phage lysate

A broth culture of Boyd's (1950) universal indicator strain Q (SL375) grown for 24 hours at 37°C. was spread as a lawn over nutrient agar plates. After drying, the plates were spotted with 0.02 ml. amounts of ten-fold serial dilutions of the phage filtrate in broth, after the method of Miles and Misra (Miles, Misra and Irwin, 1938). The plaque count was made after 6 hours' incubation.

The transduction procedure

The freeze-dried stock culture of the virulent strain of non-fimbriate S. typhimurium to be transduced was grown in peptone water for 6 hours, and this resulted in a bacterial concentration of approximately 4 x 10^8 organisms

per ml. Five tubes were set up as follows:-

(i) 10^8 organisms (1ml. of a 1 in 4 dilution in peptone water of the culture) plus 0.5 ml. of the prepared phage filtrate (F)

(ii) 10^8 organisms plus 5 ml. of the phage filtrate (F)

(iii) 10^8 organisms plus 0.5 ml. of the phage filtrate (NF)

Phage sterility controls

(iv) 2 ml. of phage filtrate (F) plus 8 ml. of peptone water

(v) 2 ml. of phage filtrate (NF) plus 8 ml. peptone water.

Tubes (iv) and (v) were incubated for 96 hours at 37°C. and if growth occurred in these tubes the experiment was abandoned. The tubes (i), (ii) and (iii) were incubated for 48 hours and, after plating out on blood agar, they were centrifuged. The deposits were examined for the presence of haemagglutinin. In a successful experiment only deposits from tubes (i) and (ii) gave a positive haemagglutination test. Ten colonies each of subcultures from tubes (i), (ii) and (iii) were picked into separate tubes of broth. After suitable periods of incubation the cultures were examined for pellicle formation, and identity was checked by serology and haemagglutination. Shadowed preparations of representative strains were examined for the presence of fimbriae by electron microscopy. A positive culture from tube (i) was selected in preference to one from (ii) since it had been

exposed to fewer phage particles. A typical non-haemagglutinating culture from tube (iii) was taken as the non-fimbriate control for the subsequent comparative virulence experiments since it had the same cultural history as the fimbriate transductant, but remained permanently non-fimbriate.

The selected strains of the fimbriate transductants and the non-fimbriate parent strains were then passaged through mice and freeze-dried stock cultures were prepared by the method described above.

Preparation of streptomycin-resistant mutants

The freeze-dried stock culture of the strain of S. typhimurium was subcultured in 50 ml. of broth overnight at 37°C. in a 500 ml. conical flask on a shaking machine; an approximate concentration of 10^{10} organisms per ml. was obtained. To the overnight broth culture was added 50 ml. of broth containing 2000 units of streptomycin sulphate (Glaxo) per ml. After further incubation on the shaking machine, the broth culture was plated out on nutrient agar containing 1000 units of streptomycin per ml. Streptomycin-resistant colonies were recovered after overnight incubation. The colonies of the streptomycin-resistant fimbriate and non-fimbriate strains of S. typhimurium were smaller than those of the parent strain (about half their size). Cultures of the streptomycin-resistant strains were examined by the methods of Wilson (1930); the colony-structure appeared smooth by direct or transmitted light,

and there was no spontaneous agglutination of bacterial suspensions in saline. There were no significant differences between the parent and the streptomycin-resistant strains on nutrient agar other than the previously observed difference in colony size. There was no detectable difference in the behaviour of the two strains in agglutination tests with standard salmonella antisera obtained from Colindale. From these single-step mutant strains designated SR, a stock of freeze-dried ampoules was prepared and used for comparative infection experiments of streptomycin-treated mice.

Methods employed for the detection of fimbriae

(i) Haemagglutination test

Freshly collected guinea-pig red blood cells were washed thoroughly in saline and a 3 per cent. suspension of cells was prepared in physiological saline. The cell suspension was discarded after a week or, if lysis occurred, sooner. The overnight broth culture was centrifuged, the supernatant was decanted and the deposit was resuspended in the residual fluid and mixed with a drop of the red cell suspension on a white glazed tile. On gentle rocking, the red cells clumped together if the test organisms were fimbriate, but failed to do so if they were non-fimbriate.

(ii) Inhibition or reversal of haemagglutination by mannose

Haemagglutination by fimbriate strains does not take place if a drop of 2 per cent. mannose solution in saline is used with a drop of the suspension of red cells;

haemagglutination caused by fimbriate organisms is reversed by the addition of a drop of the mannose solution.

(iii) Electron microscopy

Shadowed specimens and specimens negatively stained with 2 per cent phosphotungstic acid (PTA) were examined. The methods of the preparation of the specimens for electron microscopy are described below (p. 109).

The methods of preparation of mice, bacterial inoculum and its administration were essentially those described by Campbell (1961).

Preparation of mice for administration of bacterial inoculum

Streptomycin treatment of mice

Eight hours after the removal of food and water the mice were given orally 80 mg. of streptomycin (Glaxo) in 0.1 ml. of distilled water. After 18 hours, the intestinal tract was clear of enterobacteria (this was checked by the absence of growth in broth inoculated with faeces) and the appropriate dose of streptomycin-resistant S. typhimurium was administered orally in 0.1 ml of 1 per cent. Na_2HPO_4 solution, as described below.

Preparation of mice for oral administration of bacterial inoculum

The mice were kept overnight without food and water.

Preparation of bacterial inoculum

Freeze-dried stock cultures of the test strains were grown overnight at 37°C. in broth. In experiments with

streptomycin-treated mice, the streptomycin-resistant stock cultures of the test strains were grown overnight in broth containing 1000 units of streptomycin per ml. The fluid cultures were centrifuged and the identity of the test strains was checked by agglutination tests with the appropriate antisera; the haemagglutinating activity of the cultures was tested with guinea-pig red blood cells. The centrifuged deposits of the overnight broth cultures were then suspended in 1 per cent. Na_2HPO_4 solution (pH 8.8), so that the approximate concentration of organisms per ml. of the fimbriate and non-fimbriate strains in the suspensions was of the order of 10^9 and 10^{10} respectively. Ten-fold dilutions of the test bacterial suspensions were then prepared in 1 per cent. Na_2HPO_4 solution so that the highest dilution contained approximately 100 organisms per ml.

Administration of bacterial inoculum to mice

The bacterial inoculum was administered either intraperitoneally or orally in parallel experiments. In the experiments with streptomycin-treated mice it was given by the oral route only. Starting with the highest dilution of the bacterial suspension the first three dilutions were injected intraperitoneally in different batches of mice and the lower dilutions were given orally to other batches of mice. The dose was 0.1 ml. of suspension. The intraperitoneal inoculation of mice was performed as described by Cruickshank (1962). For oral administration,

a combination of the methods of McLeod (1954) and Smith (1955a) was used. Six drops (equivalent to 0.1 ml.) of the bacterial suspension were delivered from a syringe fitted with a blunted No. 15 gauge needle and fed to mice that had been starved overnight. The alkali in the suspending fluid protected the organisms against the gastric acid, and the prior starving of the mice ensured a speedy passage to the intestine. The actual dose administered was estimated by the method of Miles and Misra. Dosage schedules employed in the experiments are shown in table I.

Post-infection examination of mice

The animals were examined twice daily at 9 a.m. and 4 p.m. and the deaths were recorded. The period of observation of the experiments was three weeks or more (21-25 days).

All dead mice were examined after death and their organs were cultured for S. typhimurium. One lobe of the liver, the whole spleen, and a part of the lower tract of the ileum were removed aseptically and cut into small pieces with a sterile pair of scissors and cultured overnight at 37°C. in selenite F medium. A loopful was also cultured on plates of MacConkey's medium or deoxycholate citrate agar. These were examined on the following day for pale, non-lactose-fermenting colonies. The overnight selenite broth cultures were plated on MacConkey's medium or deoxycholate citrate agar. When inoculated strain was recovered, its identity was confirmed by

serological and haemagglutination tests. In streptomycin-treated mice, the identity of the strain of S. typhimurium recovered was confirmed by inoculation on to nutrient agar plates containing streptomycin.

Calculation of LD50 and ID50 values and the mean time to death of the orally infected mice

Reed and Muench (1938) devised a simple method for estimating 50 per cent. end points. According to the authors this method "gives the effect of using at the 2 critical dilutions between which the end point lies, larger groups of animals than were actually included at these dilutions. By inclining to equalize chance variations, the method tends to define the point more nearly than would be possible if it were simply interpolated between the 2 bracketing results." Hence the 50 per cent. lethal (LD50) and infective (ID50) doses were calculated by this method.

The mean time death

The mean time to death after oral infection was calculated as the average of the times to death of the mice in the groups immediately above and below the LD50 value, i.e. within a ten-fold dilution of the LD50 dose.

Experimental Salmonellosis II

Studies by De and Chatterjee's technique with ligated loops of rabbit gut were made to determine the effect of fimbriae on enteropathogenicity.

Bacterial strains employed

(a) Salmonella typhimurium strains

A total of 21 strains (detailed particulars are shown in Tables ~~IV~~) were used in these experiments. All the strains except S. typhimurium 1566, 629 and 1436 were rhamnose-negative strains. All the pairs of fimbriate/flagellate and non-fimbriate/flagellate strains except fimbriate S. typhimurium LT2 and non-fimbriate S. typhimurium 7471 were closely related. The pairs of S. typhimurium 1289, 1287 and 1294 belong to different phage types, but the strains in each pair are identical in all respects except with regard to the presence or absence of fimbriae, which in the fimbriate variety had been transduced by phage transduction as described above. The strains had been isolated from man, pig and poultry.

(b) Escherichia coli strains

A total of 37 strains (detailed particulars are shown in Table V) were tested. It is of interest to note that this series contained Dr. Taylor's positive and negative loop control strains (both these strains C53 and C54 were found to be fimbriate). The test strains had been isolated from cases of diarrhoea but some were old stock strains and others were recent isolations. The group contained representative strains belonging to the accepted enteropathogenic serotypes of E. coli.

(c) Other strains of bacteria

In the above series of experiments, were also included two strains of S. flexneri (one Fla 1 was

fimbriate and the other Fla 2 was non-fimbriate) and one strains of V. cholerae. All these were stock strains.

Preparation of bacterial inoculum

Overnight broth cultures were grown at 37°C. The identity of the bacteria was confirmed by serology and the presence or absence of fimbriae was checked by:-

(i) a haemagglutination test made with 3 per cent. suspension of guinea-pig red cells (Duguid et al., 1955).

(ii) a test for the reversal of haemagglutination by 2 per cent. mannose solution (Duguid and Gillies, 1957), and, in representative strains,

(iii) electron microscopic examination.

Viable counts were done on the sample of the inoculum by the method of Miles and Misra. The number of organisms injected into each loop was approximately 10^8.

Quantitative loop test - Ten-fold dilutions of an overnight broth culture of test strains were prepared and the following dilutions were injected into loops:-

10^0, 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} as shown in figs. 5 and 6.

Animals

Adult rabbits whose weights ranged from $3\frac{1}{2}$ to 5 lbs. were used. The test animal was given water but no food for 18 hours prior to the operation. It was anaesthetised by an intravenous injection of sodium pentobarbitone (veterinary nembutal, 30 mg. for each 5 lbs. body weight) and open ether.

Operative procedure

The method was essentially that of De et al. (1956). The abdomen was opened by a median incision and loops of small intestine 4-6 inches long were isolated by ligatures. Into the loops were injected 0.5 ml. of the test bacterial inoculum. The abdomen was then closed by a continuous suture and Michel-clips (12 x 3 mm.). The animal was allowed to recover. Twenty-four hours after the operation, the rabbit was killed with an intravenous injection of 1 ml. of a saturated solution of magnesium-sulphate. The bowel was examined macroscopically for congestion and distension and the contents of the loop were cultured. Viable counts by the method of Miles and Misra were done on the inoculum and on the exudate from some positive loops. Initially the experiments were limited to two loops per animal. Later, for the sake of economy, this was increased to three loops per animal. The loops were approximately 9 inches apart. The experiments were repeated with each test strain in loops whose position varied from proximal ileum, mid-ileum and terminal ileum. A few control loops were injected with sterile broth.

Post-mortem examination

After killing, each animal was examined immediately. The whole of the experimental section of the intestine was removed in one piece for examination. The outer surface of the gut was seared and cultures were made from the exudate in each experimental loop and also from a few uninoculated loops. Cultures were made on MacConkey

plates and selenite broth, and incubated aerobically at 37°C. overnight.

The cultures from uninoculated portions of the gut were usually sterile, whilst cultures from the injected positive loops gave a pure growth of the test organisms; in every instance the organism was finally identified by serological methods (slide agglutination with appropriate antiserum obtained from the Standards Laboratory, Colindale) and a haemagglutination test with guinea-pig red blood cells.

Criterion of a positive loop test

An unmistakable dilatation of test loop, as compared with the uninoculated loop, was accepted as a positive loop test. This basis of positivity would include that of Taylor et al. (1958) who accepted as positive loops showing severe and very severe inflammatory reactions. In all the positive loops the gut wall was rather thin, as a result of the dilatation, and in some there were sub-peritoneal petechial haemorrhages (figs. 2, 3, 4, 5, 6). The lumen was filled with a copious exudate which in some instances was haemorrhagic.

Experimental Salmonellosis III

Comparative studies were made of the virulence of fimbriate and non-fimbriate strains inoculated orally and intraperitoneally in albino rats.

Bacteria

S. typhimurium 1289 N - a non-fimbriate parent strain.

S. typhimurium 1289 FT - a phage-transduced fimbriate strain.

Animals

Young albino rats of either sex weighing from 100 to 120 grams were obtained from the Central Breeding Station, Edinburgh. Prior to infection, samples of faeces from the rats were examined for salmonellae. The infected animals were caged separately and throughout the period of observation they were fed on a salmonella-free pellet-diet and given water ad libitum.

Preparation of bacterial inoculum

Freeze-dried stock cultures of fimbriate and non-fimbriate S. typhimurium (strains 1289 FT and 1289 N) were grown overnight in tubes containing 8 ml. broth. The broth cultures containing about 4×10^8 organisms per ml. were centrifuged at 3000 r.p.m. and after the supernatant fluid had been discarded, the centrifuged deposits were suspended in the residual fluid and this suspension was used as the oral infecting dose for each rat. Hence the oral infecting dose was $8 \times 4 \times 10^8 = 3.2 \times 10^9$ or log 9.4 organisms. For the intraperitoneal infection experiments, centrifuged deposits of the overnight broth cultures of the freeze-dried stock cultures of the fimbriate and non-fimbriate strains were suspended in 3 ml. of 1 per cent. Na_2HPO_4 solution pH 8.8 and ten-fold dilutions were prepared as in the mouse infection experiments.

Viable counts on the overnight broth cultures of the

test strains were done by the Miles and Misra method, and the identity of the test strains was checked by serology with the appropriate O and H salmonella antisera and by a haemagglutination test with guinea-pig red blood cells.

Administration of bacterial inoculum

For the oral administration experiments the rats were starved overnight and deprived of water for about 8 hours prior to feeding the bacterial inoculum. To facilitate handling, the rats were partially anaesthetised with ether and as they were recovering the dose of the inoculum was fed as drops from a syringe through a blunted 15 gauge needle. By the time the feeding was over the animals were fully conscious and on transfer to the cage they were observed to quench their thirst by drinking from the water-bottle. In the intraperitoneal infection experiments, five batches of six rats each (three of either sex) were infected by injection of 0.1 ml. of the dilutions of the bacterial suspensions. The log dose of the bacterial inocula ranged from 3.2 to 7.2 and the difference in dosage between any consecutive batch of the infected animals was log one.

The animals were examined twice daily (morning and evening) during the period of observation.

Examination of faeces from orally infected animals

From the orally infected rats, specimens of freshly passed faeces were collected each morning into tubes of selenite. The selenite broths were cultured overnight

at 37°C. and subcultured on plates of MacConkey agar or deoxycholate-citrate agar. A minimum of ten non-lactose-fermenting colonies were examined from the plates, before discarding them as negative for the inoculated strain of S. typhimurium.

Post-mortem examination

One animal each from the orally infected (fimbriate and non-fimbriate strains of S. typhimurium) groups was killed daily from the 11th to the 22nd day of infection. After a thorough macroscopic examination, liver, spleen, mesenteric and ileo-caecal groups of lymph glands, and loops of the intestine showing ulcers, were removed for culture in selenite broth. The organs and intestinal tissues were homogenised in sterile glass-tube homogenisers and cultured. The intestinal contents were also cultured in selenite broth. The heart blood was cultured in broth. The fluid cultures after overnight incubation at 37°C. were subcultured on MacConkey or deoxycholate-citrate agar plates. The plates were examined after one day in the manner described earlier for examination of faecal specimens.

The intraperitoneally infected survivors were killed on the 30th day of infection and the liver and spleen were cultured for the inoculated strain of S. typhimurium.

Experimental Salmonellosis IV

Experiments were made in albino rats to study:-

(a) the localisation of sites of penetration of the

gastro-intestinal mucosa by S. typhimurium;

(b) the passage of fimbriate and non-fimbriate S. typhimurium organisms down the gastro-intestinal tract, and

(c) the mode of penetration of the intestinal mucosa leading to infection of the blood stream and internal organs of the animals.

Bacterial strains employed in study

S. typhimurium 1289FT - phage-transduced fimbriate strain.

S. typhimurium 1289N - non-fimbriate parent strain.

Animals

Albino rats were obtained from the Central Breeding Station, Edinburgh. They weighed between 190-360 grams.

Preparation of bacterial inoculum

Overnight broth cultures of the organisms at 37°C. were centrifuged and suspended in 0.5 ml. of 1 per cent. Na_2HPO_4 solution (pH 8.8) and used as the inoculum; the approximate viable bacterial count was 4×10^9 organisms.

The identities of the organisms were checked by serological and haemagglutination tests as previously described.

Preparation of animals and administration of bacterial inoculum

(i) The animals were anaesthetised to facilitate handling and administration of the bacterial inoculum. The animal was first induced with ether and then anaesthesia was

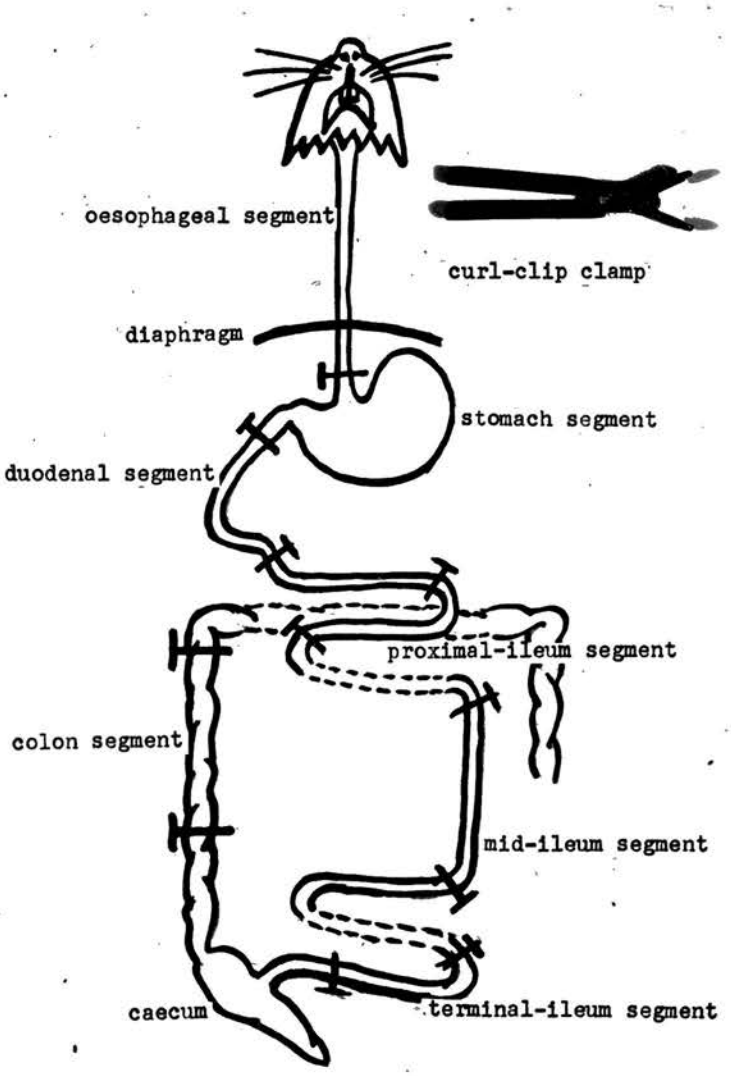

Fig. 1. Experimental salmonellosis IV. Diagram shows the division of the gastro-intestinal tract of the albino rats into segments for studies:-

(a) to localise the sites of penetration of the gastro-intestinal mucosa by S. typhimurium;

(b) to investigate the passage of fimbriate and non-fimbriate S. typhimurium organisms down the gastro-intestinal tract after oral administration, and

(c) to determine the mode of penetration of the intestinal mucosa by S. typhimurium to enter the blood stream and organs of the animals.

maintained by the administration of veterinary nembutal intraperitoneally. The dose of veterinary nembutal was 0.2 ml. of a 1 in 4 dilution of nembutal solution (1 c.c. containing 60 mg. of nembutal) for every 100 g. of body weight of the animal. The animal was usually anaesthetised and ready for operation in about 10 minutes' time.

(ii) For experimentation, the gastro-intestinal tract was divided into various segments as shown in fig. 1.

The gastro-intestinal tract was exposed by a median incision and curl-clips were used to clamp off the appropriate segment under investigation. In applying the clamp, care was taken not to occlude any blood vessel. The bacterial inoculum was introduced into the oesophageal segment through a catheter; but into the rest of the segments it was introduced by direct injection using a 21 gauge needle which was inserted obliquely through the anti-mesenteric border as described by Cregan and Hayward (1958). In studying the passage of bacteria down the gastro-intestinal tract, a bacterial inoculum was fed to the overnight starved animals using Campbell's technique (1961), while they were still only lightly under the effects of anaesthesia.

The observations were made for periods varying from 5 minutes to 2 hours. Heart blood samples were collected at varying intervals and cultured for the inoculated organism. At the end of the experiment, liver, spleen, mesenteric glands (in some experiments) and contents of the

intestinal segment at the end of the experiments, were cultured for the inoculated bacteria and their identity was established by serology and the haemagglutination test for fimbriae.

In a number of experiments, the contents of intestinal segment were aspirated for culture prior to the introduction of the bacterial inoculum to determine the presence or absence of organisms in the normally empty small intestine.

Experimental Salmonellosis V

Studies were made of the effects of fimbriate and non-fimbriate S. typhimurium organisms on human polymorphonuclear leucocytes.

The following bacterial strains were used:-

Experiment I S. typhimurium LT2 - fimbriate strain (F).
S. typhimurium 7471 - non-fimbriate strain (N).

Experiment II S. typhimurium 1287 - phage-transduced fimbriate strain (FT).
S. typhimurium 1287 - non-fimbriate parent strain (N).

Experiment III S. flexneri Fla 1 - fimbriate (living) strain (F).
S. flexneri Fla 1 - fimbriate (heat-killed by exposure to 56°C. for 30 min.) strain (F).
S. flexneri Fla 2 - non-fimbriate (living) strain (N).
S. flexneri Fla 2 non-fimbriate (heat-killed at 56°C. for 30 min.) strain (N).

Experiment IV S. typhimurium 1289 - phage-transduced fimbriate strain (FT).

S. typhimurium 1289 - phage-transduced strain (FT) (heat-killed at 56°C. for 30 min.)

S. typhimurium 1289 - non-fimbriate parent strain (N).

S. typhimurium 1289 - non-fimbriate strain (N) (heat-killed at 56°C. for 30 min.)

Overnight broth cultures of these test strains were centrifuged and washed thrice in saline. The strains were tested for the presence or absence of fimbriae by the haemagglutination test.

2. Method of preparation of leucocyte suspension (McLeod and McLeod, 1961).

A volume of 10 ml. of fresh human blood was run into a mixture of (a) 1 ml. of 10 per cent. dextran in 5 per cent. dextrose solution and (b) 1 ml. of a 1 per cent. solution of the disodium salt of ethylene-diamine-tetra-acetic acid (EDTA) in 1.4 per cent. NaCl solution. This mixture was left standing at room temperature for 45 - 60 min. A 5 ml. volume of the plasma which had separated during this period was removed and centrifuged at a low speed (about 500 r.p.m.) to precipitate the contained leucocytes which were subsequently resuspended in 0.5 ml. of the supernatant plasma-EDTA-dextrose mixture.

3. **Test**

Three drops of leucocyte suspension were added to

each sample of washed centrifuged bacterial deposit obtained from overnight broth cultures of fimbriate and non-fimbriate strains. To each test tube containing the test mixture was added 1 ml. of 0.85 per cent. sodium chloride solution and 1 ml. of a 1 in 3000 aqueous solution of methylene blue. After mixing, the mixtures were centrifuged for 5 min. at a relatively low speed (500 r.p.m.). The supernatant fluid was decanted and the deposit, resuspended in the residual fluid and (standing at room temperature), was examined microscopically under oil-immersion at varying intervals of time for the presence of dead and living leucocytes (see below). A total of 25 dead and living leucocytes were counted during each microscopic examination. A leucocytic control was put up with each experiment in which the test bacterial suspension was replaced by an approximate volume of normal saline.

4. Criterion of living and dead leucocyte (McLeod and McLeod 1961).

A granular leucocyte that remained colourless in the weak solution of methylene blue in hypotonic salt solution and showed active oscillation of the granules was considered to be living. Cells that stained frankly but still showed sluggish oscillation of their granules were considered to be moribund.

Cells that showed no movements of the granules when in hypotonic salt solution were dead; such cells were heavily stained.

Experimental Salmonellosis VI

Electron-microscope studies

1. Demonstration of fimbriae and flagella

The test strains of Salmonella typhimurium, Shigella flexneri and Escherichia coli were examined in broth cultures grown overnight at 37°C.

Preparation of bacterial suspension

The bacterial cultures were centrifuged at 3000 r.p.m. for 30 min. and resuspended in 0.3 per cent. formalin in sterile distilled water. After three washings the bacterial suspensions were made up to approximately the opacity of Brown's standard no. 1 (Cruickshank, 1962) in the formolised distilled water.

Shadow casting

One drop of bacterial suspension, prepared as described above, was placed on a carbon-collodion membrane sandwich (3 per cent. collodion in amyl-acetate) copper grid and dried overnight in a desiccator. The specimen grid was lightly shadowed with a 40:60 Au:Pd. alloy at angle of 15°. The metal alloy was evaporated in vacuo by employing an Edwards High Vacuum Evaporating Unit. (The angle of shadowing is of considerable qualitative importance. Ordinarily one wishes to avoid production of very long shadows, as these tend to mask details of structure in the shadowed areas.)

Negative staining with phosphotungstic acid (PTA)

The negative stain was prepared from a 2 per cent.(w/v)

solution of PTA in distilled water. Undissolved material was removed by centrifugation and the pH was adjusted to 7.4 by the addition of drops of 4 per cent. (w/v) potassium hydroxide solution.

The bacterial suspension was prepared as for shadowing. The specimen for electron microscopy was prepared by the spreading technique of Bradley and Kay (1960). This technique has the advantage of requiring less material than the spray droplet method. Two or three drops of the PTA stain were mixed with an equal volume of the bacterial suspension to make a large drop on a glass slide. The carbon-collodion sandwich grid was touched on the surface of this drop, and the excess was removed by touching the grid on a clean filter paper, thus leaving only a very thin film of the staining mixture on the grid.

2. Demonstration of adhesion of fimbriate S. typhimurium to human leucocytes, red cells and platelets

The leucocyte suspension was prepared by the method of McLeod and McLeod (1961) and the bacterial suspension was prepared as for the demonstration of fimbraie and flagella. To 0.5 ml. of the leucocyte suspension was added one drop of the bacterial suspension and this was centrifuged at a low speed (500 r.p.m.). The deposit was resuspended in 0.3 per cent. formaldehyde in distilled water and was centrifuged again and resuspended in the formalin solution. This procedure was repeated thrice and finally the deposit was resuspended in formalin

solution to give a faint turbidity visible to the naked eye. Carbon-collodion sandwich grids were then mounted with the preparation and they were dried in a desiccator. Both shadowed and unshadowed grids were viewed in the electron microscope.

3. Preparation and electron-microscopic examination of ultrathin sections of S. typhimurium

A fimbriate strain of S. typhimurium 1289FT was grown overnight at 37°C. in broth. The culture was centrifuged and washed thrice in the sodium-phosphate solution (D) described below:

Fixation of bacterial cells

This was carried out by the method of Millonig (1962).

Preparation of the fixative

The following solutions were used to prepare the fixative:

Solution A: 2.26 per cent. NaH_2PO_4. H_2O. pH 7.3

Solution B: 2.52 per cent. NaOH

Solution C: 5.4 per cent. glucose

Solution D: 41.5 ml. sol. A + 8.5 ml. sol. B

The fixative consisted of 45 ml. sol. D. + 5 ml. sol. C + 0.5 g. OsO_4 (osmium-tetra oxide). The sodium hydroxide added to the isotonic monosodiumphosphate (sol D) forms isotonic disodium phosphate, and by adding more or less of it, the pH is easily adjusted at every value between 5.4 and 8.0 without changing the tonicity. The pH of the fixative used was 7.3. The single solutions

and the fixative are stable for several weeks, if stored in clean pyrex bottles in the refrigerator.

Fixation of bacterial cells in 1 per cent. osmium-tetra oxide

The washed bacteria were resuspended in the chilled fixative (1 per cent. solution of osmium-tetra oxide) and the mixture was held in the cold room for 4 hours. Thereafter the bacteria were washed thrice in the sodium phosphate buffer. The washed centrifuged deposit was suspended in the residual buffer and well mixed in 0.5 ml. of 2 per cent. agar solution cooled to 45°C., to prepare agar blocks by the method of Keilenberger et al. (1958). This involved spreading the agar mixture (containing the fixed bacteria) on a clean, sterile watch glass. When well set, the agar was cut into small blocks (about 1.0 cu. mm.) embedded in araldite by the usual method (Glauert and Glauert, 1958) after dehydration.

Dehydration of agar blocks and intestinal tissue

Complete dehydration of specimens is essential prior to embedding, because the material used in the preparation of blocks is not water soluble. The dehydration was carried out using a graded series of alcohols, as follows:-

10 per cent. ethanol in water.....	3 changes, each of $\frac{1}{2}$ hour duration
100 per cent. ethanol in water.....	3 changes, each of $\frac{1}{2}$ hour duration
epoxy propane.....	3 changes, each of $\frac{1}{4}$ hour duration.

Embedding

Araldite was selected as the embedding material for the following reasons (Paese, 1960):-

(i) Its use eliminated to a large measure the methacrylate artifact. Araldite decomposes little if at all when bombarded by electrons at the normal operating intensities of an electron microscope. Thus the macromolecular geometry presumably is unaffected by surface force.

(ii) The other important reason for using araldite was to avoid "polymerisation damage" which seems to be a danger only with methacrylate embedding.

Epoxy resin "Araldite" Embedding

The araldite embedding medium was developed by Glauert, Rogers and Glauert (1956). The araldite mixture is readily soluble in ethanol and acetone. The reaction between the epoxy resin (Araldite M) and the hardener dodecenyl-succinic anhydride (964 B) is slow at room temperature and is acceleratedby the addition of an amine accelerator (964C). The hardness of the final block is controlled by the addition of a suitable amount of the plasticizer (dibutylphthalate). The four components were mixed in the following proportions in the final embedding mixture (Glauert and Glauert, 1958).

Araldite	10.0 ml.
Hardener (dodecenyl-succinic anhydride)964 B.	10.0 ml.
Plasticizer (dibutyl-phthalate).....	1.0 ml.
Amine accelerator 964C.....	0.5 ml.

Araldite embedding schedule

1. After dehydration, the specimens were placed in epoxy propane. The tissues were passed through two changes of epoxy propane for 15 min. each.

2. The epoxy propane was then replaced with the araldite mixture and the tissues were left in this, overnight at room temperature.

3. The specimens were then removed from the above soaking mixture and placed exactly at the bottom of a dry gelatin capsule (size 00). The capsules were then filled immediately with fresh araldite mixture. Care was taken not to introduce any bubbles of air at this stage. The final embedding was for 48 hours at 56°C. The blocks were sectioned after a subsequent period of 10 days, at room temperature.

Method of study of the mode of penetration of S. typhimurium through the gastro-intestinal mucosa of albino rats

Preliminary experiments established that S. typhimurium most readily and easily penetrated the mucosa of the duodenum and the proximal ileum of the gastro-intestinal tract of the albino rats. It was therefore decided to use the duodenum for the electron microscopic studies. The animals were anaesthetised with nembutal and the abdomen was opened and the duodenum was segmented as described for the earlier experiments (fig. 1) on the localisation of sites of penetration of the gastro-intestinal mucosa of albino rats. The duodenal segments were injected with a

suspension of the fimbriate S. typhimurium strain. It was anticipated that the fimbriae would help in subsequent identification of the strain in the intestinal tissue on electron microscopic examination. Each segment was infected by the injection (into the lumen) of approximately 10^9 organisms in 0.5 ml. of 1 per cent. Na_2HPO_4 solution from a 21 gauge needle through the anti-mesenteric border. The duration of exposure of the mucosa to bacterial penetration was varied in different animals and it ranged over periods of 5, 15 and 40 min. At the end of these periods, the segments were flushed with saline and a small piece of tissue was excised from the anti-mesenteric border of the distal part of each segment. The tissues were quickly transferred to a chilled 1 per cent. osmium-tetra oxide solution and they were cut into smaller pieces with a new razor blade while immersed in the fixative. The pieces were then placed in fresh fixative solution. The schedules for dehydration and araldite embedding were identical to those already described for the preparation of the agar blocks.

Fixation of animal tissue

Since alterations in fine structure are known to begin immediately after death, all possible steps were taken to ensure that osmium-tetra oxide fixative reached all parts of the specimen as soon as possible. Osmium-tetra oxide penetrates tissues very slowly. To facilitate proper fixation, as suggested by Kay and Cosslett (1961),

the specimen was cut into small pieces which were not more than 1.0 mm. thick in one dimension.

Preparation of 1 per cent. buffered osmium tetroxide fixative with sucrose

Caufield (1957) found that the addition of sucrose resulted in a greater proportion of well-fixed tissue in each block, and there was less coagulation of fibrillar and granular material.

The fixative was prepared by mixing 2 per cent. osmium tetroxide solution (12.5 ml.), veronal-acetate buffer (5.0 ml.) distilled water (2.5 ml.), and 0.1 N HCl (5.0 ml.). The pH was adjusted to 7.4 with the HCl. Finally 0.045 g. of sucrose per ml. of fixative was added.

The small bits of tissue about 1 mm. thick were placed in the chilled 1 per cent. osmium tetroxide fixative for 1½ to 2 hours. Thereafter the processes of dehydration and araldite embedding were performed as already described with reference to the preparation of bacteria for sectioning.

The preparation of thin sections

General remarks

Ultrathin sectioning has been possible for over a decade now. Pease and Baker (1948) showed that ultrathin sectioning was possible at reasonable cutting speeds if a suitable embedding medium was used. Newman et al. (1949) introduced butylmethacrylate. These workers were also the first to employ the principle of thermal expansion to drive a microtome. Microtomy made a very significant advance

with the advent of the technique of floating away sections on fluid surfaces from the knife's edge. This was perhaps first employed by Claude (1948), but the technique was exploited by Gettner and Hillier (1950). In spite of this there were limitations and difficulties and the discovery by Latta and Hartmann (1950) that fracture edges of glass could be used as knives was a very useful observation. The commercial version of the Porter-Blum microtome was first described in 1953 and became generally available in that year. The ultramicrotome developed by Sjöstrand (1953) also became available about the same time. The latest addition is the automatic LKB ultratome from Sweden which is useful for serial sectioning. The introduction of these instruments completed the technical "breakthrough" and the electron microscopy of ultrathin sections of tissue and bacteria has since prospered.

Preparation of glass knives

Until recently, ultrathin sectioning was being done almost exclusively with glass knives. Now diamond knives, originally developed by Fernandez-Moran (1956) are available commercially, though their cost is still considerable. Tissue sections cut with a diamond knife are no better or worse than those cut with glass, so the choice is dictated by expense and convenience (Pease, 1960). With some knowledge and skill, it is easy to prepare ones own knives using a glazier's hardened wheel-cutter and a pair of glazier's pliers to break the glass. Almost any type of

glass of suitable thickness (0.25 inch) will make satisfactory knives.

Breaking the glass strips

This was done using a slight modification of Cameron's (1956) method. After making a small initial score mark on the sheet of glass (12 x 8 in. and 0.25 inch in thickness) placed on a flat table, a match stick was placed on the table under the score mark and the sheet was pressed firmly down by applying pressure with the palms of the hand resting on blocks of wood placed on either side of the score mark. With practise it was found that the glass broke readily and accurately.

Making the glass knives

The strips of glass were wiped free from dust with a clean piece of cloth and care was taken not to place the fingers on the cutting face of the strip. The strip was placed on a flat table and a score mark was made at an angle of 45°. This mark stopped at least 0.25 inches from the cutting face. The knife was broken along the score mark by Weiner's method (1959). This simple method of breaking glass uniformly consists of fastening a narrow (1/16 - 1/8 inch) strip of adhesive tape over the middle of the lower lip of the pliers. To break glass this strip was oriented just below the score line, with the lip about $\frac{1}{2}$ inch in from the edge of the glass. The tape was parallel to the score line in the glass, the lips of the pliers were at right angles to it. Then the pliers were

tightly squeezed together. In this manoeuvre the tape serves as a pressure point or fulcrum, and the crack starts and develops smoothly from here.

The knives were made on the day that they were required. The quality of the knife was estimated by examining it with a binocular microscope. Knives whose edges were reasonably horizontal and free from checks and in which the spurs were minimal were selected for section cutting.

Trough construction

After a satisfactory glass knife had been prepared a trough was fashioned on it by using a brush and melted paraffin wax (melting point 55°C.). The brush was dipped in the melted paraffin wax and then it was applied to the knife to make a trough about $\frac{1}{4}$ in. behind the edge.

Preparation of the specimen tip for sectioning

The gelatin capsule was removed after softening for a few minutes in warm water and then the block was clamped directly in the specimen block holder of the Porter-Blum microtome. The block was viewed through the binocular microscope and trimmed with a sharp razor. The end of the block was cut to the shape of a trapezium pyramid and it was so orientated in the specimen holder that two sides of the pyramid face were parallel to the knife edge. This facilitated detachment of the sections in ribbon and this in turn eased the collection of the sections on the grids. The final trimming of the specimen tip was carried out by using the Porter-Blum microtome and the trimming was carried into

the tissue mass. After facing the block, the walls were trimmed to a trapezoid face. The width of the block face was about 0.5 mm. wide and the length was about twice this.

Mounting the knife on to the Porter-Blum microtome

The knife with the trough was fitted into the knife holder. The clearance angle was then adjusted so that it was not more than 5°. In mounting the knife in the Porter-Blum microtome one must bear in mind that there is a vertical bearing along which the cantilever arm slides as sections are cut. This bearing is straight and vertical for only about $\frac{3}{4}$ inch and perhaps only the middle $\frac{1}{2}$ inch of this should be used. The knife was mounted with its edge in such a position that sections would be cut during that part of the cycle and not when the specimen was either too high or too low.

Cutting of sections

The knife was changed and a new one set as before, or the knife was moved so that a fresh part of the edge was opposite the specimen tip. The trough was cleaned and refilled with 10 per cent. acetone in distilled water and the meniscus was adjusted. Care was taken to adjust the near edge of the knife so that it was parallel to the cut face of the specimen block. The knife was advanced with great care until the gap between the cut face of the block and the knife edge just appeared to close when observed through the binoculars. The final gap (of about 5μ) was closed by the use of only the fine advance mechanism and in

such a way that there was no risk of cutting a first section much thicker than 500 Å. The production of a thicker first section should be avoided so that the finest quality of the cutting edge of the knife is preserved to cut subsequent thin sections. The specimen advance mechanism was set at the 0 mark.

For actual cutting of the sections it was soon realised that a suitable speed and steady rhythm are essential for successful ultrathin sectioning. The cycling motion can be divided into two phases. The first is the fairly rapid phase that begins after a section has been cut and the block has disappeared from view behind the knife; this phase ends, perhaps with a momentary pause, as the block is poised above the knife ready to come down across the edge. The second phase is the slow vertical descent of the block across the knife edge. For good sectioning, it is important that the motion in the second phase should be as steady and controlled as possible. As soon as thin sections began to come off, the binoculars were set at an angle of about 15° to the vertical and the illumination was adjusted so that a reflection was observed from the meniscus at the knife edge. This enabled the interference colour of each section to be observed; the colour is related to thickness (Peachey, 1958) as follows:

Peachey Colour Scale

Interference colour	Thickness of section (mμ)
Grey	<60
Silver	60-90

Gold	90-150
Purple	150-190
Blue	190-240
Green	240-280
Yellow	280-320

The sections, which ranged from grey to silver and pale gold, were floated on 10 per cent. acetone (reagent grade) in distilled water. They were flattened by exposure to xylene vapours as recommended by Satir and Peachey (1958) prior to their collection on copper specimen grids. The grid was laid across a ribbon or raft of sections floating in the trough. The excess fluid from the specimen copper grids was drained off on filter paper and the grids were dried and stained.

Staining of sections

Sections of araldite-embedded tissue and bacteria require [illegible] staining in order to allow adequate visualisation of their components. The sections were stained by floating the specimen grids with their surface holding the section in contact with a large drop of 2 per cent. potassium permanganate solution for half an hour. After this period the grid was washed in distilled water and dipped into a weak solution of citric acid, and washed again. Then the specimen grids were dried and viewed in the electron microscope.

Photography

The electron microscope is fundamentally a camera and

the electron micrograph is its product. Thus, this is one of the several skills in which an electron microscopist must acquire proficiency. It is very important that the pictures should be taken quickly in order to avoid contaminating films from building up on the specimens. After the focussing has been completed, one should make sure that there is no specimen drift. Other reasons for unsatisfactory pictures are unduly thick sections, unduly thick supporting films, changes in focus during preparations for photography, objective lens astigmatism, microscope instability, characteristics of specimen and sectioning artifacts.

Photographic plates

Ilford special lantern contrasty plates ($3\frac{1}{2}$" x $3\frac{1}{4}$") were used throughout the work. After the field had been focussed, the illumination was reduced and the pictures were taken at an exposure of 4 seconds.

The plate development

Since contrast is often the greatest problem in electron microscopy, the selected developer tended to emphasize contrast, but at the same time possessed suitably balanced characteristics. The developer selected was Ilford ID 36.

Experimental Salmonellosis VII

Immunity studies were made to compare the protective potencies in mice of (a) live attenuated, (b) heat-killed phenol-preserved, and (c) acetone-killed vaccines prepared from a non-fimbriate/ non-flagellate strain of S.typhimurium

and its phage-transduced fimbriate / flagellate derivative.

Cultures used to prepare vaccines

S. typhimurium 6351 - non-fimbriate/non-flagellate strains and S. typhimurium 6354, a phage-transduced fimbriate/flagellate derivative of 6351 were used to make the vaccines.

Preparation of vaccines

Heat-killed phenol-preserved and acetone-killed vaccines

Organisms of each of the two S. typhimurium strains were taken from the lyophilised state, emulsified in a few drops of broth and cultured overnight on a nutrient agar plate at 37°C. Next day using a plate microscope with oblique illumination a portion of a smooth colony was picked up and the identity of its constituent bacteria was confirmed by slide agglutination with the appropriate specific antisera. The selected colony was then subcultured on nutrient agar. The resulting growth was washed in normal saline and was used to seed nutrient agar in 4 Roux flasks which were thereafter incubated at 37°C. for 18 hours. Each step of the operation was checked by plating, examination of colonial morphology, microscopic examination of Gram-stained preparations and slide agglutination with appropriate specific antisera. The growth in the Roux flasks was harvested in distilled water (20 ml. per flask). The harvest from all the Roux flasks was pooled together and it was then divided into two equal parts. One part was

used to prepare the heat-killed phenol-preserved vaccine and the other for the acetone-killed vaccine.

Heat-killed phenol-preserved vaccine (HP vaccine)

The organisms were killed by heating at 56°C. for one hour in a waterbath, and phenol was then added to a concentration of 0.5 per cent. as preservative. Culture of a sample at this stage indicated that no viable organisms remained. The bacterial count was estimated by Brown's opacity tube method and the killed vaccine was stored at 4°C. in the cold room.

Acetone-killed vaccine (AK vaccine)

The organisms were precipitated by adding to the harvest 3 volumes of acetone. The organisms were washed with acetone and held at 37°C. for 24 hours. Culture of a sample at this stage indicated that no viable organisms remained. The precipitate was resuspended in acetone and 10 ml. quantities were transferred to freeze-drying ampoules, which were centrifuged. After pipetting off the acetone, the precipitates were freeze-dried and stocked away until required. The freeze-dried bacterial precipitate was very easily resuspended in normal saline. This method of preparation of the vaccine differed from Landy's (1953) method in two ways, viz. (i) the bacterial precipitate was not in powdered form, and (ii) dry, powdered sterile sodium chloride was not added, so weighing was not necessary. The bacterial counts of the vaccine concentrates were estimated by the use of Brown's opacity standards.

Live attenuated vaccine (SR vaccine)

A single step streptomycin-resistant mutant (resistant to 1000 units of streptomycin) was derived from the parent strain and freeze-dried cultures were prepared by the method described earlier (Experimental Salmonellosis I).

Preparation of incomplete Freund-type adjuvant mixtures

The adjuvant consisted of:-

Drakeol 6-VR	9 parts
Arlacel A	1 part

The above was sterilised by Seitz-filtration. Drakeol 6-VR (Pennsylvania Refining Co., Bulles, Pa.) is a special grade white mineral oil used for repository injections. Its viscosity at 100°F. is 59.61 s.s.u. Arlacel A (the emulsifier) was obtained from Evans, England.

Preparation of water-in-oil emulsion

Heat-killed and phenol-preserved (HP) and acetone-killed and freeze-dried (AK) vaccines were suspended in normal saline so that they contained 10^8 organisms per ml. The water-in-oil emulsions of the vaccines were prepared by mixing equal volumes of the bacterial vaccines and the prepared incomplete Freund-type adjuvant mixture. Small volumes of the bacterial suspensions were then taken up in a syringe and spurted out through a fine needle deep under the adjuvant mixture to produce an emulsion. At the end of this process, the emulsions were further mixed in a mixer.

Mouse protective potency tests

The assay procedure employed in the potency tests

involved the use of graded immunising doses and constant challenge doses. White Swiss mice (obtained from the Clinical Endocrinology M.R.C. Research Unit, Edinburgh) weighing16 to 22 grams, and segregated as to sex, were assigned to cages in groups. Assignment of animals to cages, and assignment of cages to locations in the animal room, and the order of immunisation and challenge injections, by groups, were all determined by randomisation procedures. The temperature of the test animal room was maintained at 22°C. All immunising and challenge doses were administered in standard volumes of 0.1 ml.

Active immunisation with killed vaccines

Two schedules were adopted.

(i) A single immunising dose of each vaccine containing log 3, 5 and 7 organisms respectively, suspended in 0.1 ml. normal saline, was injected subcutaneously under the skin near the root of the tail as described by Cruickshank (1962) to three balanced groups of Swiss white mice. Each of the mice in the control group was given 0.1 ml. normal saline.

(ii) Mice immunised according to a two dose immunisation schedule were given two subcutaneous injections separated by an interval of 14 days.

Active immunisation with live attenuated vaccine

One group of mice was immunised with a single subcutaneous injection as described above. Two groups of mice were immunised by oral administration of the vaccine. One of

these two groups was treated with oral streptomycin on the day prior to active immunisation. The methods of streptomycin treatment and administration of oral bacterial vaccine have been described earlier (Experimental Salmonellosis I). The immunising doses of the vaccine contained log 1, log 2 and log 3 organisms respectively.

Active immunisation with Freund-type mixtures

The mice were immunised by a single subcutaneous injection containing log 7 organisms in 0.2 ml. emulsion.

Challenge organisms and challenge procedures

The challenge organism was a heterologous S. typhimurium strain 1566F - a fimbriate and flagellate strain of phage type 1a/U57. The intraperitoneal and the oral LD50 log doses of this strain for LAC grey mice was less than 1.2 and 4.6 respectively. Overnight broth cultures prepared from freeze-dried stock ampoules of the challenge strain were diluted in 1 per cent. Na_2HPO_4 solution pH 8.8 and the immunised mice were challenged either by the oral or the intraperitoneal route. Each group of immunised mice was divided into two subgroups for challenge. The challenge dose was of two strengths and each was administered in 0.1 ml. volumes. The actual numbers of organisms in the challenge doses were determined by viable counts by the Miles and Misra method. The period of observation after challenge varied from 21 to 29 days, after which the survivors were killed. The post-mortem procedures and investigations were as described earlier (Experimental Salmonellosis I).

Serological Procedures

Preparation of O agglutinable alcoholised suspension

A smooth colony was picked from a plate culture of the non-fimbriate/non-flagellate S. typhimurium strain 6351 NF/nfl and grown on nutrient agar in a Roux bottle. The growth was harvested in the minimum amount of normal saline. This was carefully emulsified and 20 times its volume of absolute alcohol was added. It was heated at 40°-50°C. for 30 min. and then centrifuged. The deposit was suspended in saline to give an opacity equivalent to Brown's opacity standard no. 1. Chloroform was added to the suspension as preservative.

Collection of blood from mice

The mice were anaesthetised with ether and the skin over the thorax and abdomen was reflected as for autopsy. The great vessels in the axilla were incised and the blood which welled out was taken up in a sterile Pasteur pipette. The serum was collected and inactivated by heating at 56°C. for 30 min. before carrying out the agglutination tests.

Agglutination tests

Tube agglutinations were carried out by methods described in Mackie and McCartney's Handbook of Bacteriology (1962). The tube agglutination test mixtures were incubated at 37°C. for 4 hours and then kept at 4°C. in a refrigerator for 20-22 hours before final readings were made. The clumps were small and granular; observations were aided by the use of a hand-lens and good illumination.

RESULTS

Information pertaining to Table I.

Strains were obtained from Professor J.P. Duguid, and they were phage-typed at the Enteric Reference Laboratory, Colindale Avenue, London, by Dr. E.S. Anderson. Phage-typing scheme is Anderson's modification of Callow's (1959). Viable bacterial counts were estimated by the method of Miles and Misra.

* Haemagglutination tests were done with guinea-pig red cells. In all cases in which positive results were obtained the agglutination was later reversed by addition of mannose to 0.5 per cent.

Starting with the highest dilution of the prepared bacterial suspensions in 1 per cent Na_2HPO_4 solution pH8.8 the first three dilutions were administered intraperitoneally and the rest orally, through a 15 gauge needle with a blunted point from a syringe. Each successive batch of mice received log 1 greater concentration of the test organisms.

N = non-fimbriate F = fimbriate

FT = phage-transduced fimbriate

MF = Mutant (M_nCl_2) fimbriate

nfl = non-flagellate fl = flagellate

flt = phage-transduced flagellate

SR = streptomycin-resistant Mutant

TABLE I.

Experimental salmonellosis I. COMPARATIVE VIRULENCE STUDIES OF FIMBRIATE AND NON-FIMBRIATE S. typhimurium strains for MICE INOCULATED BY THE ORAL AND INTRAPERITONEAL ROUTES. GENERAL INFORMATION PERTAINING TO THE S. TYPHIMURIUM STRAINS EMPLOYED AND THE EXPERIMENTS CARRIED OUT DURING THE COURSE OF THESE STUDIES.

Expt. No.	S. typhimurium strain	Source of strain	Rhamnose fermentation	Phage type old/new scheme	Motility	Haemagglutination *	Organisms per ml. in suspensions inoculated.	Range of bacterial inoculum in log doses: Intraperitoneal	Range of bacterial inoculum in log doses: Oral	Strain of mice
I	1289FT	Man England 1962	Neg.	Ivar 2/U41	+	+++	3.4×10^{9}	1.5-3.5	4.5-8.5	LAC grey
	1289N		"		+	-	3.8×10^{9}	1.6-3.6	4.6-9.6	
II	1294FT	"	"	2b/14a	+	+++	1.5×10^{9}	1.2-3.2	4.2-8.2	"
	1294N		"		+	-	5×10^{9}	0.7-2.7	3.7-8.7	
III	1287FT	Man N.Ireland 1962	"	2a/13	+	+++	1.5×10^{9}	1.2-3.2	4.2-8.2	"
	1287N				+	-	4.5×10^{9}	0.7-2.7	3.7-8.7	
IV	1289FT/SR	Man England 1962	"	Ivar 2/U41	+	+++	1.5×10^{8}	-	0.17-4.17	"
	1289N/SR		"		+	-	1.2×10^{8}	-	0.08-4.08	
V	1294FT/SR	"	"	2b/14a	+	+++	3.7×10^{8}	-	0.7-4.7	"
	1294N/SR		"		+	-	3.1×10^{8}	-	0.5-4.5	
VI	1566F	Pig Indiana	Pos.	1a/U57	+	+++	1.5×10^{9}	1.2-3.2	4.2-8.2	"
	1566N		"		+	-	6×10^{9}	0.8-2.8	3.8-8.8	
VII	6351N/nfl	Fowl	Neg.	2a/13	-	-	2.5×10^{9}	0.4-2.4	3.4-8.4	Swiss white mice
	6354FT/flt		"		+	+++	2.7×10^{9}	1.4-3.4	4.4-8.4	
VIII	6351N/nfl		"		-	-	6.3×10^{10}	1.8-3.8	4.8-9.8	
	6352FT/nfl		"		-	+++	8.3×10^{9}	1.9-3.9	4.9-8.9	
	6353N/flt		"		+	-	7.3×10^{9}	1.7-3.7	4.7-8.7	
IX	629MF	Fowl	Pos.	2c/14	+	+++	1.7×10^{9}	1.2-3.2	4.2-8.2	"
	629N		"		+	-	9.8×10^{9}	1.0-3.0	4.0-9.0	
X	Sw573N/nfl	Man 1962	Neg.	2a/13	-	-	1.0×10^{10}	1.0-3.0	4.0-9.0	"
	Sw576N/fl		"		+	-	1.1×10^{9}	1.0-3.0	4.0-8.0	
	Sw576FT/flt		"		+	+++	0.5×10^{9}	0.7-2.7	3.7-7.7	
XI	1436MF/fl	Meat Switzerland 1962	Pos.	4/U38	+	+++	1.8×10^{9}	1.3-3.3	4.3-8.3	"
	1436N fl		"		+	-	3.8×10^{10}	1.6-3.6	4.6-9.6	

RESULTS

Experimental salmonellosis I. Comparative virulence of fimbriate and non-fimbriate strains of S. typhimruim for mice inoculated orally and intraperitoneally.

Eleven sets of experiments were carried out in which the virulence of a non-fimbriate/flagellate or the non-fimbriate/non-flagellate parent S. typhimurium strain was compared with that of (a) its fimbriate/flagellate variant; or (b) fimbriate/non-flagellate variant; or (c) non-fimbriate/flagellate variant. The infecting intraperitoneal doses ranged from about log 1 to about log 3 and the oral doses ranged from about log 4 to about log 8 for the fimbriate strains and from about log 4 to about log 9 for the non-fimbriate strains. In experiments (no. IV and V) in which streptomycin-treated mice were employed, the animals were infected only orally and the infecting doses ranged from about log 1 to about log 4. The general information regarding the S. typhimurium strains and the experiments made with them is shown in table I. Further details of each experiment are shown in the appropriate appendices (no. 1-22) and the results of the experiments are summarised in tables II and III. In the experiments summarised in table II all the test strains are motile, i.e. in this series of experiments the comparison of virulence was between the non-fimbriate/flagellate parent strains and their fimbriate/flagellate derivatives obtained by phage-transduction or the use of mutagenic agent. In the experiments summarised in table III both the parent strains are

non-fimbriate/non-flagellate and in this series of experiments their virulence was compared with phage-transduced fimbriate/non-flagellate, non-fimbriate/flagellate and fimbriate/flagellate derivatives.

Mortality and infectivity in mice inoculated intraperitoneally and orally

It was observed in all the experiments that infection by the intraperitoneal route resulted in a higher percentage mortality and infectivity than infection by the oral route, and this despite the fact that the oral infecting doses were much larger. The percentage mortalities from intraperitoneal infection with the parent non-fimbriate/flagellate strains and their derivative fimbriate/flagellate strains ranged from 36 to 100 and 30 to 100 respectively (table II), while the corresponding mortality figures resulting from oral infection with these strains were 13.3 to 70 and 1.6 to 80 respectively. The percentage mortalities resulting from intraperitoneal and oral infection with the parent non-fimbriate/non-flagellate strains (table III) were lower. In the two experiments carried out with S. typhimurium strain 6351 N/nfl the percentage mortalities after intraperitoneal and oral infection was 0 and 12.6 and 1.4 and 12.6 respectively. For strain SW 573 N/nfl the intraperitoneal and oral mortality percentages were 36.1 and 11.1 respectively. The percentage infectivity with all the S. typhimurium strains parallelled the mortality figures but at a substantially higher level.

TABLE II

Experimental salmonellosis I. Series (a). COMPARATIVE VIRULENCE OF FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM STRAINS FOR MICE INOCULATED ORALLY AND INTRAPERITONEALLY. THE TABLE SHOWS PERCENTAGE MORTALITY AND INFECTIVITY, LOG DOSES LD50 AND ID50 AND THE AVERAGE TIME TO DEATH AT THE ORAL LD50.

Expt. No.	S. typhi-murium strains*	Duration of experiment (days)	No. of batches x No. of mice per batch: infected orally	infected intraperitoneally	Results when infection was: oral: Mortality (%)	oral: Infectivity (%)	oral: LD50 (log)	oral: ID50 (log)	intraperitoneal: Mortality (%)	intraperitoneal: Infectivity (%)	intraperitoneal: LD50 (log)	intraperitoneal: ID50 (log)	Average time to death (days) at oral LD50	Ratio result FT/N organism inoculated orally: LD50 (log)	Ratio result FT/N organism inoculated orally: ID50 (log)
I	1289FT	25	5x12	3x8	36.6	45	7.2	7.1	70	95	1.9	1.7	12.2	1.07	1.12
	1289N		6x12	3x8	56.9	61.1	6.6	6.3	66.6	75	2.1	1.9	14.4		
II	1294FT	21	5x12	3x12	18.3	60	7.8	5.7	72.2	97.6	< 1.2	< 1.2	8.6	1.03	1.05
	1294N		6x12	3x12	31.9	62.5	7.4	5.4	75	91.6	< 0.7	< 0.7	12.7		
III	1287FT	22	5x12	3x12	45	63.3	6.4	5.4	91.6	94.4	< 1.2	< 1.2	9.2	1.05	0.98
	1287N		6x12	3x12	53.1	61.1	6.1	5.6	94.4	100	< 0.7	< 0.7	13.1		
IV	1289FT/SR	22	5x6		33.3	66.6	2.9	1.3					16.2	0.84	1.08
	1289N/SR		5x6		20	70	3.5	1.2					12.8		
V	1294FT/SR	24	5x6		16.6	73.3	4.7	1.3					20	1.04	1.53
	1294N/SR		5x6		13.3	80	> 4.5	0.85					> 24		
VI	1566F	23	5x12	3x8	80	81.6	4.6	4.5	100	100	< 1.2	< 1.2	13.8	0.92	0.90
	1566N		6x12	3x8	70	73.6	5.0	4.8	100	100	< 0.8	< 0.8	10.7		
IX	629MF	24	5x12	3x12	1.6	53.3	> 8.2	5.9	30.5	100	2.9	< 1.2	> 24	0.95	1.03
	629N		6x12	3x12	19.4	72.2	8.6	5.2	36.1	100	2.5	< 1.0	15.2		
XI	1436MF	22	5x12	3x12	31.6	76.6	7.3	4.9	83.3	97.2	< 1.3	< 1.3	13.6	1.17	0.86
	1436N		6x12	3x12	33.3	73.6	7.8	5.7	84.1	94.4	< 1.6	< 1.6	15.3		

* Note all the test strains in this series are flagellate

N = non-fimbriate F = fimbriate FT = phage-transduced fimbriate SR = streptomycin-resistant mutant

MF = fimbriate, obtained by the use of $MnCl_2$ as a mutagenic agent < = less than > = more than

The average time to death at oral LD50

This ranged from 9.2 to >24 days for the parent non-fimbriate/flagellate strains and from 8.6 to 20 days for their fimbriate/flagellate derivatives. The non-fimbriate/non-flagellate parent strains 6351 N/nfl and SW 573 N/nfl appear to have a rather low degree of virulence for white mice. The mean times to death for these strains were 23 days and more than 22 days respectively.

50 per cent. lethal (LD50) and infective (ID50) log doses

The intraperitoneal LD50 and ID50 results

The details are shown in tables II and III. The ID50 log dose by either the intraperitoneal or oral routes of infection was invariably smaller than the corresponding LD50 log dose irrespective of the characteristics of the infecting S. typhimurium strain, i.e. whether it was non-fimbriate/non-flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate or fimbriate/flagellate.

The intraperitoneal LD50 and ID50 log doses for the parent non-fimbriate/flagellate strains and their fimbriate/flagellate derivatives ranged from about 10 organisms (less than log 1) to 1000 organisms (about log 3). However, the intraperitoneal LD50 log doses for the parent non-fimbriate/non-flagellate strains (for 6351 N/nfl it was found to be more than log 3.8 and for strain SW 573 N/nfl it was found to be log 2.5) were found to be higher than those of their derivatives (table III), but their ID50 log doses were more nearly identical.

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	1289N		"		+	-	3.8×10^9	1.6-3.6	4.6-9.6	
II	1294FT	"	"	2b/14a	+	+++	1.5×10^9	1.2-3.2	4.2-8.2	"
	1294N		"		+	-	5×10^9	0.7-2.7	3.7-8.7	
III	1287FT	Man N.Ireland 1962	"	2a/13	+	+++	1.5×10^9	1.2-3.2	4.2-8.2	"
	1287N				+	-	4.5×10^9	0.7-2.7	3.7-8.7	
IV	1289FT/SR	Man England 1962	"	Ivar 2/U41	+	+++	1.5×10^8	-	0.17-4.17	"
	1289N/SR		"		+	-	1.2×10^8	-	0.08-4.08	
V	1294FT/SR	"	"	2b/14a	+	+++	3.7×10^8	-	0.7-4.7	"
	1294N/SR		"		+	-	3.1×10^8	-	0.5-4.5	
VI	1566F	Pig Indiana	Pos.	1a/U57	+	+++	1.5×10^9	1.2-3.2	4.2-8.2	"
	1566N		"		+	-	6×10^9	0.8-2.8	3.8-8.8	
VII	6351N/nfl	Fowl	Neg.	2a/13	-	-	2.5×10^9	0.4-2.4	3.4-8.4	Swiss white mice
	6354FT/flt		"		+	+++	2.7×10^9	1.4-3.4	4.4-8.4	
VIII	6351N/nfl		"		-	-	6.3×10^{10}	1.8-3.8	4.8-9.8	
	6352FT/nfl		"		-	+++	8.3×10^9	1.9-3.9	4.9-8.9	
	6353N/flt		"		+	-	7.3×10^9	1.7-3.7	4.7-8.7	
IX	629MF	Fowl	Pos.	2c/14	+	+++	1.7×10^9	1.2-3.2	4.2-8.2	"
	629N		"		+	-	9.8×10^9	1.0-3.0	4.0-9.0	
X	SW573N/nfl	Man 1962	Neg.	2a/13	-	-	1.0×10^{10}	1.0-3.0	4.0-9.0	"
	SW576N/fl		"		+	-	1.1×10^9	1.0-3.0	4.0-8.0	
	SW576FT/flt		"		+	+++	0.5×10^9	0.7-2.7	3.7-7.7	
XI	1436MF/fl	Meat Switzerland 1962	Pos.	4/U38	+	+++	1.8×10^9	1.3-3.3	4.3-8.3	"
	1436N/fl		"		+	-	3.8×10^{10}	1.6-3.6	4.6-9.6	

RESULTS

Experimental salmonellosis I. Comparative virulence of fimbriate and non-fimbriate strains of S. typhimruim for mice inoculated orally and intraperitoneally.

Eleven sets of experiments were carried out in which the virulence of a non-fimbriate/flagellate or the non-fimbriate/non-flagellate parent S. typhimurium strain was compared with that of (a) its fimbriate/flagellate variant; or (b) fimbriate/non-flagellate variant; or (c) non-fimbriate/flagellate variant. The infecting intraperitoneal doses ranged from about log 1 to about log 3 and the oral doses ranged from about log 4 to about log 8 for the fimbriate strains and from about log 4 to about log 9 for the non-fimbriate strains. In experiments (no. IV and V) in which streptomycin-treated mice were employed, the animals were infected only orally and the infecting doses ranged from about log 1 to about log 4. The general information regarding the S. typhimurium strains and the experiments made with them is shown in table I. Further details of each experiment are shown in the appropriate appendices (no. 1-22) and the results of the experiments are summarised in tables II and III. In the experiments summarised in table II all the test strains are motile, i.e. in this series of experiments the comparison of virulence was between the non-fimbriate/flagellate parent strains and their fimbriate/flagellate derivatives obtained by phage-transduction or the use of mutagenic agent. In the experiments summarised in table III both the parent strains are

non-fimbriate/non-flagellate and in this series of experiments their virulence was compared with phage-transduced fimbriate/non-flagellate, non-fimbriate/flagellate and fimbriate/flagellate derivatives.

Mortality and infectivity in mice inoculated intraperitoneally and orally

It was observed in all the experiments that infection by the intraperitoneal route resulted in a higher percentage mortality and infectivity than infection by the oral route, and this despite the fact that the oral infecting doses were much larger. The percentage mortalities from intraperitoneal infection with the parent non-fimbriate/flagellate strains and their derivative fimbriate/flagellate strains ranged from 36 to 100 and 30 to 100 respectively (table II), while the corresponding mortality figures resulting from oral infection with these strains were 13.3 to 70 and 1.6 to 80 respectively. The percentage mortalities resulting from intraperitoneal and oral infection with the parent non-fimbriate/non-flagellate strains (table III) were lower. In the two experiments carried out with S. typhimurium strain 6351 N/nfl the percentage mortalities after intraperitoneal and oral infection was 0 and 12.6 and 1.4 and 12.6 respectively. For strain SW 573 N/nfl the intraperitoneal and oral mortality percentages were 36.1 and 11.1 respectively. The percentage infectivity with all the S. typhimurium strains parallelled the mortality figures but at a substantially higher level.

TABLE II

Experimental salmonellosis I. Series (a). COMPARATIVE VIRULENCE OF FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM STRAINS FOR MICE INOCULATED ORALLY AND INTRAPERITONEALLY. THE TABLE SHOWS PERCENTAGE MORTALITY AND INFECTIVITY, LOG DOSES LD50 AND ID50 AND THE AVERAGE TIME TO DEATH AT THE ORAL LD50.

Expt. No.	S.* typhi-murium strains	Duration of experiment (days)	No. of batches x No. of mice per batch: infected orally	infected intraperitoneally	Results when infection was: oral — Mortality (%)	oral — Infectivity (%)	oral — LD50 (log)	oral — ID50 (log)	intraperitoneal — Mortality (%)	intraperitoneal — Infectivity (%)	intraperitoneal — LD50 (log)	intraperitoneal — ID50 (log)	Average time to death (days) at oral LD50	Ratio result FT/N organism inoculated orally: LD50 (log)	Ratio result FT/N organism inoculated orally: ID50 (log)
I	1289FT	25	5x12	3x8	36.6	45	7.2	7.1	70	95	1.9	1.7	12.2	1.07	1.12
	1289N		6x12	3x8	56.9	61.1	6.6	6.3	66.6	75	2.1	1.9	14.4		
II	1294FT	21	5x12	3x12	18.3	60	7.8	5.7	72.2	97.6	< 1.2	< 1.2	8.6	1.03	1.05
	1294N		6x12	3x12	31.9	62.5	7.4	5.4	75	91.6	< 0.7	< 0.7	12.7		
III	1287FT	22	5x12	3x12	45	63.3	6.4	5.4	91.6	94.4	< 1.2	< 1.2	9.2	1.05	0.98
	1287N		6x12	3x12	53.1	61.1	6.1	5.6	94.4	100	< 0.7	< 0.7	13.1		
IV	1289FT/SR	22	5x6		33.3	66.6	2.9	1.3					16.2	0.84	1.08
	1289N/SR		5x6		20	70	3.5	1.2					12.8		
V	1294FT/SR	24	5x6		16.6	73.3	4.7	1.3					20	1.04	1.53
	1294N/SR		5x6		13.3	80	> 4.5	0.85					> 24		
VI	1566F	23	5x12	3x8	80	81.6	4.6	4.5	100	100	< 1.2	< 1.2	13.8	0.92	0.90
	1566N		6x12	3x8	70	73.6	5.0	4.8	100	100	< 0.8	< 0.8	10.7		
IX	629MF	24	5x12	3x12	1.6	53.3	> 8.2	5.9	30.5	100	2.9	< 1.2	> 24	0.95	1.03
	629N		6x12	3x12	19.4	72.2	8.6	5.2	36.1	100	2.5	< 1.0	15.2		
XI	1436MF	22	5x12	3x12	31.6	76.6	7.3	4.9	83.3	97.2	< 1.3	< 1.3	13.6	1.17	0.86
	1436N		6x12	3x12	33.3	73.6	7.8	5.7	84.1	94.4	< 1.6	< 1.6	15.3		

* Note all the test strains in this series are flagellate

N = non-fimbriate F = fimbriate FT = phage-transduced fimbriate SR = streptomycin-resistant mutant

MF = fimbriate, obtained by the use of $MnCl_2$ as a mutagenic agent < = less than > = more than

The average time to death at oral LD50

This ranged from 9.2 to >24 days for the parent non-fimbriate/flagellate strains and from 8.6 to 20 days for their fimbriate/flagellate derivatives. The non-fimbriate/non-flagellate parent strains 6351 N/nfl and SW 573 N/nfl appear to have a rather low degree of virulence for white mice. The mean times to death for these strains were 23 days and more than 22 days respectively.

50 per cent. lethal (LD50) and infective (ID50) log doses

The intraperitoneal LD50 and ID50 results

The details are shown in tables II and III. The ID50 log dose by either the intraperitoneal or oral routes of infection was invariably smaller than the corresponding LD50 log dose irrespective of the characteristics of the infecting S. typhimurium strain, i.e. whether it was non-fimbriate/non-flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate or fimbriate/flagellate.

The intraperitoneal LD50 and ID50 log doses for the parent non-fimbriate/flagellate strains and their fimbriate/flagellate derivatives ranged from about 10 organisms (less than log 1) to 1000 organisms (about log 3). However, the intraperitoneal LD50 log doses for the parent non-fimbriate/non-flagellate strains (for 6351 N/nfl it was found to be more than log 3.8 and for strain SW 573 N/nfl it was found to be log 2.5) were found to be higher than those of their derivatives (table III), but their ID50 log doses were more nearly identical.

TABLE III

Experimental salmonellosis I. Series (b). COMPARATIVE VIRULENCE OF NON-FIMBRIATE/NON-FLAGELLATE STRAINS OF *S. TYPHIMURIUM* AND THEIR FIMBRIATE/FLAGELLATE VARIANTS FOR MICE INOCULATED ORALLY AND INTRAPERITONEALLY. THE TABLE SHOWS PERCENTAGE MORTALITY AND INFECTIVITY, LOG DOSES LD50 AND ID50 AND THE AVERAGE TIME TO DEATH AT THE ORAL LD50.

Expt. No.	*S. typhimurium* strain	Flagella	Fimbriae	Duration of experiment (days)	No. of batches x no. of mice per batch: infected orally	infected intraperitoneally	Results when infection was: oral: Mortality (%)	oral: Infectivity (%)	oral: LD50 (log)	oral: ID50 (log)	intraperitoneal: Mortality (%)	intraperitoneal: Infectivity (%)	intraperitoneal: LD50 (log)	intraperitoneal: ID50 (log)	Average time to death (days) at oral ID50
VII	6351N/nfl	-	-	23	6x12	3x12	1.4	12.6	>8.4	>8.4	0	58.4	>2.4	<0.4	>23
VIII	"	-	-	23	"	"	12.6	22.4	9.8	9.1	12.6	94.4	>3.8	<1.8	13.3
	6352FT/nfl	-	+		5x12	"	23.3	61.6	8.4	6.5	72.2	91.6	<1.9	<1.9	12.9
	6353N/flt	+	-		"	"	21.6	94.5	8.2	6.4	97.2	100	<1.7	<1.7	11.8
VII	6354FT/flt	+	+	23	"	"	32.9	61.6	6.8	5.9	77.7	97.2	<1.4	<1.4	15.8
X	SW573N/nfl	-	-	22	6x12	"	11.1	47.2	>9.0	6.6	36.1	88.6	2.5	<1.0	>22
	SW576N/fl	+	-		5x12	"	10	56.6	>8.0	5.7	63.9	91.7	<1.0	<1.0	>22
	SW576FT/flt	+	+		"	"	3.3	45	>7.7	5.9	44.4	83.3	1.9	<0.9	>22

N = non-fimbriate FT = phage-transduced fimbriate nfl = non-flagellate

fl = flagellate flt = phage-transduced flagellate > = more than < = less than

The oral LD50 and ID50 results

(i) In three experiments (table II, expt. no. I, II and III), the LD50 log doses for parent non-fimbriate/flagellate strains were less than those for their fimbriate/flagellate derivatives. The log doses differences were 0.6, 0.4 and 0.3 respectively, i.e. less than 10 fold. In three other experiments (tables II and III, expt. no. IV, VI and XI) the situation was quite the reverse but log dose differences were again only 0.6, 0.5 and 0.4 respectively, i.e. again less than 10 fold. Finally, in two experiments (table II, expt. no. V and IX) the LD50 log doses were approximately identical.

(ii) In five experiments (table II, expt. no. I, II, IV, V and IX) ID50 log doses for the parent non-fimbriate/flagellate strains were less than those for the corresponding fimbriate/flagellate strains; the log dose differences were 0.8, 0.3, 0.1, 0.45 and 0.7 respectively, i.e. less than 10 fold. In the three other experiments (tables II and III, expt. no. III, VI and XI), the fimbriate/flagellate derivatives had a smaller ID50 log dose; the log dose differences were 0.2, 0.3 and 0.8 respectively, i.e. less than 10 fold.

(iii) In two experiments performed with streptomycin-treated mice in the above series (table II, expt. no. IV and V), the oral LD50 and ID50 (log doses) were considerably lowered for both the parent non-fimbriate/flagellate strains and their fimbriate/flagellate derivatives. The oral LD50

and ID50 with streptomycin-treated mice were very nearly identical to intraperitoneal LD50 and ID50 log doses for the non-streptomycin-treated mice.

(iv) In experiments (table III, expt. no. VII, VIII and X) with the parent non-fimbriate/non-flagellate strains (6351 N/nfl and SW 573 N/nfl) the LD50 and ID50 log doses were greater than those for their fimbriate/non-flagellate (more than 10 fold), or non-fimbriate/flagellate(more than 10 fold), or fimbriate/flagellate (about 1000 fold with strain 6354 FT/flt) derivatives. The log dose differences between the parent and their derivative strains in this series were as follows:-

(Log 1 = 10, log 2 = 100, log 3 = 1000, and so on)

Strains (table III, expt. no. VII and VIII)	Oral dose differences	
	LD50 (log)	ID50 (log)
Log doses for strain 6354 fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6351 non-fimbriate/non-flagellate by	3	3.2
Log doses for strain 6353 non-fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6351 non-fimbriate/non-flagellate by	1.6	2.7
Log doses for strain 6352 fimbriate/non-flagellate are less (i.e. it is more virulent) than those of strain 6351 non-fimbriate/non-flagellate by	1.4	2.6
Log doses for strain 6354 fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6352 fimbriate/non-flagellate by	1.6	0.6
Log doses for strain 6354 fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6353 non-fimbriate/flagellate by	1.4	0.5
Log doses for strain 6353 non-fimbriate/flagellate are only slightly less (as in table II) than those of strain 6352 fimbriate/non-flagellate by	0.2	0.1

S. typhimurium strains (table III, expt. no. X)	Oral dose differences	
	LD50 (log)	ID50* (log)
Log doses for strain SW 576 fimbriate/ flagellate are less than those of strain SW 573 non-fimbriate/non-flagellate by	The strains have a rather low degree of virulence for mice	0.7
Log doses for strain SW 576 fimbriate/ flagellate are less than those of strain SW 576 non-fimbriate/flagellate by		0.2
Log doses for strain SW 576 non-fimbriate/ flagellate are less than those of strain SW 573 non-fimbriate/non-flagellate by		0.9
* ID50 dose differences are less than 10 fold		

Here again as for strains in table II there were practically no differences in the LD50 and the ID50 log doses of the non-fimbriate/flagellate and fimbriate/flagellate derivatives of the parent non-fimbriate/non-flagellate strains 6351 and SW 573, i.e. the ratio of the oral infection LD50 and ID50 doses for mice (table II) with the fimbriate/ flagellate and the non-fimbriate/flagellate S. typhimurium is always close to unity.

Experimental salmonellosis II

The effect of fimbriation on the reaction produced by organisms of S. typhimurium and E. coli strains of enteropathogenic serotypes in ligated loops of the rabbit gut (De and Chatterjee's technique)

Mortality during operative procedure

Only 4 (5.0 per cent.) out of a total of 80 rabbits

TABLE IV

Experimental salmonellosis II. COMPARATIVE STUDIES OF THE EFFECTS OF INJECTION OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF S. TYPHIMURIUM INTO LIGATED LOOPS OF RABBIT GUT (ENTEROPATHOGENICITY TEST). 0.5 ml. OF BROTH CULTURE OF EACH TEST STRAIN WAS INJECTED INTO A SEPARATE TEST LOOP. IN EXPERIMENTS NOS. 1 to 5 AND 8 and 9 THE INOCULUM WAS AN 18 hr. GROWTH IN BROTH, and IN EXPERIMENTS NOS. 6 and 7 THE INOCULUM WAS A 48 hr. GROWTH. THE TEST ANIMALS WERE KILLED 24 HOURS AFTER THE OPERATION.

Expt. No.	S.typhimurium test strain	Result: No. positive/No. of loops tested			
		Proximal loop (nearest the stomach)	Middle loop	Distal loop (nearest the caecum)	Total positive loops / No. of loops tested
1	LT2(F)	1/1	1/1		2/2
	7471(N)	1/1	0/1	1/1	2/3
2	1289FT	1/1	0/1		1/2
	1289N	0/1	0/1	0/1	0/3
3	1287FT		1/2		1/2
	1287N	1/2		1/2	2/4
4	1294FT	0/1	0/1		0/2
	1294N	1/1	0/1	0/1	1/3
5	1566F	2/2	2/2	0/1	4/5
	1566N	1/1	0/1	0/1	1/3
6	629MF	1/1	0/1	1/1	2/3
	629N	1/1	1/1	1/1	3/3
7	6351NF/nfl	1/1	0/1	1/1	2/3
	6352FT/nfl	1/1	1/1	1/1	3/3
	6353NF/flt	1/1	1/1	1/1	3/3
	6354FT/flt	1/1	1/1	1/1	3/3
8	573N/nfl	1/1		1/1	2/2
	576FT/flt	1/1		1/1	2/2
	576N/fl	1/1		1/1	2/2
9	1436MF	1/1		1/1	2/2
	1436NF	1/1		1/1	2/2
Total proportion of positive loops		19/22 (85.7%)	8/17 (47.0%)	13/18 (70.6%)	40/57 (69.1%)
Total proportion of positive loops in expts. with fimbriate/flagellate strains		8/9 (88.8%)	5/9 (55.5%)	4/5 (80%)	17/23 (73.9%)
Total proportion of positive loops in expts. with fimbriate/Non-flagellate strains		1/1 (100%)	1/1 (100%)	1/1 (100%)	3/3 (100%)
Total proportion of positive loops in expts. with non-fimbriate/flagellate strains		8/10 (80%)	2/7 (28.6%)	6/10 (60.0%)	16/27 (59.2%)
Total proportion of positive loops in expts. with non-fimbriate/non-flagellate strains		2/2 (100%)	-	2/2 (100%)	4/4 (100%)

N & NF = non-fimbriate nfl = non-flagellate F = fimbriate
fl = flagellate FT = phage-transduced fimbriate flt = phage-transduced flagellate
MF = fimbriate obtained by use of M_nCl_2 as a mutagenic agent

employed in this series of experiments died during or immediately after the operation. All of the deaths occurred in the early stages of the research programme and were due to overdosage of anaesthetic. With experience, the whole operative procedure was found to be rather simple, safe and was easily executed without assistance.

Studies with S. typhimurium strains

A total of 57 loops in 22 rabbits were infected with 21 S. typhimurium strains belonging to six different phage types. Nine of the test strains were fimbriate/flagellate, one was fimbriate/non-flagellate, nine were non-fimbriate/flagellate and two were non-fimbriate/non-flagellate. A total of 40 (69.1 per cent.) infected loops showed positive dilatation. The results are summarised as follows:-

Characteristics of strain of S. typhimurium tested	No. giving positive loop test	Total number of loops tested	Percentage positive loops
Fimbriate/flagellate	17	23	73.9
Fimbriate/non-flagellate	3	3	100
Non-fimbriate/flagellate	16	27	59.2
Non-fimbriate/non-flagellate	4	4	100

The detailed results are shown in table IV, and it is evident that highest percentage of positive loops was obtained amongst those situated proximally, i.e. nearest to the stomach end of the gastro-intestinal tract. Positive loops in this situation invariably showed a more severe

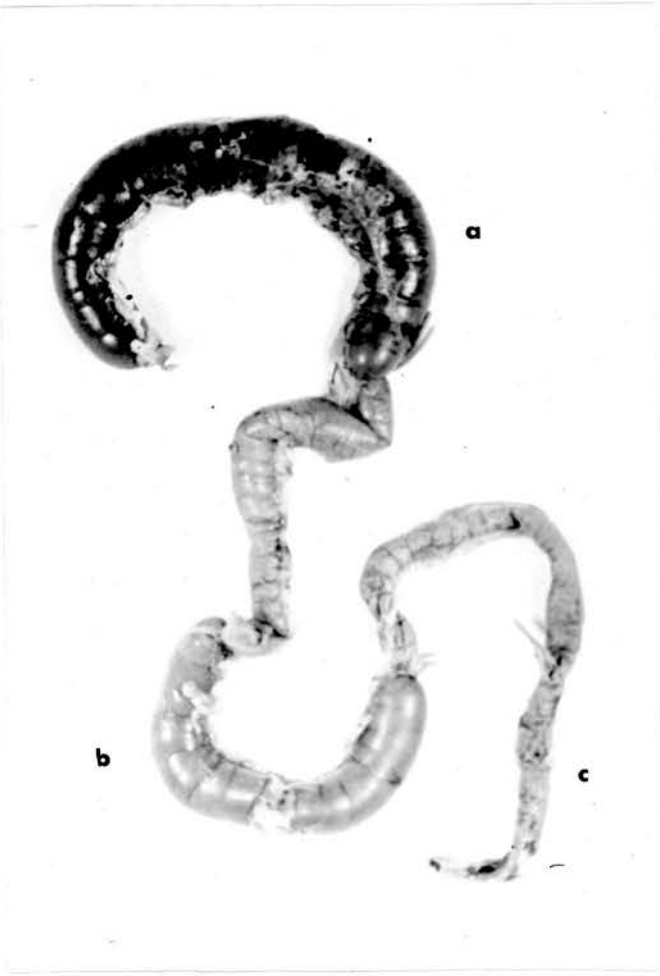

Fig. 2 Experimental salmonellosis II. A comparative study the fimbriate and the non-fimbriate bacteria injected into ligated loops of rabbit gut. Loop - (a) - situated in the proximal part of the small intestine and loop - (c) situated in the terminal part nearest to the caecum were infected with S. typhimurium 1566N (non-fimbriate/flagellate strain) while the loop (b) was infected with S. typhimurium 1566F (fimbriate/flagellate strain). The inoculum was 0.5 ml. of overnight broth culture. The test loops were separated by uninoculated loops. Of the inoculated loops, (a) and (b) showed positive dilatation of the gut and loop (c) was negative. Of the two positive loops, the one (a) situated nearest the stomach showed a greater dilatation, more marked congestion with haemorrhages in the gut wall, and the exudate was haemorrhagic.

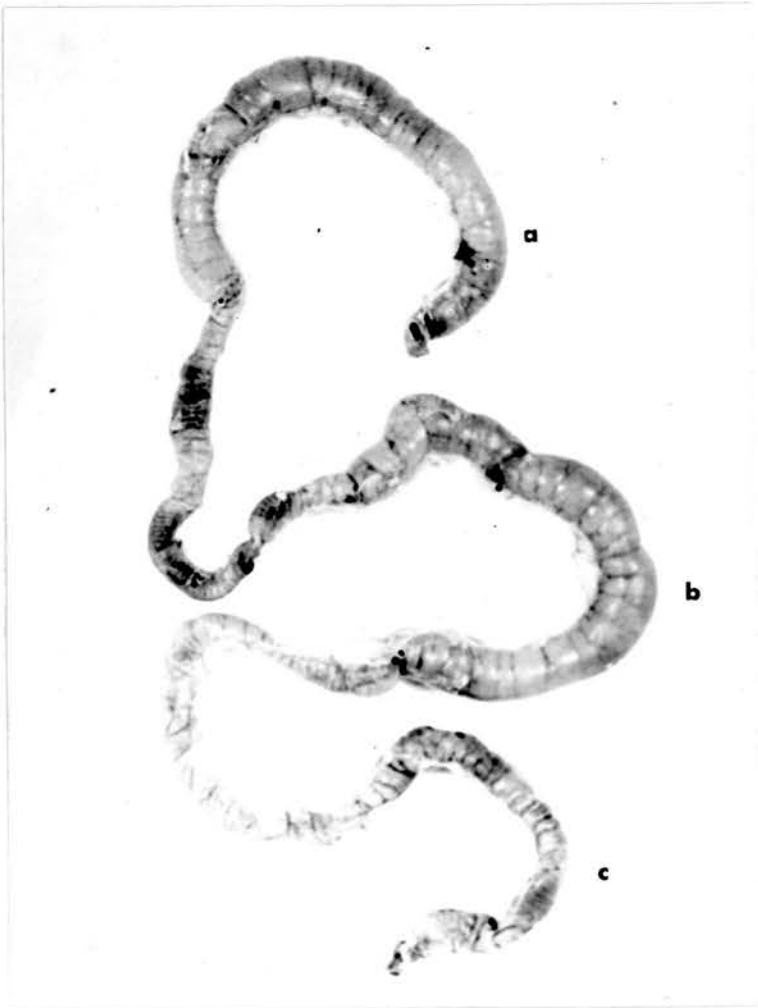

Fig. 3 Experimental salmonellosis II. Ligated loops of rabbit gut. The loops (a), (b) and (c) were inoculated with 0.5 ml. of overnight broth culture of S. typhimurium 1566F (fimbriate/flagellate strain). The test loops are separated by uninoculated loops. The proximal test loop (a) and the middle test loop (b) show positive dilatation, while the distal test loop (c) shows no dilatation of the gut.

TABLE V (Contd.)

Expt. No.	E. coli strains and their sero-types*	Source of strains	Flagella	Fimbriae	Non-fimbrial haemagglutinin	Results - No. of positive loops/No. tested			Total positive loops	
						proximal loop (nearest stomach)	middle loop	distal loop (nearest caecum)	No. tested	
27	C118 - 0119	recent infantile diarrhoea	-	-	-	0/1	0/1	0/1	0/3	0%
28	C15 - 0127 - B5	old stock strain	-	+	-		0/2		0/2	
29	C20 - 0127: B5	recent infantile diarrhoea	-	-	-	1/1		0/1	1/2	
30	C71 - 0127: B5	" mild infantile diarrhoea	+	-	-		0/2		0/2	
31	C73 - 0127: B5	" " " "	+	-	-	0/1	0/1	0/1	0/3	
32	C76 - 0127: B5	" " " "	-	-	-			0/2	0/2	1/25
33	C84 - 0127: B5	" " " "	+	-	-	0/1	0/1	0/1	0/3	4%
34	C108 - 0127: B5	" " " "	+	-	-	0/1	0/1	0/1	0/3	
35	C128 - 0127: B5	" moderate " "	+	-	-	0/1		0/1	0/2	
36	C73 - 0127: B5	" mild " "	+	-	-	0/1	0/1	0/1	0/3	
37	C84 - 0127: B5	" " " "	+	-	-	0/1	0/1	0/1	0/3	
	Total proportion of positive loops					(10/35) 28.5%	(4/31) 12.9%	(7/39) 17.9%	(21/105) 20%	
	Total proportion of positive loops in experiments with fimbriate strains					(4/12) 33.3%	(1/7) 14.3%	(3/14) 21.4%	(10/33) 30.3%	
	Total proportion of positive loops in experiments with non-fimbriate strains					(6/23) 26.0%	(3/24) 12.5%	(4/25) 16%	(13/72) 18.0%	
	Total proportion of positive loops in experiments with non-fimbriate/non-flagellate strains					(5/12) 41.6%	(2/10) 20%	(4/14) 28.6%	(11/36) 30.9%	
	Total proportion of positive loops in experiments with Fimbriate/flagellate strains					(2/6) 33.3%	(1/4) 25%	(1/7) 14.3%	(4/17) 23.5%	

C and B = strains obtained from Professor J.P. Duguid
E = strains obtained from Dr. J. Taylor

* This information was supplied by Professor J.P. Duguid

TABLE V

Experimental salmonellosis II. A COMPARATIVE STUDY OF THE EFFECTS OF INJECTION OF FIMBRIATE AND NON-FIMBRIATE ORGANISMS OF ENTEROPATHOGENIC SEROTYPES OF *E. COLI* INTO LIGATED LOOPS OF RABBIT GUT. 0.5 ml. OF BROTH CULTURE OF THE TEST STRAIN WAS INOCULATED INTO THE TEST LOOP. IN EXPTS. NOS. 10-15, 34 and 35 THE INOCULUM WAS A 48 HR. GROWTH, AT 37°C. IN THE REST OF THE EXPERIMENTS THE INOCULUM WAS AN 18 HR. GROWTH. THE TEST ANIMALS WERE KILLED 24 HR. AFTER THE OPERATION.

Expt. No.	*E. coli* strains and their sero-types*	Source of strains	Flagella	Fimbriae	Non-fimbrial haemagglutinin	Results - No. of positive loops/No. Tested			Total positive loops	
						proximal loop (nearest stomach)	middle loop	Distal loop (nearest caecum)	No. tested	
1	C6 - 026: K60	old stock F41 from F.Orskov	-	-	-	0/1	0/1	0/1	0/3	
2	C53 - 026: B6	Taylor's positive loop control	-	+	-	1/1		1/2	2/3	
3	C83 - 026	recent infantile diarrhoea	-	-	-	0/1	0/1	0/1	0/3	
4	C93 - 026	" " "	-	-	-	0/1	0/1	0/1	0/3	
5	C109 - 026: K60	" mild infantile diarrhoea	-	-	-	0/1	0/1	0/1	0/3	
6	C127 - 026: K60	" very severe infantile diarrhoea	-	-	-	1/1		1/1	2/2	13/40
7	C129 - 026: K60	" " " diarrhoea	-	-	-	0/1	0/1	0/1	0/3	32.5%
8	E126 - 026: B6	" infantile diarrhoea	-	+	-	1/1		1/1	2/2	
9	E431 - 026: B6	" " "	-	-	-	1/1	0/1	1/1	2/3	
10	C83 - 026	" " "	-	-	-	1/1	0/1	1/1	2/3	
11	C93- 026	" " "	-	-	-	0/1	1/1	0/1	1/3	
12	C95 - 026	" " "	+	-	-	1/1	1/1	0/1	2/3	
13	C68 - 026	" " "	+	+	+	0/1	0/1	0/1	0/3	
14	C96 - 026	" " "	+	+	-	0/1	0/1	0/1	0/3	
15	C5 - 025	recent infantile diarrhoea	-	-	+	1/1	1/1	1/1	3/3	100%
16	C148 - 055: K59	recent severe illness	?	+	-	0/1		0/1	0/2	
17	E317 - 055: B5	" infantile diarrhoea	+	-	-	0/1	0/4	0/1	0/6	0/13
18	E444 - 055: B5	" " "	-	+	-	0/2	0/1	0/2	0/5	0%
19	C54 - 0111: B4	old stock Taylor's negative loop control	+	+	-	0/1		0/2	0/3	
20	C121 - 0111: B4	recent infantile diarrhoea	+	+	-	1/1	1/1	1/1	3/3	
21	C147 - 0111: K58	" severe illness	?	+	-	0/1		0/1	0/2	
22	E121 - 0111: B4	" infantile diarrhoea	+	+	-	1/1	0/1		1/2	4/21
23	E171 - 0111: B4	" " "	+	+	-	0/1		0/2	0/3	19.5%
24	B40 - 0111: B4	old stock strain	+	-	+	0/1		0/1	0/2	
25	C142 - 0111	recent infantile diarrhoea		-	-	0/1	0/1	0/1	0/3	
26	C146 - 0111	" " "		-	+	0/1	0/1	0/1	0/3	

Contd.

reaction; they were more dilated, showed haemorrhages in the gut wall, and the exudate was often haemorrhagic (figs. 2, 4, 5 and 6).

Studies with strains of enteropathogenic serotypes of E. coli

A total of 105 loops in 39 rabbits were infected with thirty-seven E. coli strains in serotypes 026 (14), 025 (1), 055 (3), 0111 (8), 0119 (1) and 0127 (10). This series included strains that were fimbriate/flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate and non-fimbriate/non-flagellate. A total of 21 (20 per cent.) of the test loops showed positive gut dilatation reactions. The results were recorded as follows:-

Characteristics of strain of E. coli tested	No. giving positive loop test	Total number of loops tested	Percentage positive loops
Fimbriate/flagellate	4	17	23.5
Fimbriate/non-flagellate	4	12	33.3
Non-fimbriate/flagellate	2	31	6.4
Non-fimbriate/non-flagellate	11	36	30.9

Forty loops were injected with strains of E. coli serotype 026; of these, 13 (32.5 per cent.) produced positive gut dilatation. Four (19.5 per cent.) of 21 loops injected with E. coli serotype 0111 were positive and one (4 per cent.) of 25 loops injected with E. coli serotype 0127 were positive. Three loops injected with a single strain of

Table VI

Experimental Salmonellosis II. COMPARATIVE STUDIES OF THE EFFECT OF FIMBRIATION IN *S. TYPHIMURIUM* AND *E. COLI* STRAINS ON THE MINIMAL DOSE OF ORGANISMS REQUIRED TO PRODUCE A POSITIVE LOOP TEST #. LOOPS NEAREST THE STOMACH (1) AND CAECUM (6) RECEIVED UNDILUTED CULTURE (0.5ml.). THE INTERVENING LOOPS RECEIVED 0.5ml. DOSES OF GRADED DILUTIONS AS INDICATED. THE BACTERIAL INOCULUM WAS AN 18 HR. BROTH CULTURE OF THE ORIGINAL TEST STRAIN OR RABBIT-PASSAGED STRAIN. THE TEST ANIMALS WERE KILLED 24 HR. AFTER THE OPERATION.

Test strain*	Flagella	Fimbriae	Viable count of test culture (orgs. per ml.)	Diluent used to prepare bact. suspension	Reaction in loop (no.) inoculated with bacterial culture at dilution (1) undiluted	(2) 10^{-7}	(3) 10^{-5}	(4) 10^{-3}	(5) 10^{-1}	(6) undiluted	Minimal log dose giving a positive loop test
S. typhimurium strains											
6351NF/nf1	-	-	4.5×10^{8}	Ringer soln.	+	-	-	-	-	+	8.7
6352FT/nf1	-	+	4.8×10^{8}	1 percent Na_2HPO_4 soln. pH 8.8	+	-	-	-	-	+	8.7
6353NF/flt	+	-	4.6×10^{8}	Peptone water	+	-	-	-	+	+	7.8
6354FT/flt	+	+	4.8×10^{8}	"	+	-	-	+	+	+	8.7
629N	+	-	2.2×10^{9}	1 percent Na_2HPO_4 soln.	+	-	-	-	+	+	8.3
629NF	*	+	1.3×10^{8}	"	+	-	-	+	+	+	6.1
573NF/nf1	-	-	4.8×10^{8}	"	+	-	-	+	+	+	6.7
576NF/f1	+	-	5.6×10^{9}	"	+	-	-	-	-	+	8.7
576FT/flt	+	+	5.9×10^{9}	"	+	-	-	-	+	+	7.8
573NF/nf1/P	-	-	1.9×10^{9}	"	+	-	-	-	-	-	9.3
576FT/flt/P	+	+	2.3×10^{9}	"	+	-	-	-	+	+	8.4
1436F	+	+	1.2×10^{9}	"	+	-	-	-	+	+	8.1
1436NF	+	-	5×10^{9}	"	+	-	-	-	+	+	8.7
1436F/P	*	+	1.2×10^{9}	"	+	-	+	+	+	+	4.1
1436NF/P	+	-	5.2×10^{9}	"	+	-	-	+	+	+	6.7
1436F/PP	+	+	7.5×10^{9}	"	+	-	-	+	+	+	6.9
1436NF/PP	+	-	7.5×10^{9}	"	+	-	-	-	+	+	8.9
E. coli strains											
C6/026	-	-	1.3×10^{9}	"	-	-	-	-	-	-	Neg. at 9.1
C127/026	-	-	1.2×10^{9}	"	+	-	-	-	+	+	8.1
C143/055	-	+	2.8×10^{9}	"	-	-	-	-	-	-	Neg. at 9.4
C147/0111	-	+	2×10^{9}	"	-	-	-	-	-	-	" " 9.5
C128/0127	+	-	7.3×10^{9}	"	-	-	-	-	-	-	" " 9.9
C127/026/P	-	-	7.4×10^{9}	"	+	-	-	-	-	+	9.9

N & NF = non-fimbriate FT = phage-transduced fimbriate nf1 = non-flagellate
flt = phage-transduced flagellate MF = fimbriate by use of $MnCl_2$ as a mutagenic agent

\+ P = once passaged through rabbit gut; PP = twice passaged through rabbit gut.

E. coli serotype 025 were all positive. Finally, 13 loops injected with three strains of E. coli serotype 055 and 3 loops injected with a single strain of E. coli serotype 0119 were all negative. The detailed results are shown in table V. It is again evident that, as in the series with S. typhimurium strains, the highest percentage of positive loops occurred in the group of test loops situated nearest the stomach end of the gastro-intestinal tract.

Minimal dose of organisms of S. typhimurium and E. coli required to produce a positive gut dilatation and the effect of prior rabbit-gut-passage on the size of the dose required

Table VI shows that the S. typhimurium and E. coli test strains included non-fimbriate/non-flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate and fimbriate/flagellate organisms. Ten experiments were carried out with un-passaged S. typhimurium strains and five experiments with un-passaged E. coli strains. The minimal log dose producing positive gut dilatation ranged from 5 to 9 with the un-passaged S. typhimurium strains and it was about 9 with the un-passaged E. coli strains. After passage, the minimal log dose for a positive loop reaction was, with S. typhimurium, from 4 to 9; and with E. coli, 9.9. Figures 5 and 6 illustrate some of the typical results obtained in these experiments. However, it is apparent from table VI that the minimal log dose producing a positive gut loop was less for the fimbriate/flagellate S. typhimurium than the parent non-fimbriate/

TABLE VII

Experimental Salmonellosis II.

COMPARATIVE STUDIES OF THE EFFECT OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF *S. TYPHIMURIUM* and *E. COLI* INJECTED INTO LIGATED RABBIT GUT. VIABLE BACTERIAL COUNTS ON EXUDATES FROM POSITIVE LOOPS WERE DONE BY MILES AND MISRA METHOD. THE INITIAL INOCULUM OF 0.5 ml. OF THE TEST STRAIN CONTAINED APPROXIMATELY 10^8 ORGANISMS. THE TEST ANIMALS WERE KILLED 24 hrs. AFTER THE OPERATION.

Test strain	Flagella	Fimbriae	Position of the test loop	Volume of exudate recovered from loop (ml.)	Viable count (orgs. per ml.) of exudate	Total organisms in exudate recovered from positive loops
S. typhimurium strains						
1566F	+	+	Proximal	15	7.4×10^9	1.1×10^{11}
1566N	+	-	Middle	20	4.5×10^9	9×10^{10}
629NF	+	+	Proximal	15	5.4×10^8	8.1×10^9
629N	+	-	Middle	10	7.4×10^8	7.4×10^9
6351N/nf1	-	-	Proximal	10	1.8×10^8	1.8×10^9
6352FT/nf1	-	+	Middle	10	4.3×10^8	4.3×10^9
6353N/f1t	+	-	Proximal	11	7.1×10^8	7.8×10^9
"	+	-	Distal	10	6.7×10^8	6.7×10^9
6454FT/f1t	+	+	Proximal	12	7.8×10^8	9.3×10^9
"	+	+	Distal	10	7.2×10^8	7.2×10^9
E. coli strains						
C20/0127	-	+	Proximal	10	3.7×10^9	3.7×10^{10}
C53/026	-	-	Proximal	20	5×10^9	1×10^{11}
C83/026	-	-	Proximal	15	5.8×10^9	8.7×10^{10}
C95/026	-	-	Proximal	18	6.7×10^9	1.2×10^{11}

N = non-fimbriate F = fimbriate nf1 = non-flagellate
FT = phage-transduced fimbriate f1 = flagellate f1t = phage-transduced flagellate

flagellate strains. The log dose differences were as follows:-

Strains not passaged in rabbit gut

6354 FT/flt	less than	6352	NF/fl	by log	2	(100 fold)
629 MF/fl	" "	629	N/fl	" "	3	(1000 fold)
SW576 FT/flt	" "	SW576	NF/flt	" "	0.9	(less than 10 fold)
1436 MF/fl	" "	1436	NF/flt	" "	2	(100 fold)
SW573 NF/nfl	" "	SW576	FT/flt	" "	1.1	(more than 10 fold)

Strains passaged once in rabbit gut

1436 MF/fl	less than	1436	NF/fl	by log	2	(100 fold)
SW576 FT/fl	" "	SW573	Nf/nfl	" "	0.9	(less than 10 fold)

Strains passaged twice in rabbit gut

1436 MF/fl less than 1436 NF/fl by log 3 (1000 fold)

With E. coli strains the presence or the absence of fimbriae and flagella and their prior passage through the rabbit gut made no apparent difference to the minimal dose of the organisms required to produce a positive gut dilatation test.

Bacterial counts on exudates from positive loops

The exudates showed a 10 to 100 fold increase in the organisms inoculated into the loops. The details are shown in table VII.

Experimental Salmonellosis III

Intraperitoneal and oral infection of albino rats with S. typhimurium.

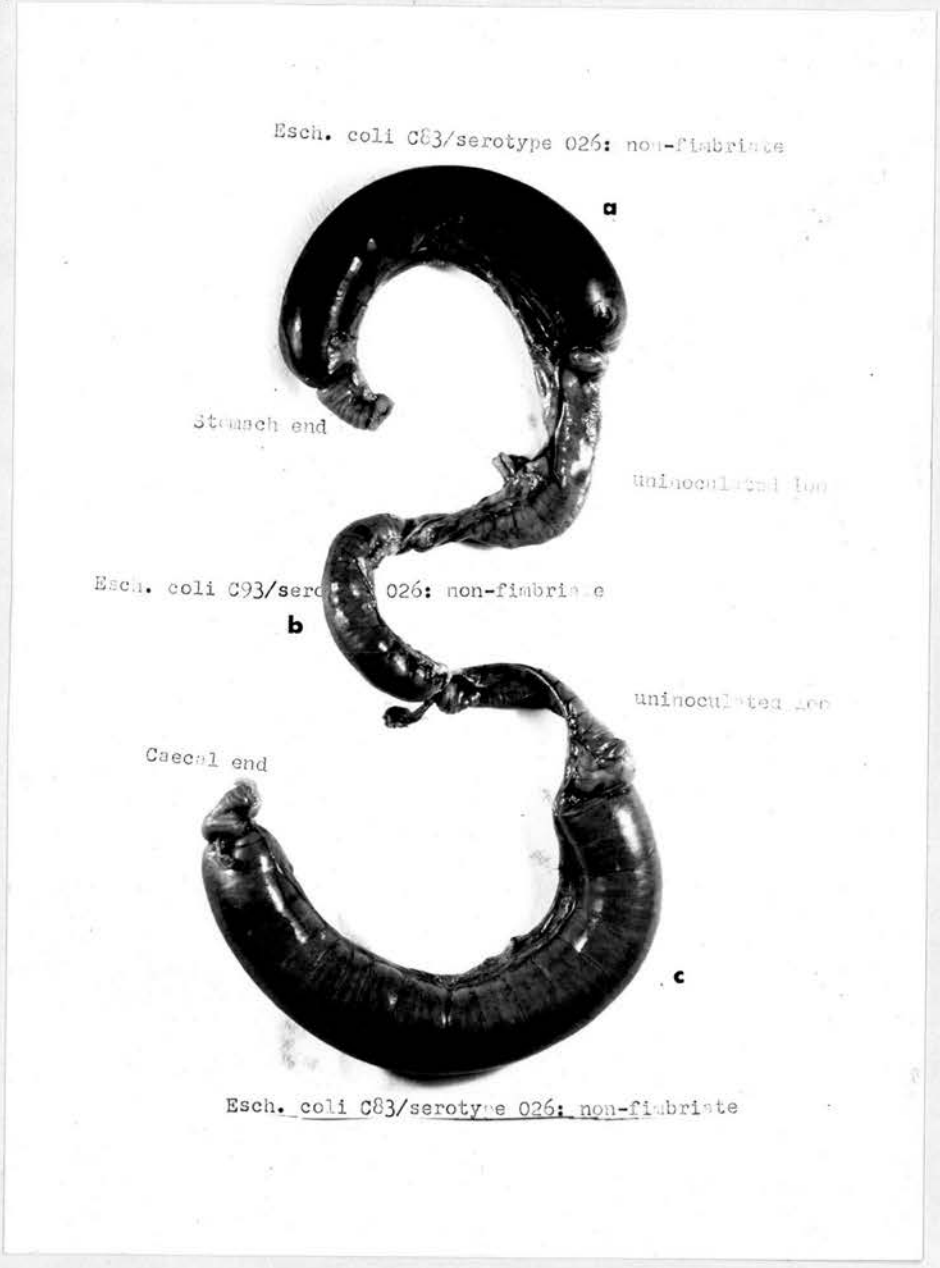

Fig. 4 Experimental salmonellosis II. Ligated loops of rabbit gut. The test loops (a), (b) and (c) which are separated by uninoculated loops were infected with 0.5 ml. of overnight broth culture of non-fimbriate/non-flagellate E. coli serotype 026 strains. The test loops (a) and (c) show marked dilatation, while test loop (b) shows a mild dilatation only.

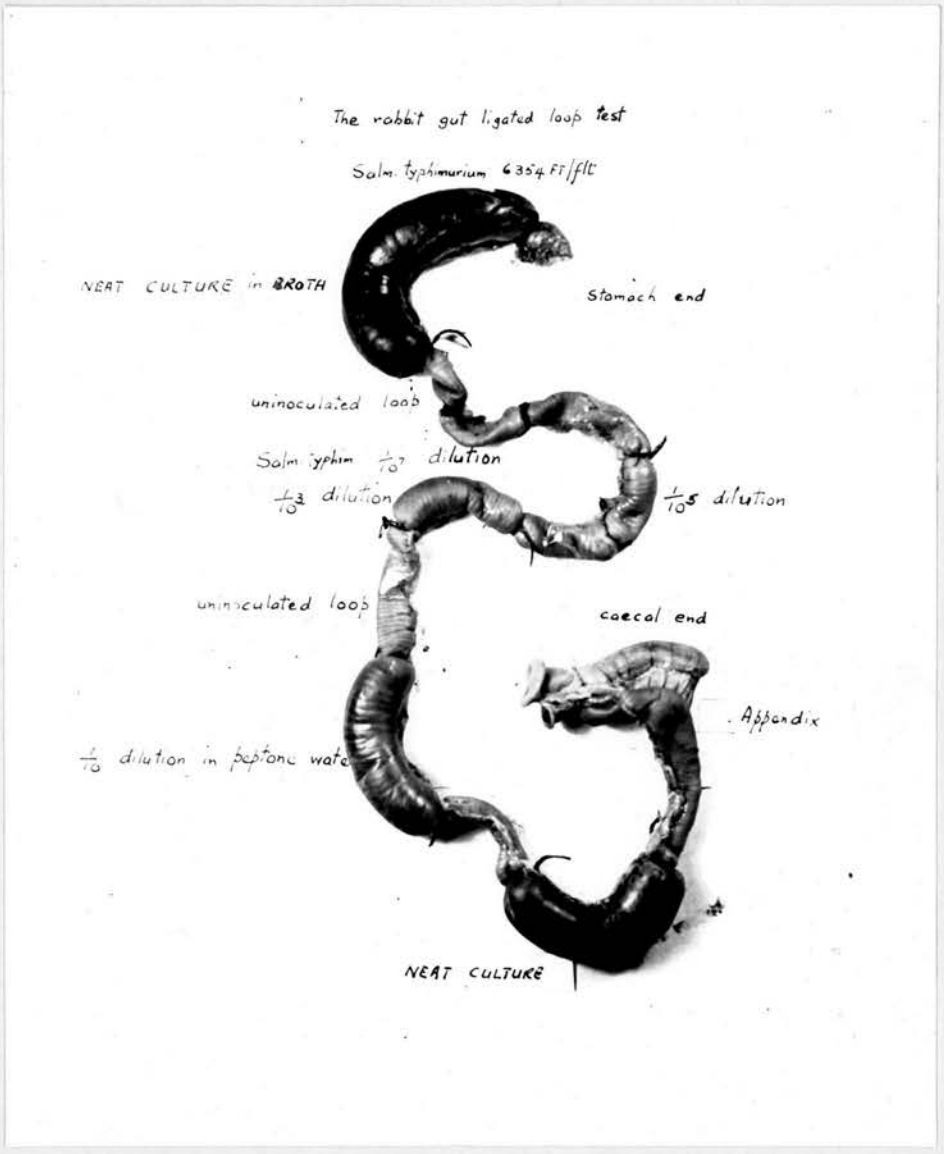

Fig. 5 Experimental salmonellosis II. Ligated rabbit gut loops. Quantitative loop test. Ten-fold dilutions of an overnight broth culture of Salm. typhimurium 6354FT/flt were prepared in 1 per cent. Na_2HPO_4 solution and the following dilutions as shown in the picture were injected into the loops: 10^0 (undiluted culture), 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}.

Loops inoculated with 10^0, 10^{-1} and 10^{-3} dilutions of the test strain resulted in positive dilatation of the gut. The highest dilution of the inoculum to give a positive gut test contained 10^5 viable organisms (dilution $1/10^3$).

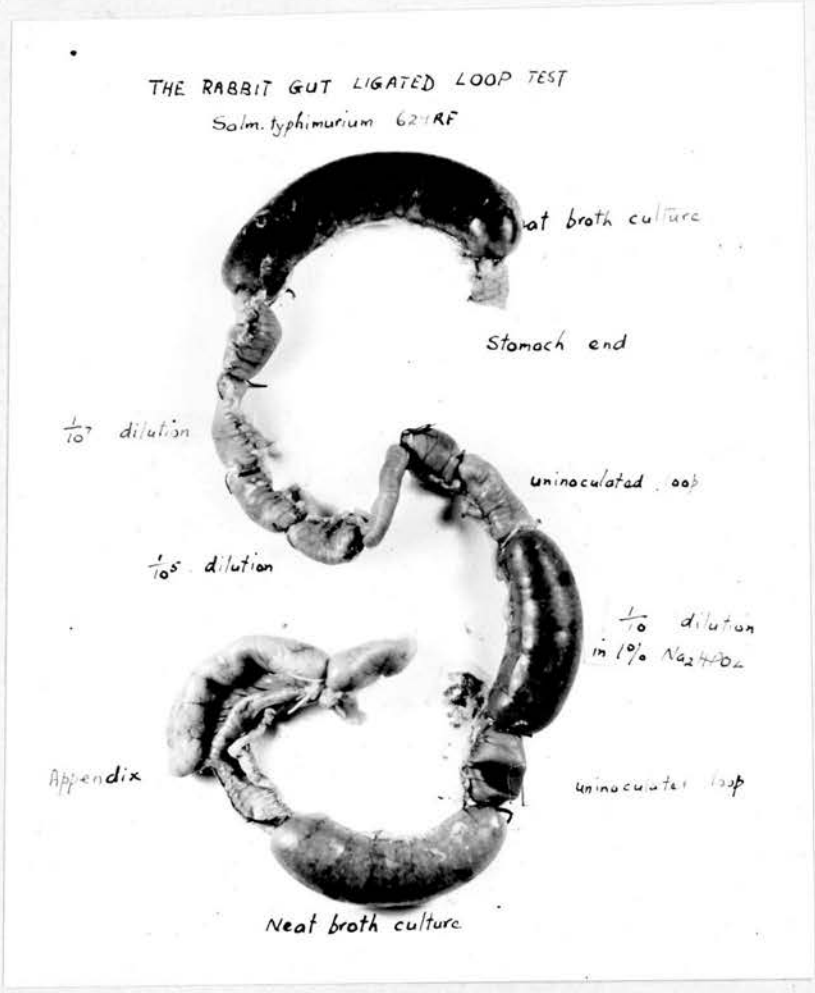

Fig. 6 Experimental salmonellosis II. Ligated gut loops. Quantitative loop test. Similar results as with Salm. typhimurium 6354FT/f1t were obtained with Salm. typhimurium 629MF/f1.

Intraperitoneal infection of albino rats

Two series of five groups of six rats each (equal numbers of male and female animals) were given intraperitoneal inoculations of bacterial suspensions (in 1 per cent. Na_2HPO_4 at pH 8.8) of the parent non-fimbriate and the phage-transduced fimbriate S. typhimurium strain 1289. The log dose of organisms of each strain administered to the two groups of rats ranged from log 3.2 to log 7.2 at log 1 intervals.

Between the fourth to the seventh day a number of animals showed signs of illness (loss of the usual activity, ruffled hair, anorexia, a few had conjunctivitis and some showed signs of diarrhoea) but the illness was soon followed by recovery in most of the animals. There were only four deaths. Of the four deaths, one was caused by infection with the non-fimbriate S. typhimurium strain and the other three were caused by infection with the fimbriate strain. The deaths occurred on the 10th, 12th and 13th days of infection with the fimbriate strain and on the 16th day of infection with the non-fimbriate strain. All of the deaths occurred in the groups of animals receiving the largest dose of organisms.

At the end of 30 days of observation only one of the survivors from series initially infected with the non-fimbriate strain was found to be infected, while five of the survivors from the series infected with the fimbriate strain were found to be infected. Details of mortality

and infectivity are shown in appendix 23. Both the LD50 and ID50 of the non-fimbriate S. typhimurium were greater than log 7.2 organisms and of the fimbriate strain they were log 8.2 and log 7.5, respectively.

Oral infection of albino rats

Ten rats were infected orally with a phage-transduced fimbriate strain of S. typhimurium 1289 and 6 rats were infected orally with the non-fimbriate parent strain. The dose administered to each animal in the groups was of the order of log 9. Only one animal in each of the groups died during the period of observation which varied from 11 to 22 days (table VIII).

Infection of internal organs of albino rats infected orally with fimbriate or non-fimbriate S. typhimurium strains

One infected animal from each of the groups (one infected with the fimbriate strain and the other with the non-fimbriate strain) referred to above was sacrificed from the eleventh day of infection onwards. On post-mortem examination the following observations were made:-

1) The mesenteric and ileo-caecal lymph glands generally, and less often the spleen, were enlarged.

2) Ulcers were seen in the duodenum and the small intestine of most of the animals; in a few, even the colon was ulcerated. The ulcers were easily visible when viewed from the external surface of the intestine.

3) No abscesses were seen in the liver or spleen of any of the animals sacrificed.

TABLE VIII

Experimental salmonellosis III

RESULTS OF CULTURES OF HEAT BLOOD AND INTERNAL ORGANS OF ALBINO RATS INFECTED ORALLY (LOG DOSE = 9 ORGANISMS) WITH PHAGE-TRANSDUCED FIMBRIATE AND NON-FIMBRIATE PARENT S. TYPHIMURIUM 1289 AND SACRIFICED BETWEEN THE 11th AND 22nd DAYS OF THE EXPERIMENT.

Test Strain	Serial No. expt. animal	Duration of infection when animal was sacrificed	Results of culture from					
			Heart Blood	Liver	Spleen	Mes. Lymph Glands	Intest. Tissue and Ulcer	Intest. Cont.
S. typhimurium 1289FT/f1 phage-transduced fimbriate	R 1	11 days	-	+	+	+	+	-
	R 2	14 days	-	-	-	+	+	-
	R 3	14 days	-	+	+	+	+	-
	R 4	15 days	-	-	+	+	-	-
	R 5	16 days	-	-	-	+	+	+
	R 6	17 days	-	-	+	+	-	-
	R 7	18 days	-	-	-	+	-	-
	R 8	20 days	-	-	-	+	+	+
	R 9	21 days	-	-	-	+	+	-
	R 10	22 days	-	-	-	+	-	-
S. typhimurium 1289N/f1 parent non-fimbriate strain	R 11	12 days	-	-	+	+	+	-
	R 12	14 days	-	-	+	+	-	-
	R 13	15 days	-	-	-	+	-	-
	R 14	16 days	-	-	+	+	+	-
	R 15	17 days	-	-	+	+	-	-
	R 16	18 days	-	-	-	+	-	-
Percentage positive cultures from animals infected with fimbriate strain			0	20	40	100	60	20
Percentage positive cultures from animals infected with non-fimbriate strain			0	0	66	100	33	0
Percentage positive cultures from animals infected with both strains			0	12.5	50	100	50	12.5

N = non-fimbriate strain FT = phage-transduced fimbriate strain
+ = the inoculated strain of S. typhimurium was recovered
- = the inoculated strain of S. typhimurium was not recovered
f1 = flagellate

The liver, spleen, mesenteric lymph glands, the ulcerated intestinal tissue, intestinal contents, and heart blood from each of the sacrificed animals was cultured for S. typhimurium. The details of the results of culture are shown in table VIII.

Specimens of heart blood from all of the animals in the experiment were sterile on culture.

Liver specimens from only two animals were positive for S. typhimurium. Both these animals had been infected with the fimbriate strain and they had been sacrificed on the 11th and 14th days of infection.

Spleens from four animals in each group were positive on culture for the relevant infecting strain of S. typhimurium.

The mesenteric glands from all animals in both groups were positive on culture for the relevant infecting strain of S. typhimurium.

Eight specimens of intestinal tissue with ulcers were positive for S. typhimurium on culture. Six (6/10 = 60 per cent.) of these were from animals infected with the fimbriate S. typhimurium and two (2/6 = 33 per cent.) were from animals infected with the non-fimbriate strain.

S. typhimurium was isolated from the intestinal contents of two animals only. Both these animals had been infected with the fimbriate strain and the animals had been sacrificed on the 16th and 20th day of infection.

Excretion of S. typhimurium in the faeces of orally

TABLE IX.

Experimental salmonellosis III.

RESULTS OF CULTURE OF FAECAL SPECIMENS FROM ADULT RATS BEFORE AND AFTER ORAL INFECTION (WITH APPROXIMATELY LOG 9 ORGANISMS) WITH A PHAGE-TRANSDUCED FIMBRIATE OR NON-FIMBRIATE STRAIN OF S. TYPHIMURIUM 1289.

Test Strain	Serial No. expt. animal	Results of culture of faecal specimens collected on day*																					
		-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
S. typhimurium 1289FT/fl phage-transduced fimbriate strain	R 1	-	+	+	+	-	-	+	-	-	-	-	-K											
	" 2	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-K								
	" 3	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-K								
	" 4	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-K							
	" 5	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-K						
	" 6	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-K					
	" 7	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-K				
	" 8	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-K		
	" 9	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-K	
	" 10	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-			-K
S. typhimurium 1289N/fl non-fimbriate parent strain	" 11	-	+	-	-	+	-	-	-	-	-	-	-K											
	" 12	-	+	-	+	-	+	-	+	-	-	+	-	-	-	+K								
	" 13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-K							
	" 14	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+K						
	" 15	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+K					
	" 16	-	+	-	-	+	-	+	-	-	-	+	-	-	+	+	-	-	-	+K				
Percentage positive cultures from all animals infected with fimbriate strains		0	80	40	30	30	30	20	50	20	0	0	0	11.1	0	33.3	0	16.6	20	25	0	0	0	0
" " non-fimbriate		0	50	16.6	16.6	50	16.6	16.6	16.6	16.6	0	33.3	0	0	40	60	0	33.3	50	100	0	0	0	0
" " either strain		0	68.7	31.25	25	37.5	25	18.75	37.5	18.75	0	12.5	0	7.1	14.3	42.8	0	22.2	28.6	40				

* Animals were infected on Day 0 N = non-fimbriate FT = phage-transduced fimbriate fl = flagellate

K = killed; + = strain of S. typhimurium administered orally was recovered

- = strain of S. typhimurium administered orally was not recovered

infected albino rats

All faecal specimens collected prior (table IX - results of culture of faecal specimens collected on -1 day) to the oral infection of the albino rats were negative for S. typhimurium and other salmonellae.

The details of daily examination of faecal specimens from each of the animals for the inoculated strain of S. typhimurium are shown in table IX.

Culture of the faeces of one of the animals (table IX no. R 13) infected with the non-fimbriate S. typhimurium strain proved consistently negative. However, this animal was killed on the 15th day of the experiment and a culture of the mesenteric lymph glands yielded the inoculated non-fimbriate S. typhimurium strain (table VIII no. R 13). Faecal specimens collected from all of the other animals infected with the fimbriate or non-fimbriate S. typhimurium strains during the period of observation (ranging from 1 to 18 days following infection) were positive on at least one occasion and in general were intermittently and irregularly positive on culture for the relevant infecting S. typhimurium strain.

Experimental Salmonellosis IV

Studies in albino rats to determine:-

(a) the sites of penetration of the gastro-intestinal mucosa by S. typhimurium;

(b) the mode of passage of S. typhimurium (fimbriate and non-fimbriate strains) through the gastro-intestinal tract;

(c) the mode of penetration of the mucosa by organisms gaining access to the blood stream and organs of the animals.

Culture of intestinal contents of segments prior to introduction of bacterial inoculum

The small intestine of the rats contained no solid faeces except in the terminal part. Of a total of twelve specimens taken from various parts of the small intestine of different rats and cultured aerobically, no growth was obtained from nine specimens. The details are shown in table X.

TABLE X

Results of culture of 12 samples of intestinal contents taken at various levels of the small intestine from rats prior to introduction of bacterial inoculum

Region of small intestine from which the contents were sampled	No. of samples yielding	
	No growth on culture	Positive culture
Duodenum	5	1 (Proteus)
Proximal ileum (jejunum)	2	0
Mid-ileum	1	1 (Proteus)
Terminal-ileum	1	1 (Coliform)

Localisation of sites of penetration of the gastro-intestinal mucosa by the injection of the test organisms into segments of the gastro-intestinal tract

The appearance of the inoculated bacteria in the blood

TABLE XI

Experimental salmonellosis IV. EXPERIMENTS TO DETERMINE SITES OF PENETRATION OF MUCOSA OF GASTRO-INTESTINAL TRACT OF ALBINO RATS BY FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM STRAINS. THE BACTERIAL INOCULUM INJECTED INTO THE TEST GASTRO-INTESTINAL SEGMENT (FIG. 1) CONTAINED APPROXIMATELY 10^8 ORGANISMS OF THE TEST STRAIN.

Gastro-intestinal segment inoculated	Expt. No.	S.typhi-murium strain	Result of culture of								Histological changes in intestinal tissue at end of expt.	*Evidence of penetration of mucosa
			Gastro-intestinal segment content		Heart blood sampled at			Liver	Spleen	Saline Washing of peritoneal cavity		
			Prior to inoculum	At end of experiment	5 min.	15 min.	30 min.					
Oesophagus	1	1289 FT		+	NG	NG		NG	NG			NO
	2	"		+	NG	NG	NG	NG				
	3	1289 FT		+	-	-	NG	NG	NG	NG		
	4	1289 FT		+	(Heart blood samples collected at 30, 60, 90 & 105 mins. gave negative cultures, but a specimen collected after 120 mins. was positive)			+	+			YES
	5	1289 N		+	-	-	NG	NG	NG	NG		NO
Stomach	6	1289 FT		+	-	-	+	+	+	NG	NAD	YES
	7	1289 FT		+	+	+	+	+	+	NG		
	8	1289 N		Coliform organisms	-	-	NG	+	+	NG		
Duodenum	9	1289 FT	NG	+	NG			+	+			YES
	10	1289 FT	Proteus orgs.	+		+		+	+			
	11	1289 FT	NG	+			+	+	+			
	12	1289 FT	NG	+			+	+	+		NAD	
	13	1289 FT		+	+							
	14	1289 FT		+	NG	+						
	15	1289 FT	NG	+	+	+	+	+	+			
	16	1289 N	NG	+	+	+	+	+	+	NG		
Proximal part of small intestine	17	1289 FT	NG	+	-	-	NG	+	+		NAD	YES
	18	1289 FT	NG	+	+	-	NG	+	+			

Contd.

TABLE XI (Contd.)

Gastro-intestinal segment inoculated	Expt. No.	S.typhi-murium strain	Result of culture of							Histological changes in intestinal tissue at end of experiment	* Evidence of penetration of mucosa
			Gastro-intestinal segment content		Heart blood sampled at			Liver	Spleen	Saline Washing of peritoneal cavity	
			Prior to inoculum	At end of experiment	5 min.	15 min.	30 min.				
Mid part of small intestine	19	1289 FT	Proteus	+	-	-	NG	+	NG		NAD	YES
	20	1289 N	NG	+	-	-	+	+	+	NG		
Terminal part of small intestine	21	1289 FT	NG	+	-	+	+	+	+		NAD	YES
	22	1289 N	Coliform group of orgs.	+			+	+	+	NG		
Large intestine	23	1289 FT		+	-	NG	NG	NG	NG	NG	NAD	NO
	24	1289 FT			NG	NG	NG	+	+			YES
	25	1289 FT			NG	NG	NG	NG	NG			NO
	26	1289 FT			(Heart blood samples collected at 60, 90 and 105 mins. gave positive cultures.)			+	+	NG		YES
	27	1289 N	Coliform group of orgs.	+			NG (samples taken at 60 & 75 mins.)	+	NG			YES

The weight of rats employed in this study ranged from 190 to 370 g. FT = fimbriae phage-transduced
N = parent non-fimbriate strain. + = inoculated S. typhimurium strains were recovered
- = inoculated S. typhimurium strains were not recovered
NG = No growth. NAD = nothing abnormal seen. * Positive cultures for the inoculated test strain from the heart blood, or liver or spleen of the test animal.

stream and the internal organs (liver, spleen and mesenteric glands) was accepted as evidence of the penetration of the gastro-intestinal mucosa. The details of the results obtained are shown in table XI.

(a) Oesophageal segment. Five observations were made - 4 with fimbriate S. typhimurium and one with the non-fimbriate strain. Four of the observations were over a period of 30 minutes, and in some of these experiments the inoculated bacteria were recovered from the heart blood, liver or spleen. In the fifth experiment, the observations extended over a period of 2 hours and the inoculated bacteria were recovered from the heart blood, liver and spleen.

(b) Stomach. Three experiments were carried out - two with the fimbriate strain and one with the non-fimbriate. In one experiment the fimbriate strain was recovered from the heart blood in 5 minutes after injection into the segment and in the other after 30 minutes. The non-fimbriate strain was not recovered from the heart blood at the end of 30 minutes, but the organism was recovered from the liver and spleen.

(c) Duodenum. In all the 8 experiments, 7 with the fimbriate strain and one with the non-fimbriate strain, the organism was recovered from the heart blood (the time of recovery varied from 5 to 30 minutes), liver and spleen.

(d) The proximal ileum or jejunum. Two experiments were carried out, one with the fimbriate and one with the

non-fimbriate S. typhimurium strain. In the experiment with the fimbriate strain, cultures from heart blood, sampled up to 30 min. following inoculation were negative but cultures from liver and spleen were positive. In the experiment with the non-fimbriate strain, not only the liver and spleen yielded positive cultures but the organism was recovered from the heart blood after 5 minutes; cultures of blood samples taken at 15 minutes and 30 minutes were negative.

(e) Mid-ileum. Two experiments, one with the fimbriate and one with the non-fimbriate strain, were done. In the experiment with the fimbriate strain the heart blood and spleen were negative at the end of 30 minutes, but the liver was positive; while in the experiment with the non-fimbriate strain the heart blood, liver and spleen were all positive at the end of 30 minutes.

(f) Terminal ileum. Again two experiments were done; one each with the fimbriate and non-fimbriate strains. The heart blood was positive after 15 minutes with the fimbriate strain and after 30 minutes with the non-fimbriate strain; the liver and spleen yielded positive cultures in both the experiments.

(g) The large intestine. Five experiments were done - four with the fimbriate strain and one with the non-fimbriate strain. In two experiments with the fimbriate strain, cultures of samples of the heart blood, liver and spleen taken at 30 min. were all negative. In the third

TABLE XII

Experimental Salmonellosis IV.

THE OCCURRENCE OF FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM FED ORALLY TO ADULT RATS DOWN THEIR GASTRO-INTESTINAL TRACT AND PROBABLE SITE OR SITES OF PENETRATION OF THE GASTRO-INTESTINAL EPITHELIUM BY THESE ORGANISMS.

Expt. Serial No.	Nature of Strain 1289	Period of Experimentation	RESULT OF CULTURE OF SAMPLE OF														
			Contents of							Heart Blood at (min.)					Liver	Spleen	Mesenteric lymph glands
			Oesophagus	Stomach	Duodenum	Jejunum	P.ileum	T.ileum	Caecum	5	15	30	60	75			
1	FT)		+	+	+	+	NG	NG	Neg	NG	+				+	+	+
2	FT)	15 min.	+	+	NG	NG	NG	Neg	Neg	NG	NG				+	+	+
3	N)		+	NG	NG	NG	NG	Neg	Neg	NG	NG				NG	NG	
4	FT)		+	+	+	+	+	Neg	Neg	NG	NG	NG			+	NG	
5	FT)	30 min.	NG	+	+	+	+	NG	Neg	NG	NG	NG			+	+	+
6	N)		+	+	+	+	NG	+	+	NG	NG	NG			+	NG	+
7	N	40 min.	+	+	NG	+	NG	Neg	Neg	+	NG	+	NG		+	+	+
8	FT)	60 min.	+	+	NG	NG	NG	Neg	Neg	NG	+	+	+		+	+	NG
9	N)		+	+	+	+	NG	Neg	Neg	NG	NG	NG	+		+	+	+
10	FT	75 min.	+	+	+	+	NG	NG	Neg	NG	+	+	+	+	+	+	+
Total positive cultures of heart blood, liver, spleen & mesentric lymph glands										1/10	3/10	3/7	3/3	1/1	9/10	7/10	6/7
Percentage positive cultures of heart blood, liver, spleen and mesentric lymph glands										10	30	42.8	100	100	90	70	85.6

The weight of rats employed in this study ranged from 185 to 240 g. N = non-fimbriate NG = no growth
FT = phage-transduced fimbriate S. typhimurium 1289 Neg = growth obtained but inoculated S. typhimurium was not isolated

experiment, culture of the heart blood sampled at 30 min. was negative, but the liver and spleen cultures were positive. In the fourth experiment with the fimbriate strain, the heart blood, liver and spleen sampled at the end of one hour all yielded positive cultures. In the experiment with the non-fimbriate strain, the heart blood and spleen sampled 75 min. after inoculation gave negative cultures, but the liver culture was positive.

Investigation of the passage through the gastro-intestinal tract of S. typhimurium (fimbriate and non-fimbriate strains) fed orally to albino rats and probable sites of penetration of the intestinal mucosa of the tract by these organisms

Ten experiments (six with the fimbriate and four with the non-fimbriate strains) were carried out and the times of observation varied from 15 to 75 minutes. The details are shown in table XII.

(a) Within 15 minutes of ingestion, organisms from the bacterial inoculum had passed down the gastro-intestinal tract to the proximal part of the small intestine (or jejunum) of one animal, to the stomach of the second, but only to the cardiac end of the oesophagus of a third animal. No growth was obtained from cultures of intestinal contents of the mid-ileum of all three animals, the jejunum and the duodenum of two animals and from the stomach of one animal (non-fimbriate strain).

Within 30-40 minutes of ingestion, the bacteria were

recovered from as far down the intestinal tract as the mid-ileum in two animals, the caecum in one (fed non-fimbriate strain) and the jejunum of the fourth (also fed non-fimbriate strain). No growth was obtained from the mid-ileum of two animals, the duodenum of one animal and the oesophagus and the terminal-ileum of the other.

Within 60-70 minutes following ingestion the bacteria had passed to the jejunum of two animals (one fed with the non-fimbriate strain) and only as far down as the stomach of one animal. No growth was obtained from the terminal ileum of one animal, mid-ileum of all three animals and the duodenum and the jejunum of one animal.

(b) Evidence of penetration of intestinal wall. In all 5 cases in which the inoculated strain was recovered from the heart blood, cultures from liver, spleen and (with one exception) the mesenteric glands were also positive. Ingestion of the parent non-fimbriate strain of S. typhimurium by 4 rats produced a positive blood culture in 2 (within 5 min. and 60 min. of ingestion), of 6 rats fed fimbriate S. typhimurium 3 gave positive blood cultures (after 15 min. in each case). In one animal the heart blood culture taken at 5 minutes was positive, but it was negative at 15 minutes, positive again at 30 minutes and negative at 40 minutes. These results are not readily attributable to experimental variation because the blood samples were obtained uniformly and a standard culture procedure was followed in each case. It seems that bacterial penetration of the intestinal wall probably occurs intermittently and

that the organisms are cleared from the blood rather rapidly. Another interesting observation was that in two experiments (table XII, no. 4 and 6) over a period of 30 minutes, the heart blood and spleen cultures were negative but the inoculated strain of bacteria was recovered from the liver.

Experimental Salmonellosis V

Studies of fimbriae and their effects on human polymorphonuclear leucocytes.

According to Page and Good (1958) one of the most striking features of the inflammatory process is the regular sequence of morphologic events that characterise inflammation. Regardless of the nature of the irritant used to elicit inflammation, the initial event is the activation of histogenous wandering cells which appear in abundance during the first hour following introduction of the irritant. Subsequently, polymorphonuclear leucocytes invade the area from the peripheral blood and become predominant in the early hours of the inflammatory process. Fimbriate bacteria can adhere to leucocytes and it was therefore decided to study the effects of fimbriae on the viability of human polymorphonuclear leucocytes. In order that heat-inactivated organisms could be used in these studies to define the effect of fimbriae on the viability of leucocytes, it was first necessary to demonstrate the relative thermostability of the fimbrial adhesive property.

Effect of heat on fimbriae

TABLE XIII

Experimental Salmonellosis V

THE EFFECT OF HEAT ON THE HAEMAGGLUTINATING PROPERTY OF FIMBRIAE OF S. TYPHIMURIUM AND S. FLEXNERI.

Strain	Temp o C.	Period of Exposure in Hours	Haem.agglut. of 3 per cent guinea-pig red cells		Remarks
			with heated bacterial suspension	with formalised bacterial suspension	
S. flexneri Fla 1.	56	½	+++	+++	
"	60	1½	+++	+++	
S. typhimurium 1289 FT.	56	½	+++	+++	
"	56	3/4	+++	+++	
"	56	1	+++	+++	
"	60	½	+++	+++	
"	60	1	+++	+++	
"	60	1½	+++	+++	
S. flexneri Fla 2	60	1½	Neg	Neg	Non-fimbriate control strains
S. typhimurium 1289 N	60	1½	Neg	Neg	

FT = phage-transduced fimbriate strain

N = non-fimbriate strain

The exposure of thrice washed overnight broth cultures of fimbriate strains of S. typhimurium 1289 and S. flexneri Fla 1 to a temperature of 60°C. in a water-bath for up to 1½ hr. did not affect the haemagglutinating property of the fimbriae. The details are shown in table XIII.

Preparation and determination of viability of leucocytes (see Methods)

The method of McLeod and McLeod (1961) yielded very satisfactory preparations of leucocytes from fresh humen blood. The leucocyte sediment obtained was very easily dispersed and gave a smooth suspension in which a large proportion of the cells were discrete. This permitted rapid and accurate counts of the proportion of cells showing active oscillation of the granules and resistance to colouring by methylene blue.

Adhesion of fimbriate cells to leucocytes

When wet film preparations of mixtures of organisms and leucocytes were observed with the light microscope, the fimbriate S. typhimurium strains LT2, 1287 and 1289, and S. flexneri Fla 1 were seen to adhere to the leucocytes. Studies with phase-contrast microscopy and electron microscopy confirmed these observations (see electron-micrographs: figs. 17 and 18). The non-fimbriate S. typhimurium strains 7471, 1287 and 1289 and S. flexneri Fla 2 did not adhere to the leucocytes.

The effect of fimbriae on human leucocytes in vitro

The results of experiments (Materials and Methods, page

TABLE XIV

Experimental salmonellosis V. LETHAL AFFECT OF FIMBRIAE ON HUMAN LEUCOCYTES IN IN VITRO EXPERIMENTS (OBSERVATIONS UP TO ABOUT 2 HOURS AT ROOM TEMPERATURE). RESULTS SUMMARISED FROM APPENDICES 24 to 27.

Experiment No.	Test strains	Haem-agglutination	Mean percentage dead leucocytes up to about 2 hours of observation		
			Leucocyte control	with fimbriate strain	with non-fimbriate strain
1.	S. typhimurium LT2	+++	40	64	
	" " 7471(N)	-			55
2.	S. typhimurium 1287FT	+++	38	84	
	" " 1287(N)	-			53
3.	S. flexneri Fla 1	+++	18.5	48	
	* Heated S. flexneri Fla 1	+++		47	
	S. flexneri Fla 2	-			19
	* Heated S. flexneri Fla 2	-			27
4.	S. typhimurium 1289FT	+++	44	55	
	* Heated S. typhimurium 1289FT	+++		55	
	S. typhimurium 1289N	-			49
	* Heated S. typhimurium 1289N	-			55

* Heated at 56°C for 30 minutes. FT = phage-transduced fimbriate strain N = non-fimbriate strain

106) summarised in appendices no. 24 to 27, suggest that particularly during the first 1-2 hours of exposure of leucocytes to salmonella and shigella the fimbriate strains were more lethal to the leucocytes than were the non-fimbriate strains. The mean percentages of dead leucocytes produced in the in vitro experiments within two hours are shown in table XIV. It was seen that the mean percentage of dead leucocytes produced in the experiment with fimbriate strains was greater than that occurring in the leucocyte control tests and in the experiments with the non-fimbriate strains. The differences in lethal activity of the fimbriate and non-fimbriate organisms for the leucocytes are more convincingly illustrated graphically (figs. 7, 8 and 9.). However, in view of the relatively small total numbers of leucocytes counted these results should be interpreted with caution.

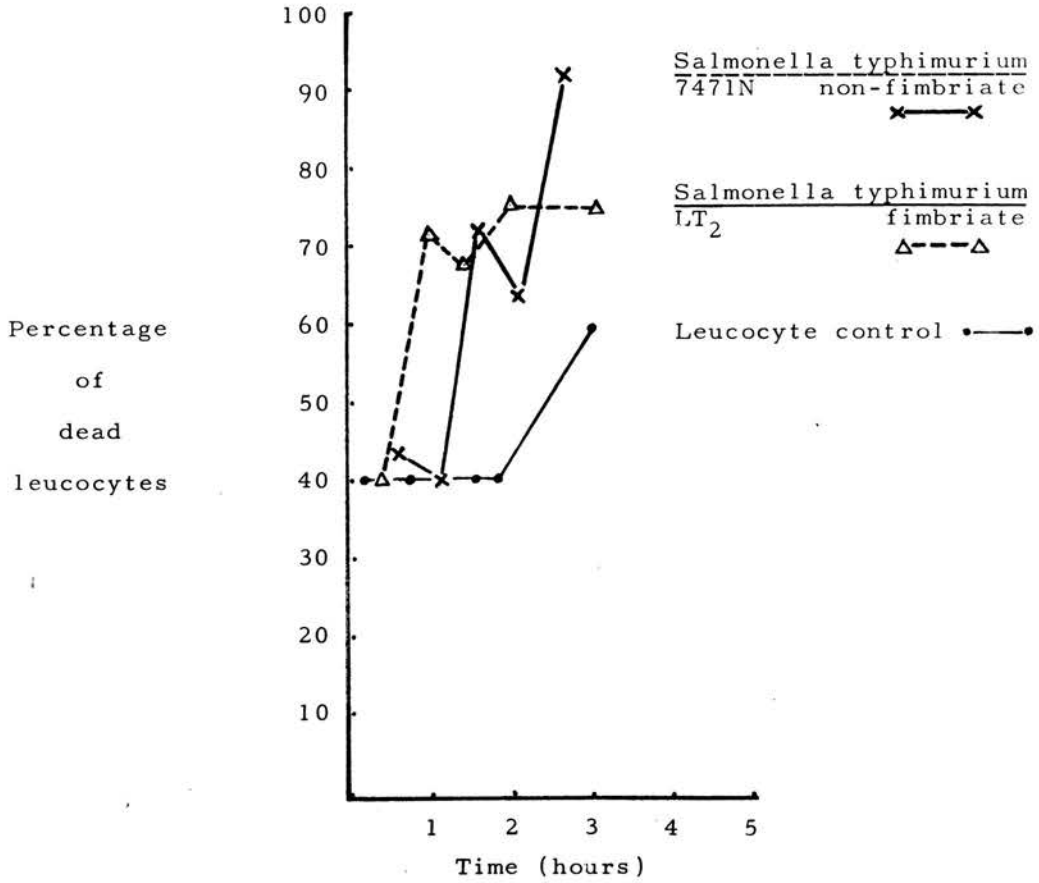

Fig. 7 Experimental Salmonellosis V. **The effect of fimbriate and non-fimbriate strains of S. typhimurium on human leucocytes. The graph shows the rate of killing of leucocytes in in vitro experiments by the permanently fimbriate strain of Salm. typhimurium LT_2, and the permanently non-fimbriate strain of Salm. typhimurium 7471.**

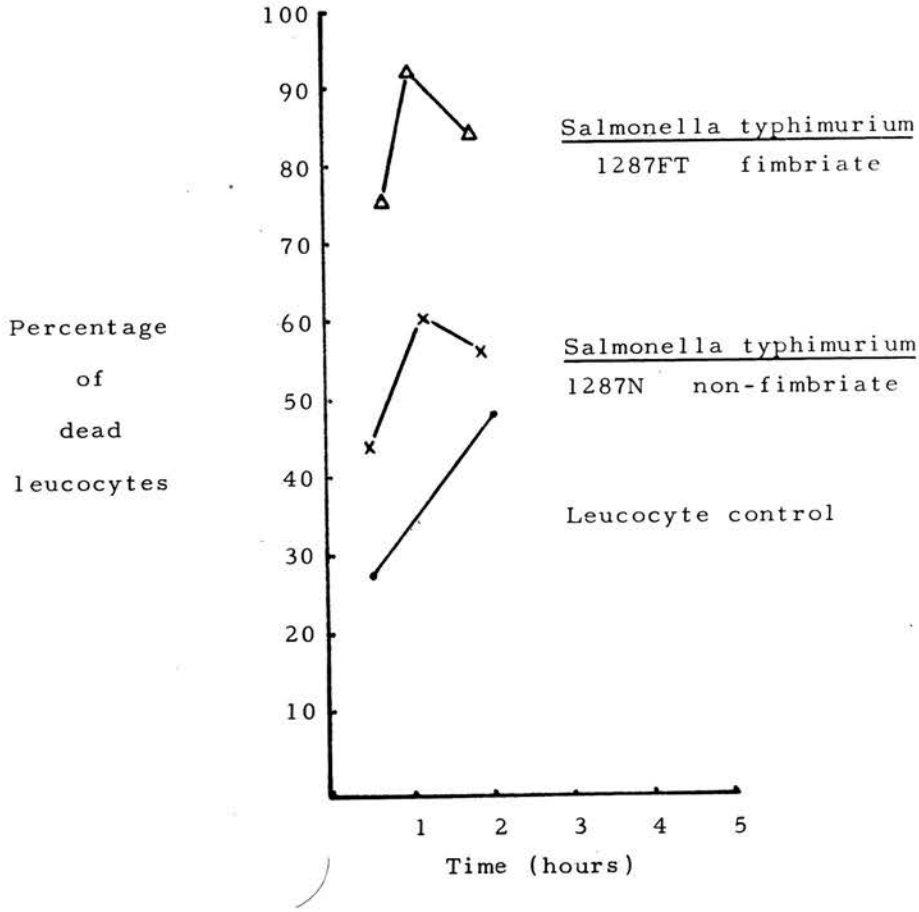

Fig. 8 **Experimental salmonellosis V. The effect of fimbriate and non-fimbriate strains of S. typhimurium on human leucocytes.** The percentage values for dead leucocytes are plotted against time. During the first two hours of exposure the phage-transduced fimbriate Salm. typhimurium was apparently more lethal to the leucocytes than was the non-fimbriate parent Salm. typhimurium 1287 strain.

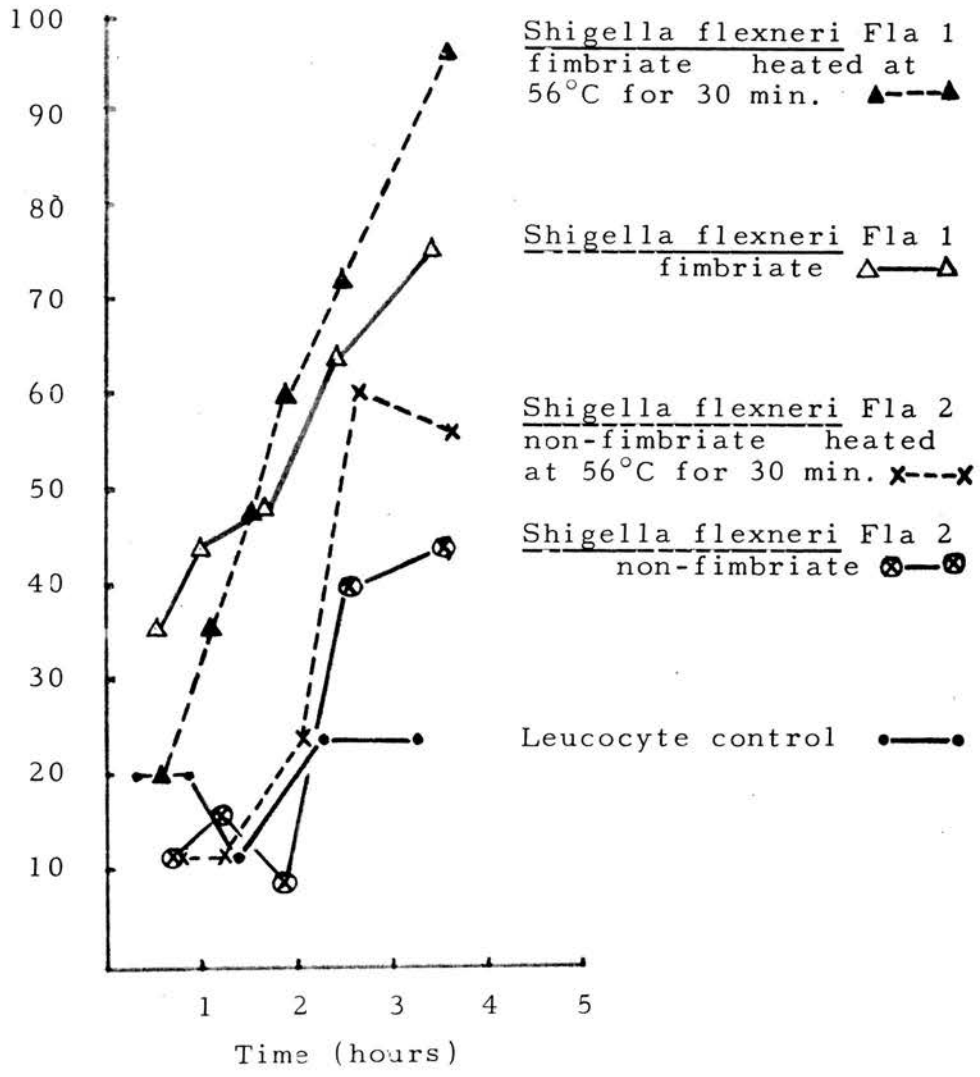

Fig. 9 Experimental Salmonellosis V. **The effect of fimbriate and non-fimbriate strains of Shig. flexneri on human leucocytes. Heated and unheated suspensions of Shigella flexneri strains Fla 1 (fimbriate) and Fla 2 (non-fimbriate) were tested. During the first two hours of observation the fimbriate strain was apparently more lethal to human leucocytes.**

Experimental Salmonellosis VI. Electron microscopic studies.

Au:Pd. SHADOWED ELECTRON MICROGRAPHS. (figs. 10 to 14)

DEMONSTRATING FLAGELLA AND FIMBRIAE

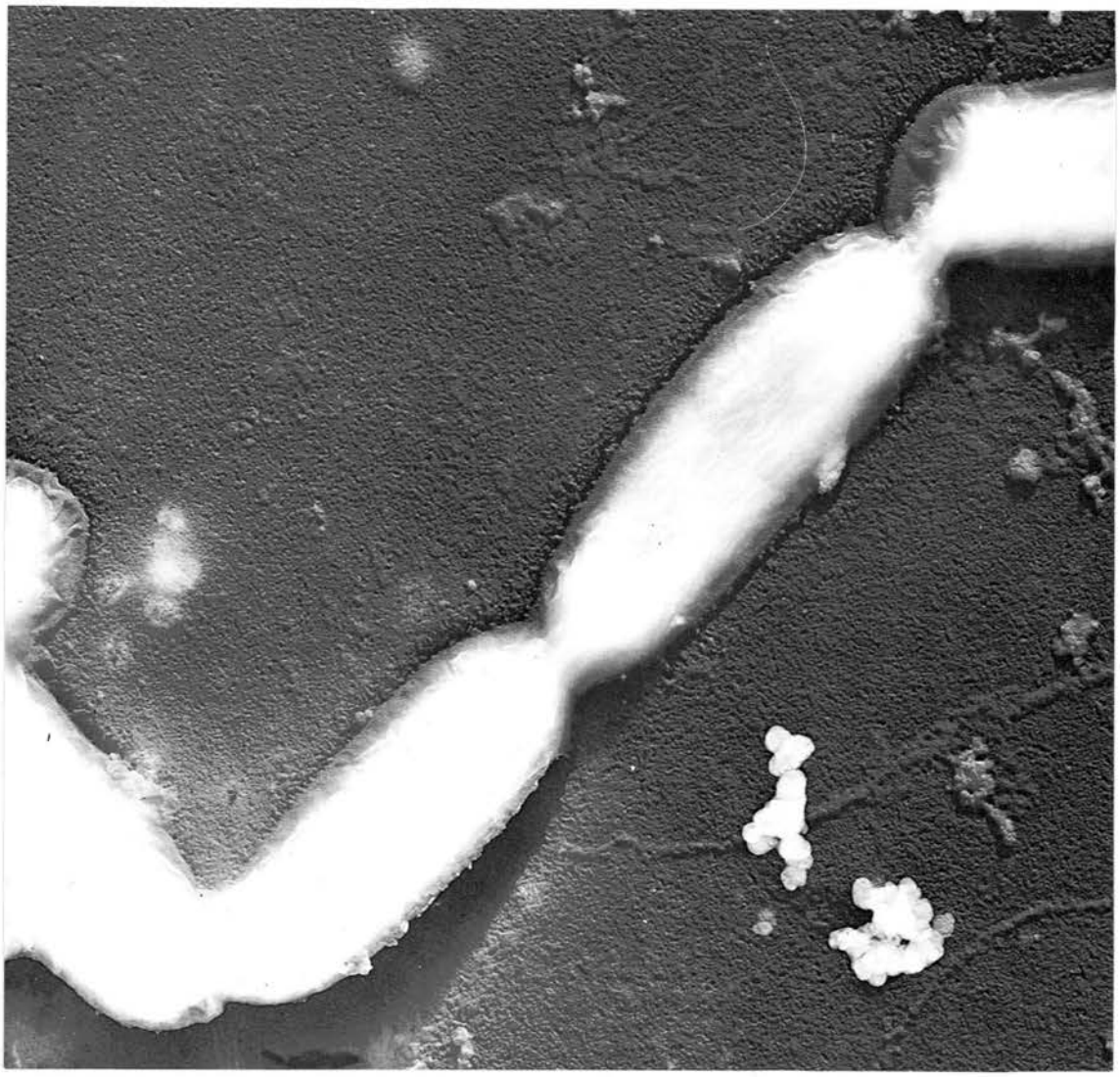

Fig. 10 Experimental Salmonellosis VI. A micrograph of non-flagellate / non-fimbriate Gram-negative bacteria: Shigella flexneri Fla 2.

Magnification 30,000

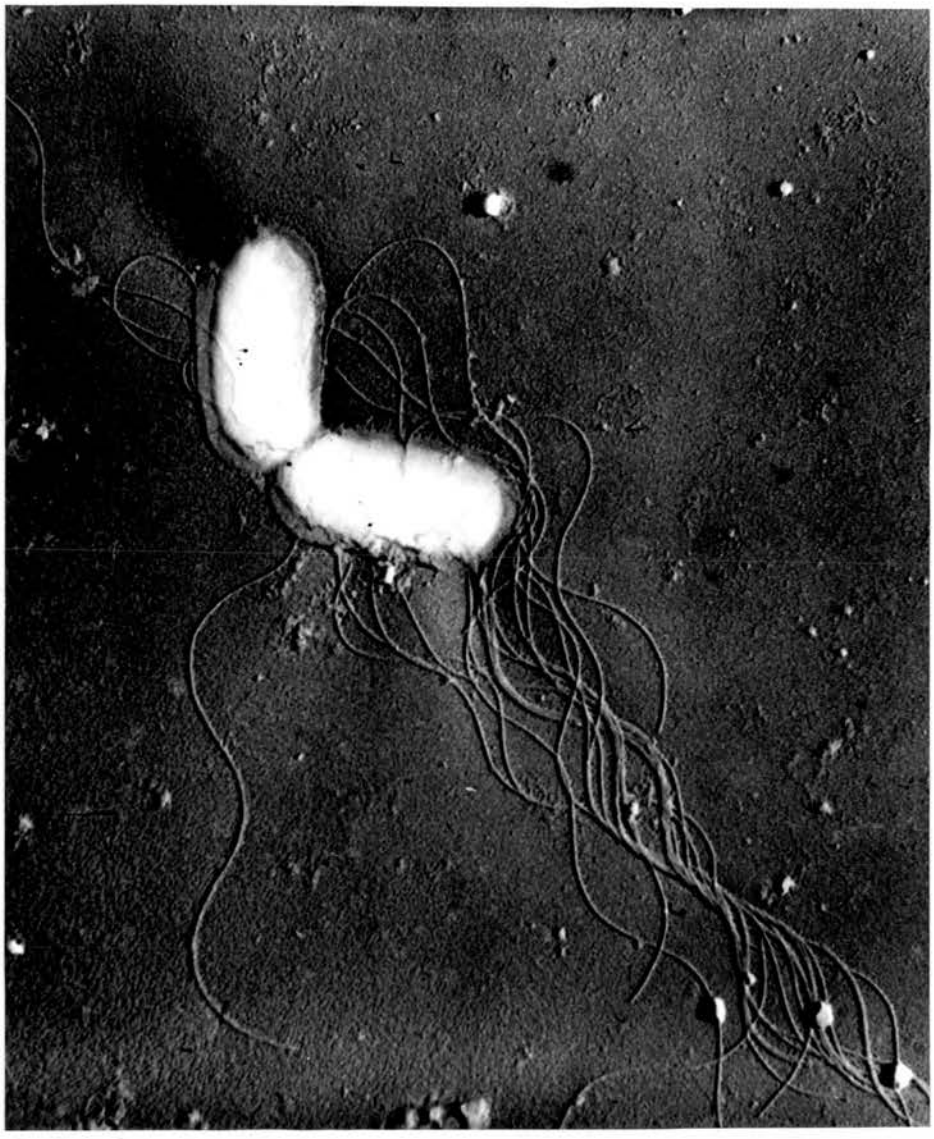

Fig. 11 Experimental Salmonellosis VI. A micrograph of flagellate but non-fimbriate Gram-negative bacteria. Salmonella typhimurium 1566N.
Magnification 16,000

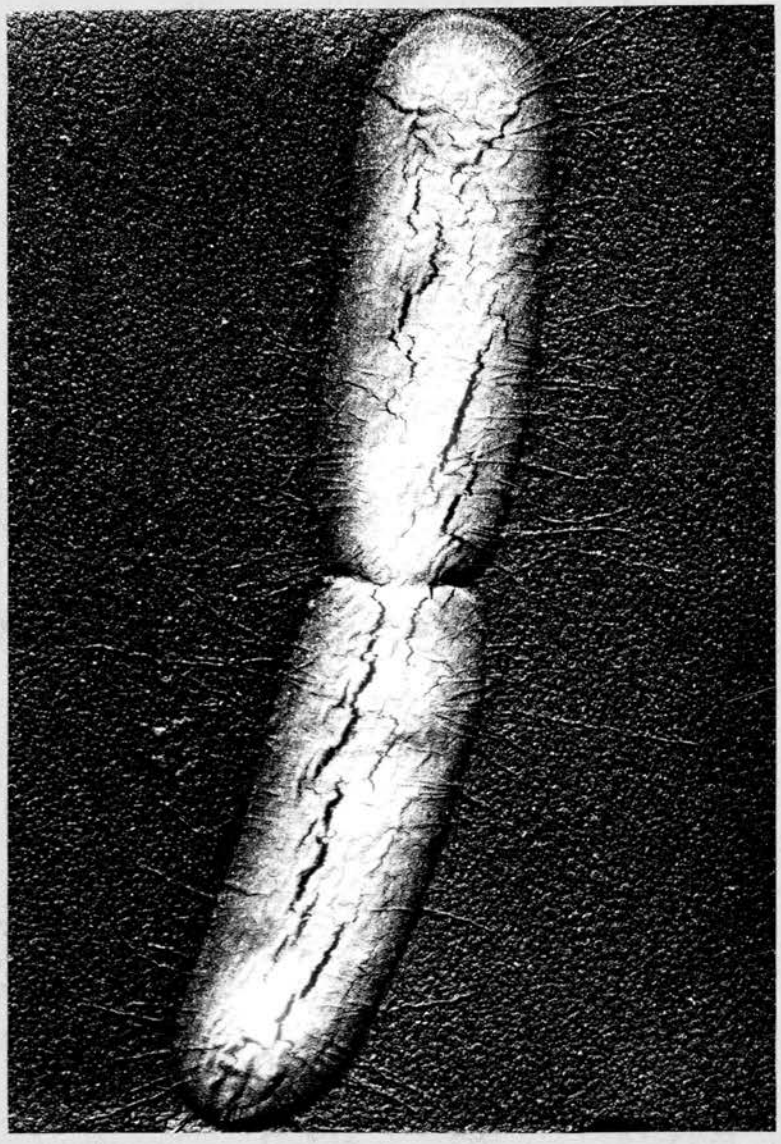

Fig. 12 Experimental salmonellosis VI. A micrograph of non-flagellate but fimbriate Gram-negative bacteria. Shigella flexneri Fla 1. Magnification 32,000.

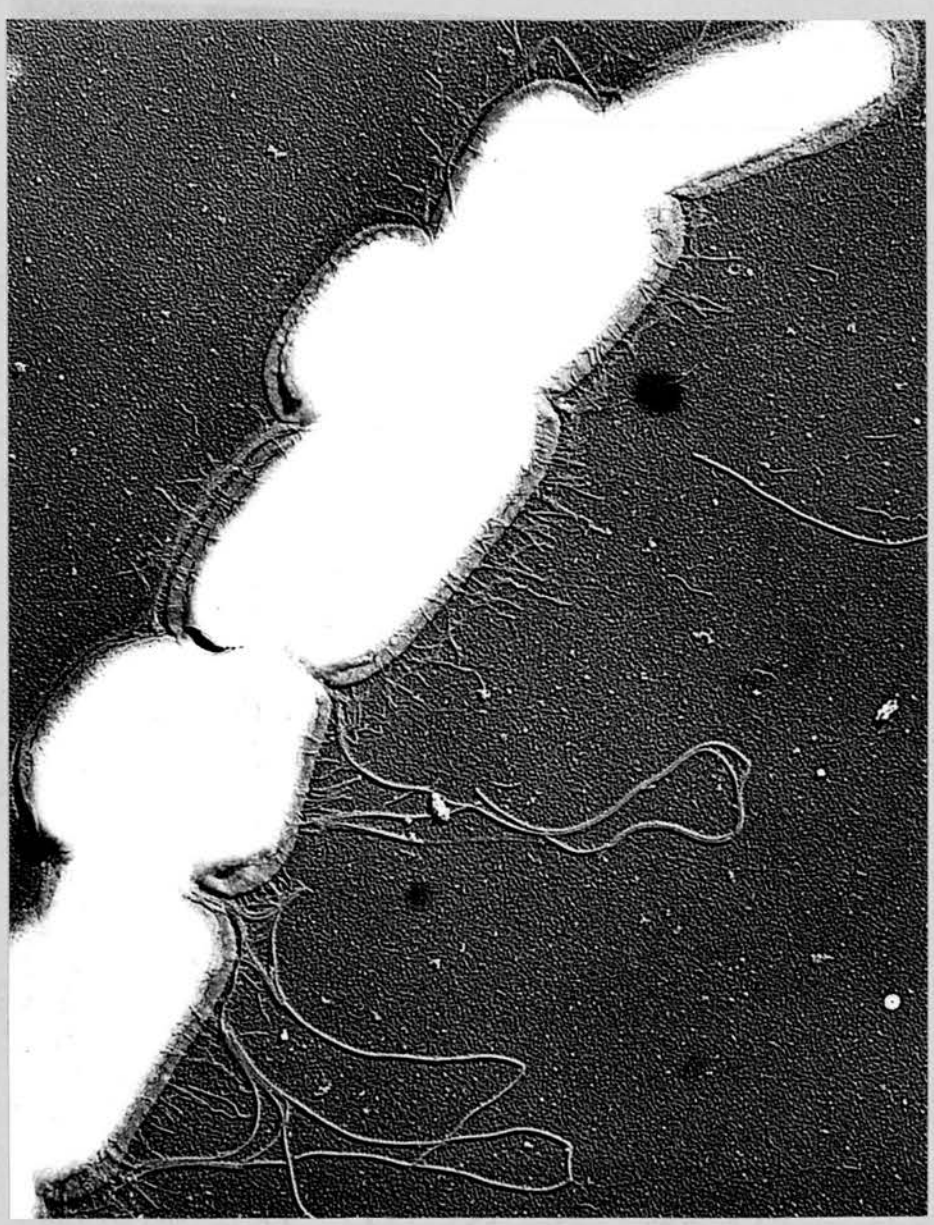

Fig. 13 Experimental salmonellosis VI. A micrograph of flagellate/fimbriate Gram-negative bacteria. Salmonella typhimurium 1566 F. Magnification 18,000.

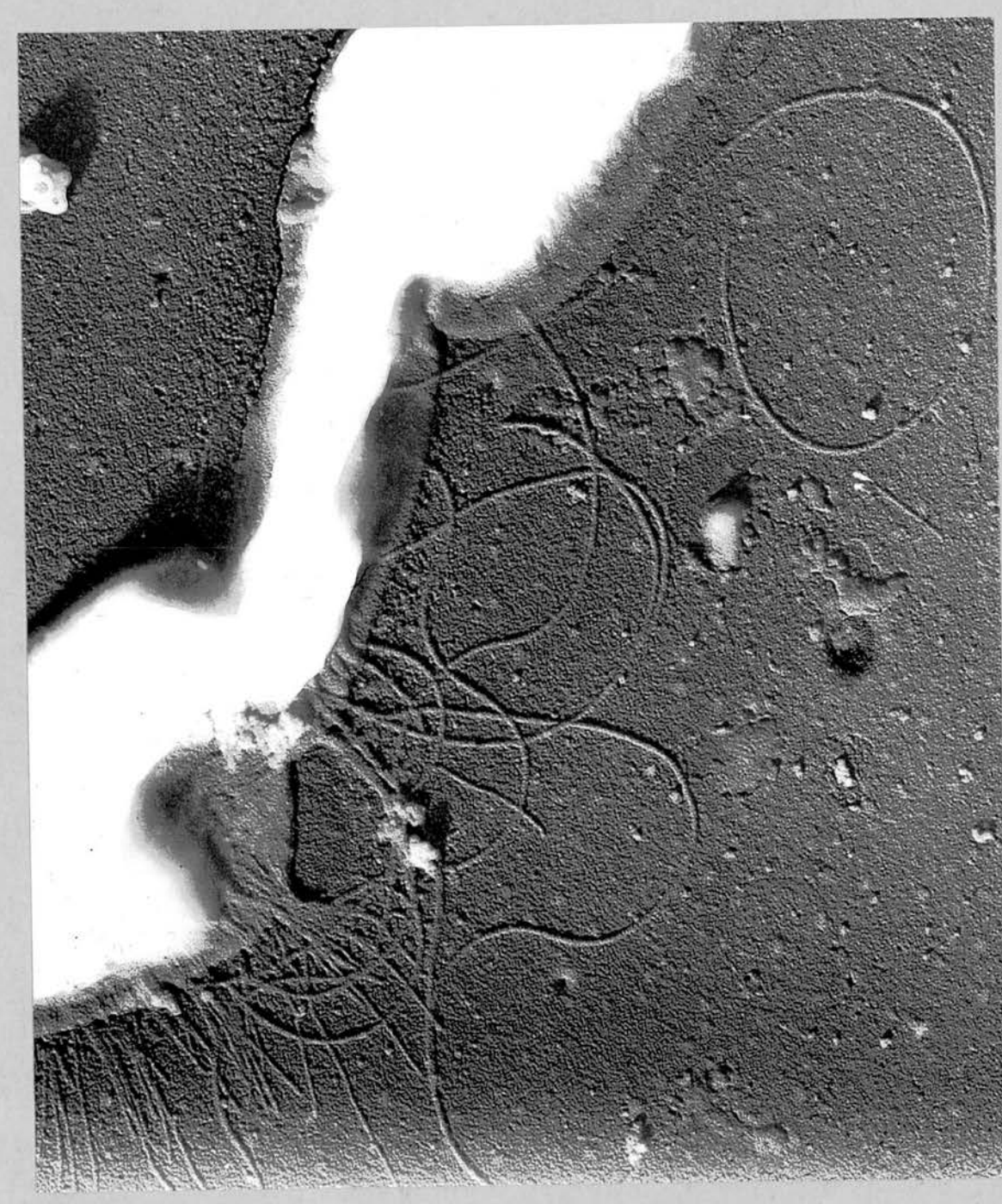

Fig. 14 Experimental salmonellosis VI. A micrograph of flagellate and fimbriate Gram-negative bacteria. Escherichia coli 026 : B6.

Magnification 48,000.

PHOSPHOTUNGSTIC ACID NEGATIVE STAINED ELECTRON MICROGRAPHS (figs. 15 a
DEMONSTRATING FLAGELLAE AND FIMBRIAE

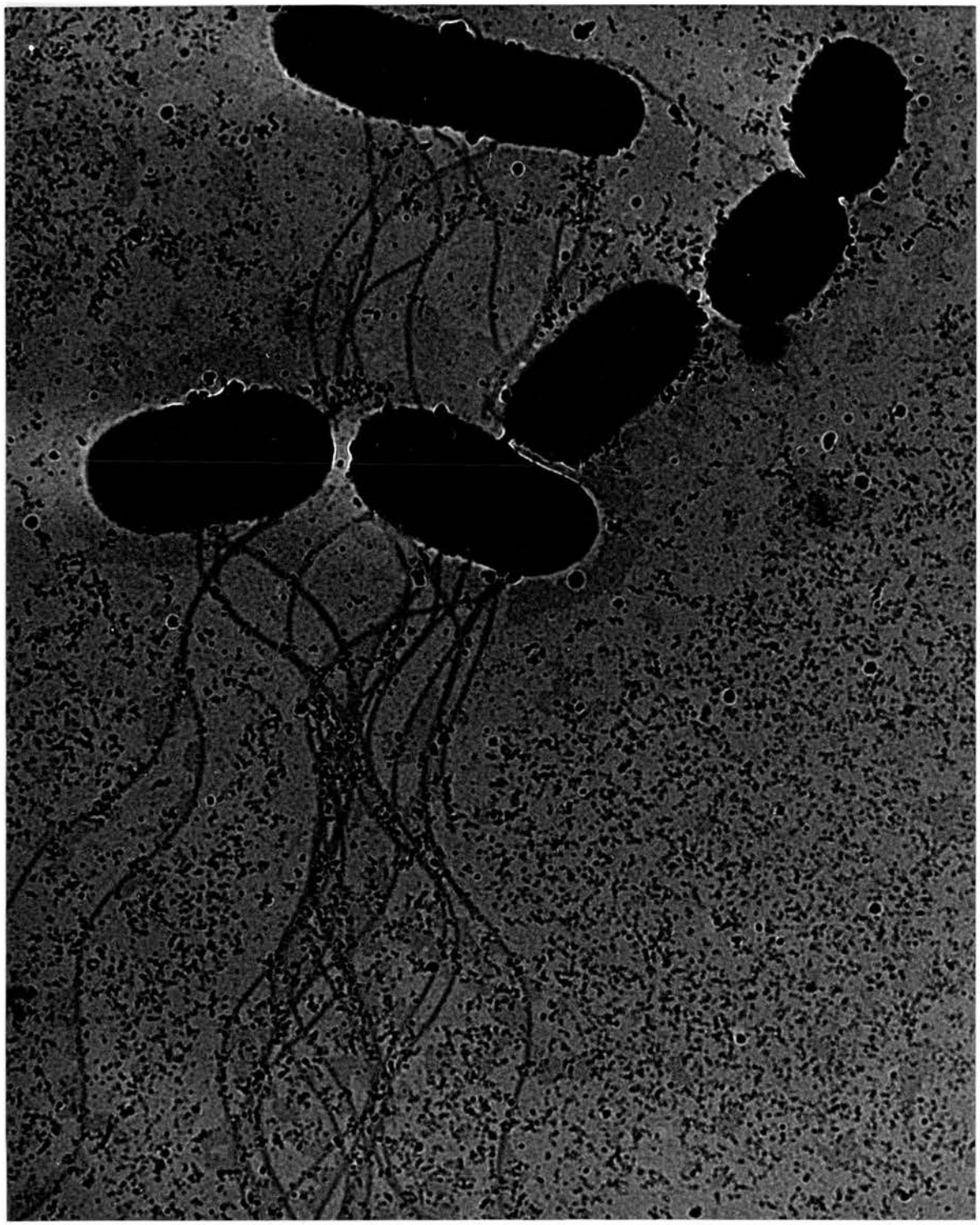

Fig. 15 Experimental salmonellosis VI. A micrograph of a flagellate strain of Salmonella typhimurium 1566N.
Magnification 21,000.

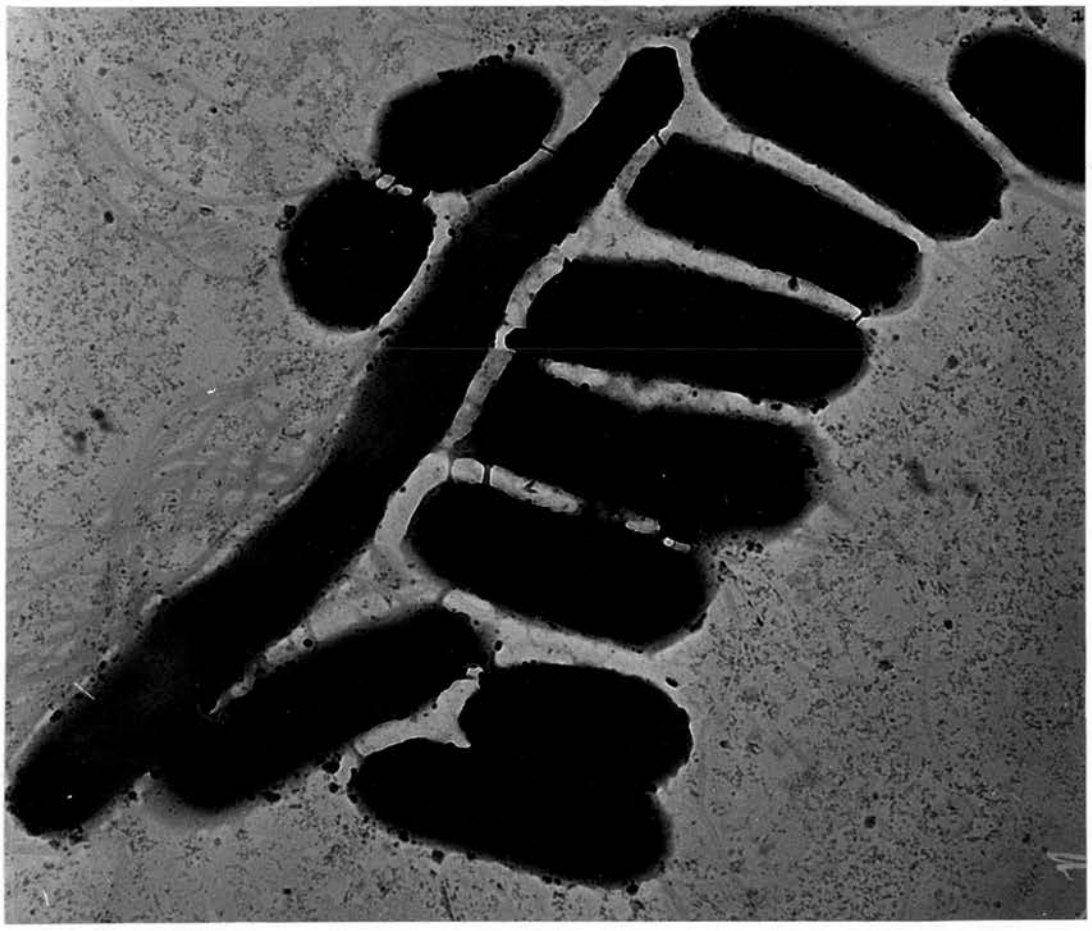

Fig. 16 Experimental salmonellosis VI. A micrograph of a flagellate and fimbriate strain Salmonella typhimurium 1566F: fl = flagellum; f = fimbria

Magnification 20,000.

UNSHADOWED AND SHADOWED ELECTRON MICROGRAPHS (figs. 17 and 18) SHOWING FIMBRIATE SALMONELLA TYPHIMURIUM ADHERING TO HUMAN LEUCOCYTES, R.B.C. AND PLATLETS.

Fig. 17 Experimental salmonellosis VI. An unshadowed micrograph showing agglutination of red blood cells by fimbriate Salmonella typhimurium 1289 FT.

Magnification 8,000.

Fig. 18 Experimental salmonellosis VI. A micrograph of a shadowed specimen showing a fimbriate Salmonella typhimurium organism adhering to a red blood corpuscle. Magnification 40,000.

Electron Microscopic studies

Araldite sections of S. typhimurium

To gain experience in the necessary techniques and to familiarise the writer with the electron microscopic appearances of the test organisms in thin sections, the following initial studies were done.

The longitudinal and transverse sections of S. typhimurium (figs. 19, 20, 21 and 22) show that the bacterium is surrounded by a rigid cell wall (c.w.) and an additional amorphous outer layer - the micro capsule (c). The cell wall is a dense layer of uniform thickness surrounding the whole cell and in sections it appears as two dense layers (fig. 21) separated by a less dense layer. The plasma membrane (p.m.) or the limiting membrane around the cytoplasm is seen separated from the cell wall by a space (fig. 21). The cytoplasm appears finely granular on the electron micrographs and some discrete granules (r) which may be ribosomes or ribonucleoprotein particles are also seen. The larger cytoplasmic granules (p.g., figs. 20 and 22) which are extremely dense contain inorganic polymetaphosphates and are said to correspond to metachromatic volutin granules (Glauert, 1962). The nuclear material (n) is located in the central part of the bacterial cell in the region (figs. 19, 20, 21 and 22) which has a lower average density than the cytoplasm. The fig. 24 is an interesting electron micrograph of a dividing S. typhimurium and illustrates the symmetry

between the two daughter cells. The spindle shaped pattern of the nuclear material seems to reflect the orderly movement of the fibrillar material during division of the nuclear material. The micrographs also show flagella and fimbriae of S. typhimurium in sections. The flagella (fl) appear hollow and in transverse section (fig. 23) the flagella show a five-fold symmetry with a dense unit at each corner of a regular pentagon as described by Glauert (1962). The fimbriae (f) are much shorter and thinner; they do not show any subunits and they appear to be long hollow cylinders.

FINE STRUCTURE OF FIMBRIATE/FLAGELLATE SALMONELLA TYPHIMURIUM

Electron micrographs (figs. 19 to 23) of thin sections of S. typhimurium.

c = capsular material; pm = plasma membrane; n = nuclear region; cw = cell wall; r = cytoplasmic granules or ribosomes; s = spindle; pg = dense granules of inorganic polymetaphosphates; fl = flagellum; f = fimbria.

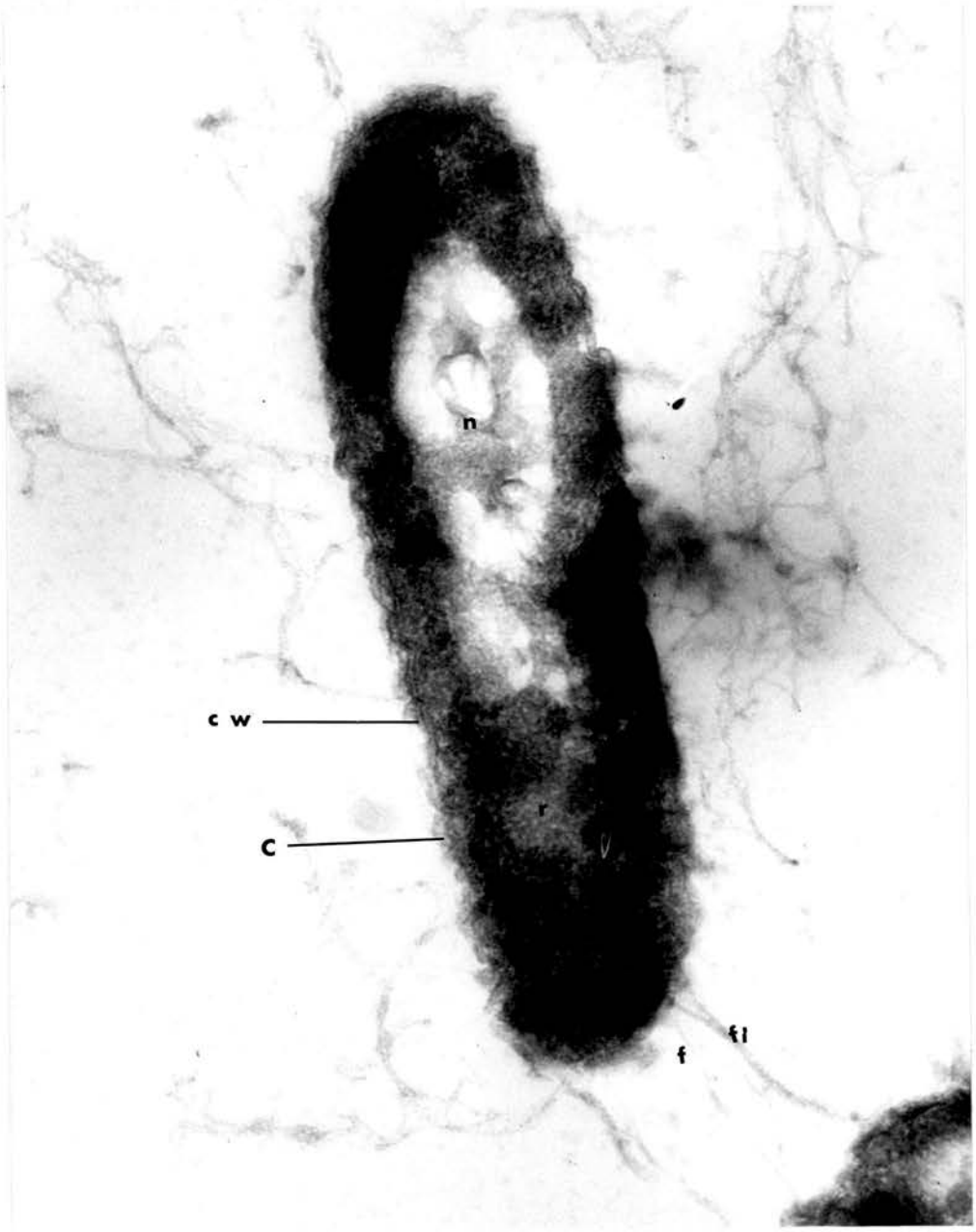

Fig. 19 Experimental salmonellosis VI. A fimbriate/flagellate S. typhimurium organism in longitudinal section.

Magnification 100,000.

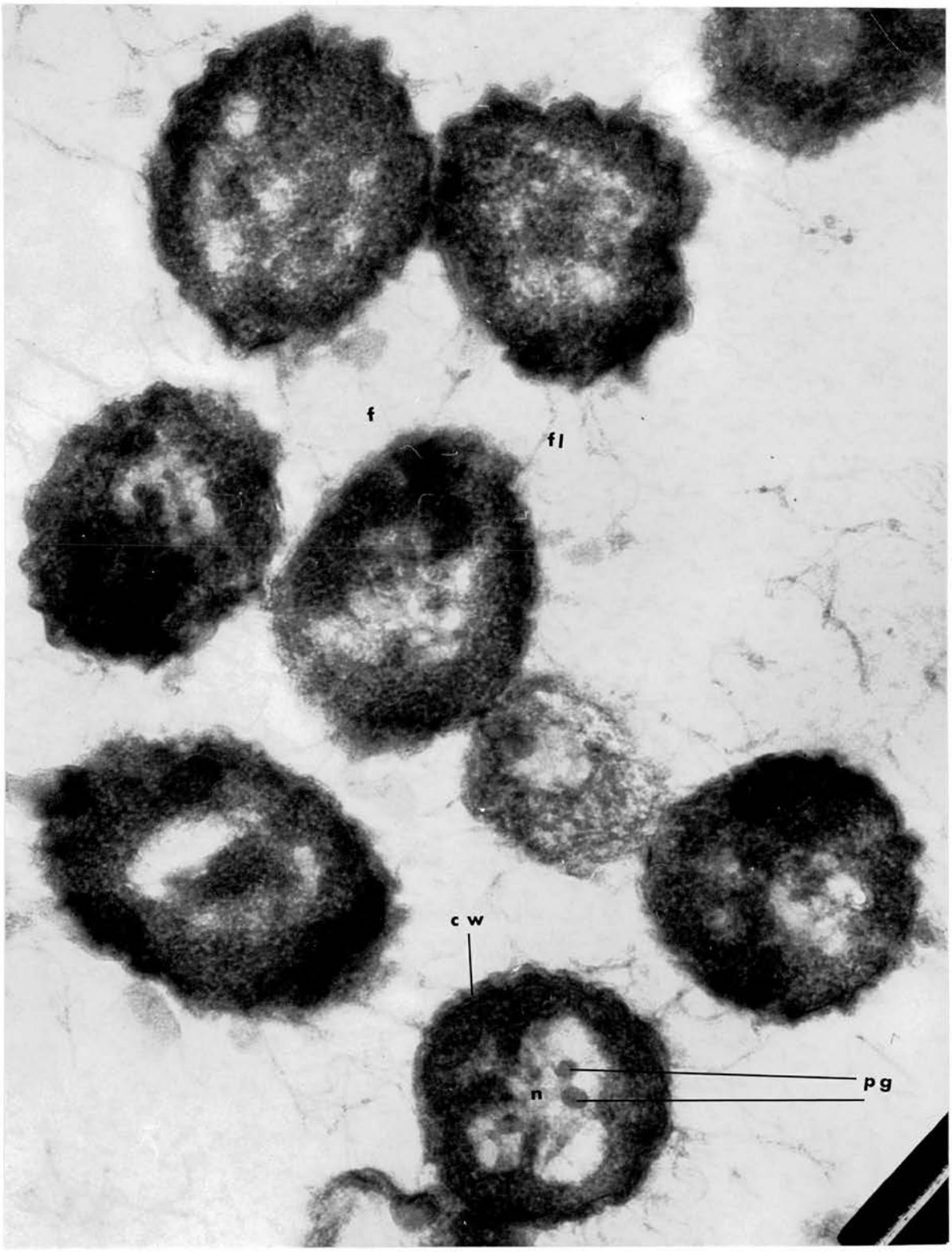

Fig. 20 Experimental salmonellosis VI. Fimbriate/flagellate S. typhimurium organisms in transverse sections.
Magnification 100,000.

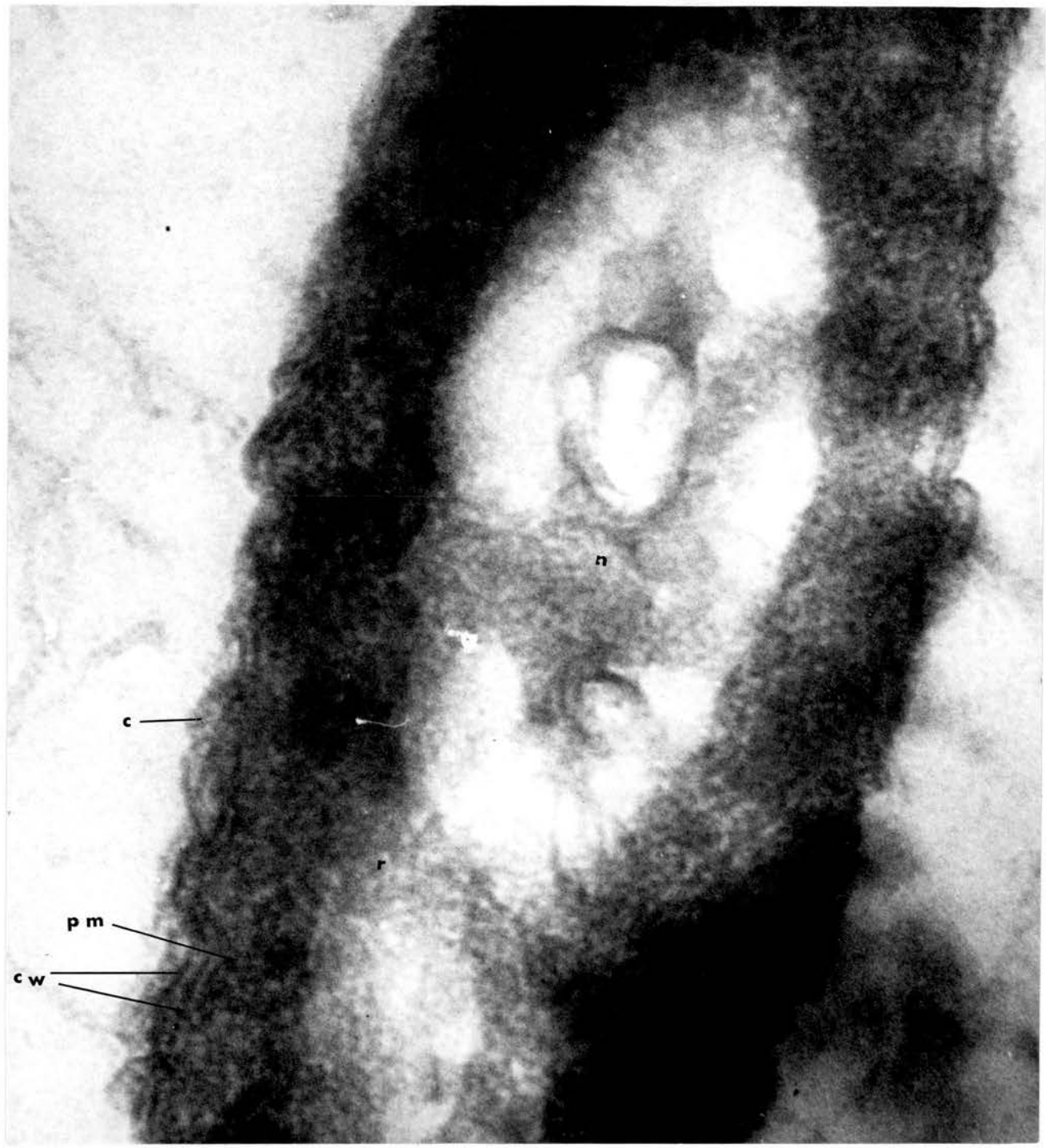

Fig. 21 Experimental salmonellosis VI. A longitudinal section of S. typhimurium. The cell wall (cw) consists of two dense membranes separated by a less dense material. The plasma membrane (pm) is separated slightly from the cell wall.

Magnification 300,000.

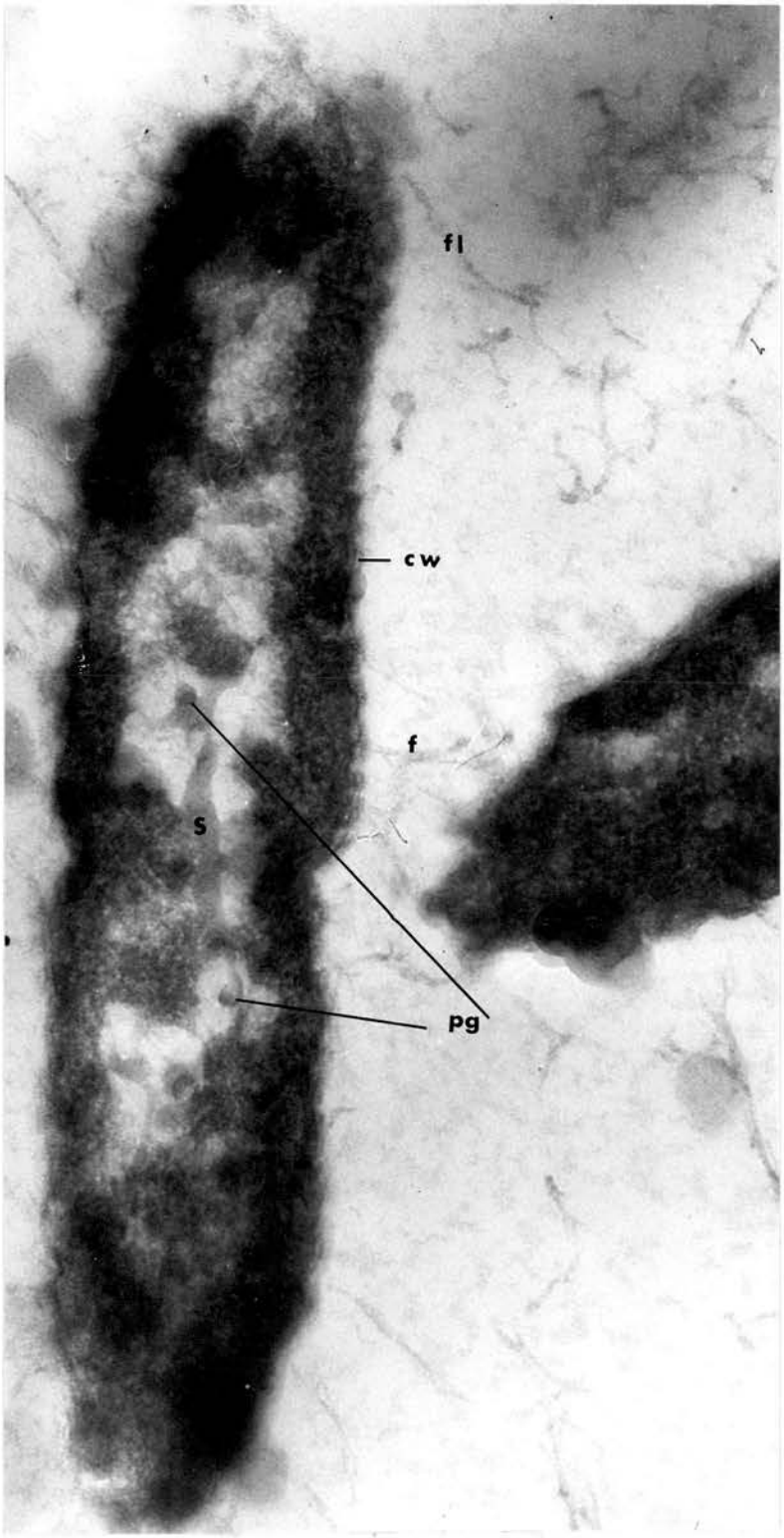

Fig. 22 Experimental salmonellosis VI. A longitudinal section of a dividing fimbriate/flagellate S. typhimurium organism. Note the symmetry of the two daughter cells.

Magnification 100,000.

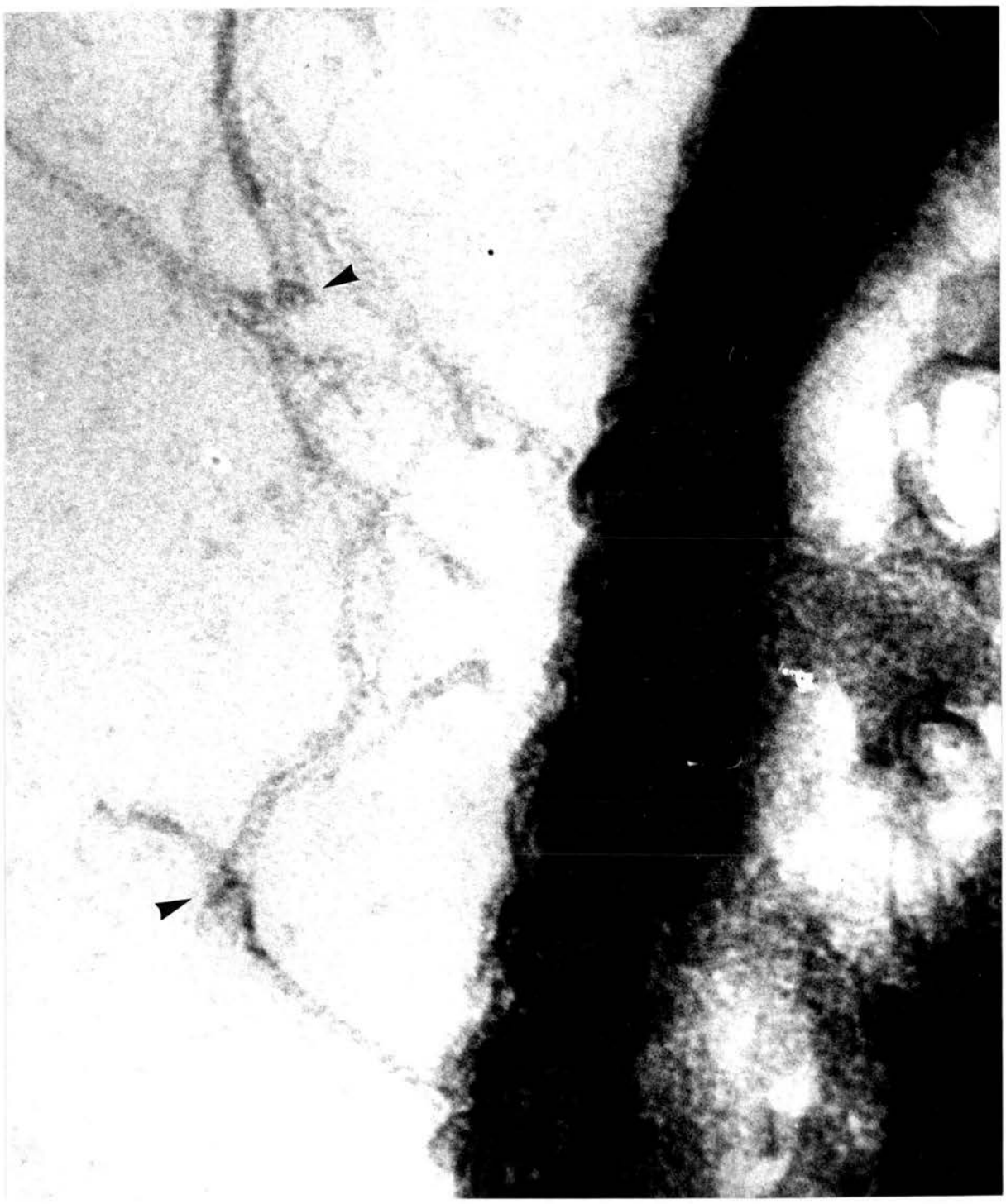

Fig. 23 Experimental salmonellosis VI. Flagella of S. typhimurium cut longitudinal and transverse sections. It has a five-fold symmetry in transverse section (arrowed).

Magnification 300,000.

Electron microscopic studies on the mode of penetration of duodenal mucosa of albino rats by S. typhimurium

Earlier experimental work in albino rats (page 152) on the localisation of possible sites of penetration of the gastro-intestinal tract by S. typhimurium had shown that the duodenum was the commonest site where penetration appeared to occur most regularly and consistently. In view of this the duodenum was chosen for the above mentioned electron-microscopic studies.

Figures 24, 25, 26, 27 and 28 are electron-micrographs of sections of normal duodenum of the albino rat. According to Palay and Karlin (1959) the ultra structures of the human and rat villus are very similar.

The normal duodenal villus of the albino rat is covered with columnar cells. Mucus-secreting goblet (GC) cells are present. The core of the villus contains a central lacteal, smooth muscle cells, a few mononuclear cells and a plexus of capillaries. The free surface of each columnar epithelial cell is formed into numerous slender microvilli (MV) which vary in length but are usually around 1μ long. This is also called the brush-border (BB). In the underlying cytoplasm, and running parallel to the free surface of each cell, is the filamentous mesh that forms the terminal web (TW). Fibres extend from the web into the micro-villi, and end laterally at the terminal bar (TB) where the cells are fixed firmly together. The lateral cell membrane (CM) of each cell interlocks in a convoluted pattern with that

of the neighbour except towards the base. Occasional lymphocytes or polymorphs are also seen. The supranuclear cytoplasm contains numerous mitochondria (M) which are compact and electron-opaque. The epithelial cells rest on a thin homogeneous basement membrane, beneath which are collagen fibres (cf). The capillaries (C) lie free in the metrix.

The duodenal segments of three albino rats were infected by the injection of approximately 10^9 organisms of a fimbriate strain of S. typhimurium (Methods, page 114). The duration of exposure of the mucosa to bacterial penetration was different in each animal and it ranged over periods of 5, 15 and 40 min. Figures 29, 30 and 31 are electron-micrographs of sections of duodenum after exposure of the duodenal mucosa to S. typhimurium organisms for 5 min., while fig. 32 is an electron-micrograph of a section from the duodenum after exposure for 40 min. Fig. 29 is an electron-micrograph of a bacterium adhering to the microvilli (MV) of the brush-border (BB). Fig. 30 shows some unidentified cellular debris adhering to the microvilli. Fig. 31 is an electron-micrograph of bacteria (B) that appear to be fimbriate (the test strain S. typhimurium was fimbriate) lying in the intercellular tissue. Fig. 32 is an electron-micrograph of transverse and longitudinal sections of bacteria (which appear to be fimbriate) lying near to the base of the epithelial cells. These sections of bacteria in the tissues are very similar to the sections

of S. typhimurium illustrated in fig. 33, but it could not be claimed that these observations afford clear evidence that the test bacteria penetrated the duodenal mucosa. Although this study was profitable in providing the writer with experience in electron-microscopic techniques, the above results did not appear to justify further work along these lines in the present project.

ELECTRON-MICROSCOPE STUDIES ON THE MODE OF PENETRATION OF THE DUODENAL MUCOSA OF ALBINO RATS BY S. TYPHIMURIUM. ELECTRON MICROGRAPHS (FIGS. 24 to 32) OF ULTRATHIN ARALDITE-EMBEDDED SECTIONS.

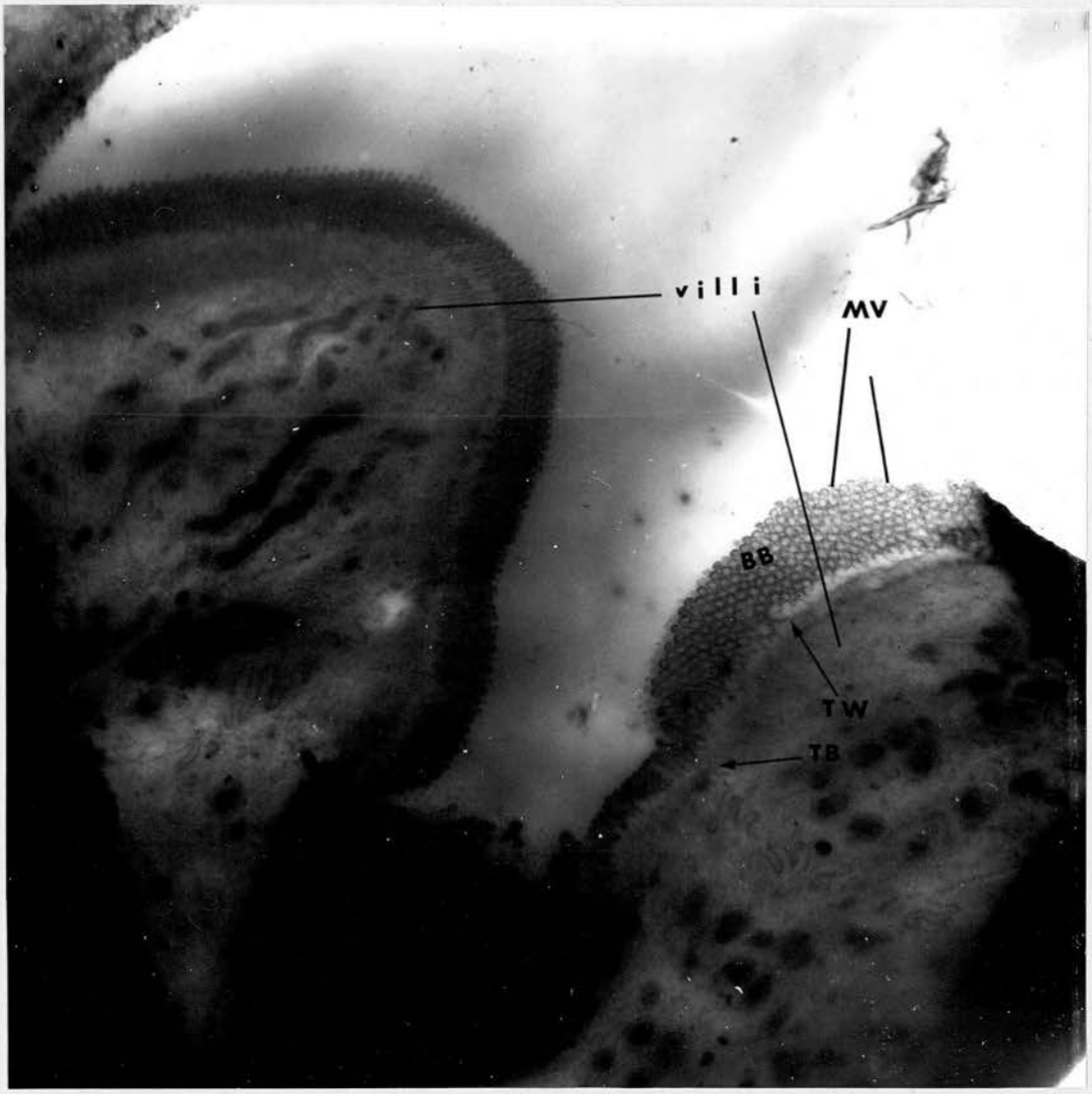

Fig. 24 Experimental salmonellosis VI. A low power electron-micrograph of tips of normal duodenal villi of albino rats. A goblet cell (GC) is present between two villi. Columnar epithelial cells are visible in each villus. The surface of the villus is covered by a brush border (BB) made up of a serried array of microvilli. Microvilli (MV), mitochondria (M), a terminal web (TW) and terminal bar (TB) are indicated.

Magnification 3000.

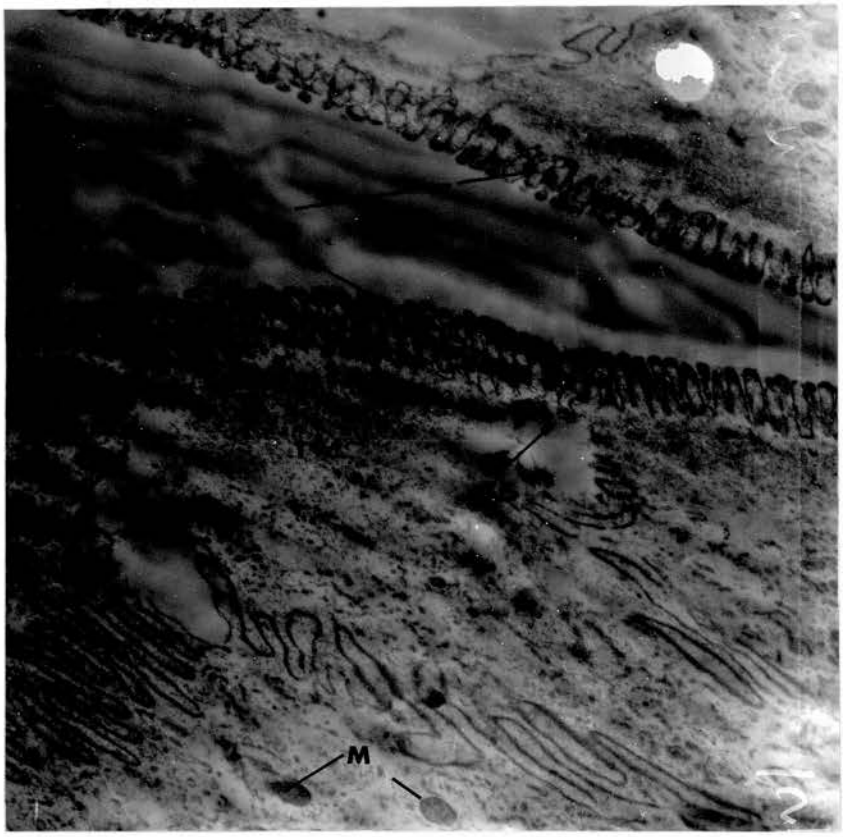

Fig. 25 Experimental salmonellosis V. Electron-micrograph of normal duodenum of albino rats showing supranuclear region of the epithelial cells, with the brush border (BB), the terminal web (TW), terminal bar (TB) and mitochondria (M) indicated.

Magnification 12,000

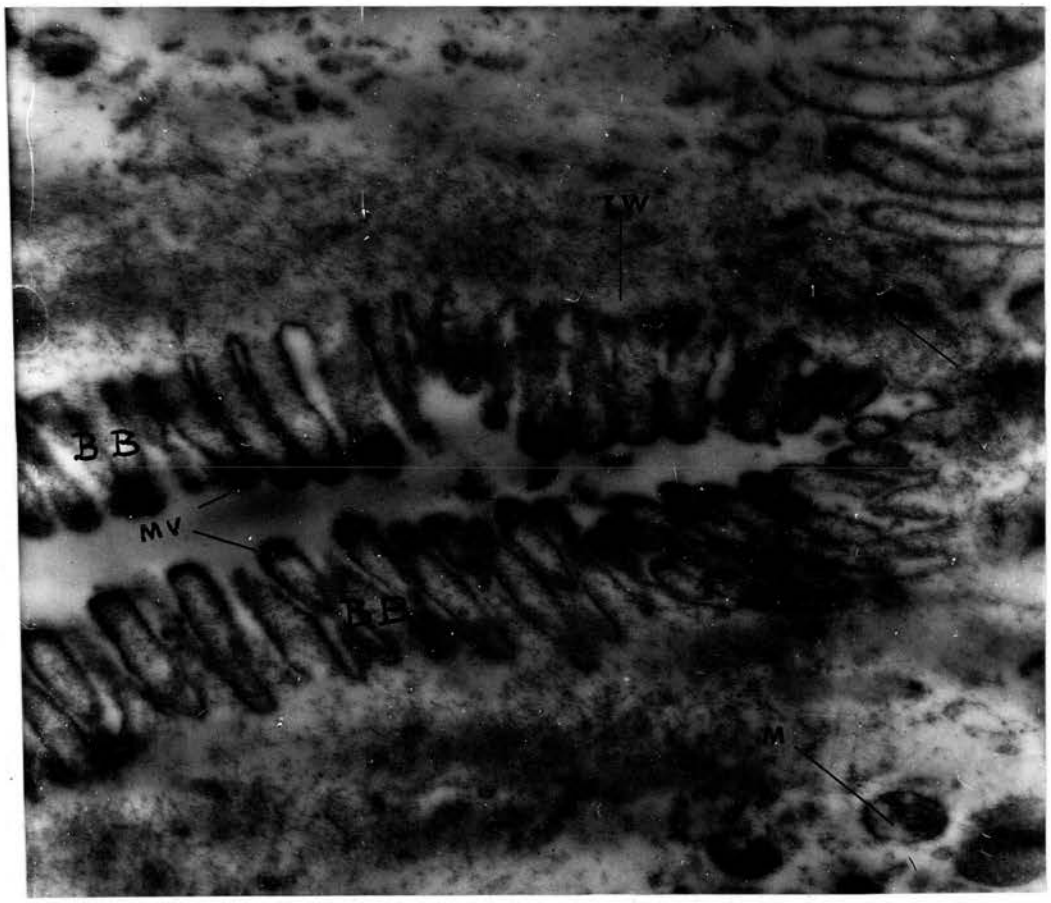

Fig. 26 Experimental salmonellosis V. Electron-micrograph of normal duodenum of albino rats showing supranuclear region of the epithelial cells, with the brush border (BB), the terminal web (TW), terminal bar (TB) and mitochondria (M) indicated.

Magnification 40,000

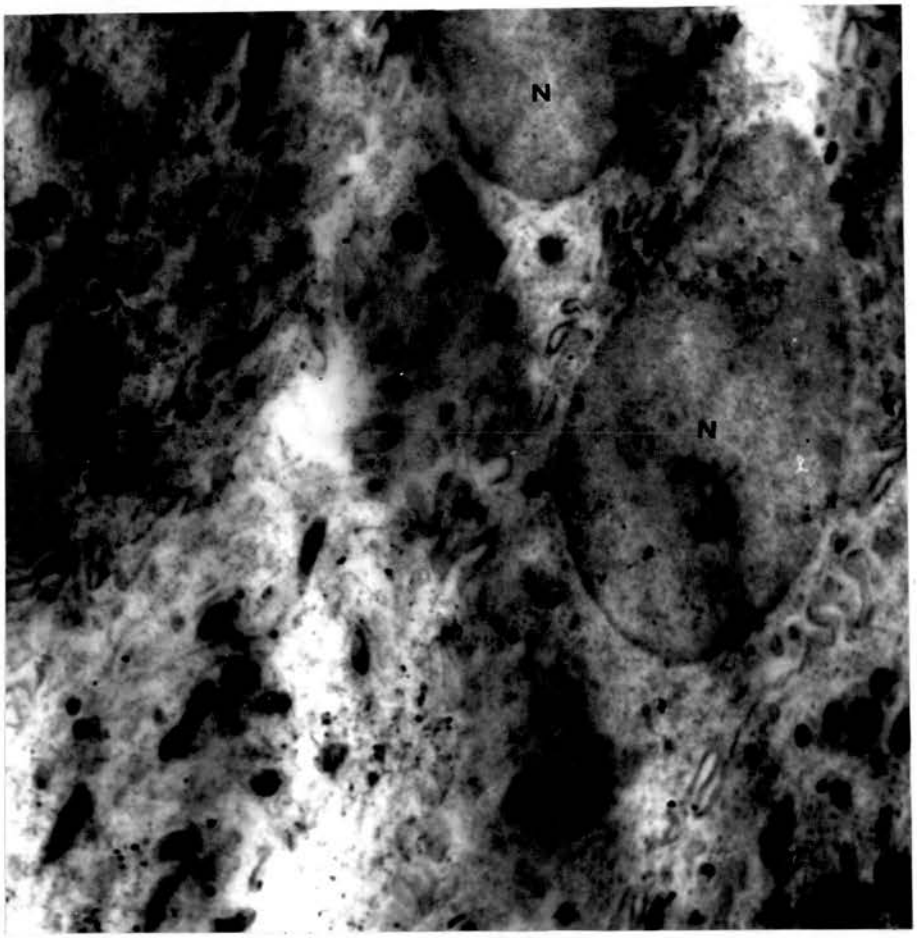

Fig. 27 Experimental salmonellosis VI. A nucleus (N) is indicated on the electron micrograph of nuclear region of epithelial cells.

Magnification 50,000.

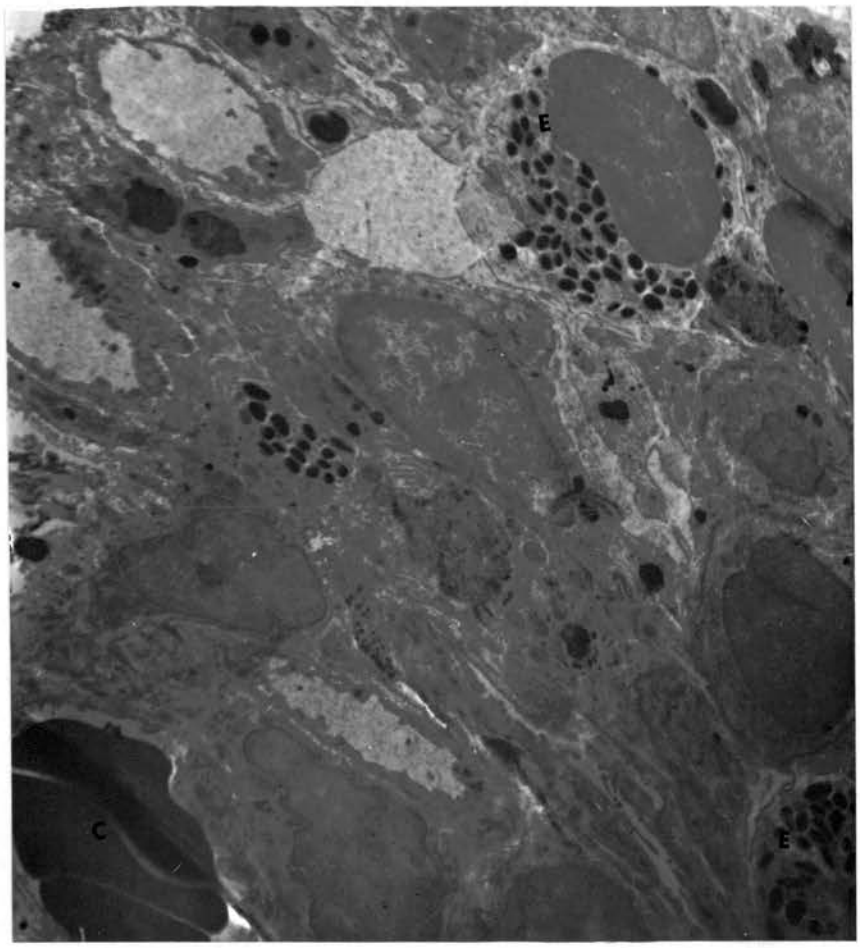

Fig. 28 Experimental Salmonellosis VI. Electron micrograph of the central core of a villus containing eosinophilic leucocytes (E) showing characteristic electron-dense granules and a blood capillary (C) containing red cells.

Magnification 15,000.

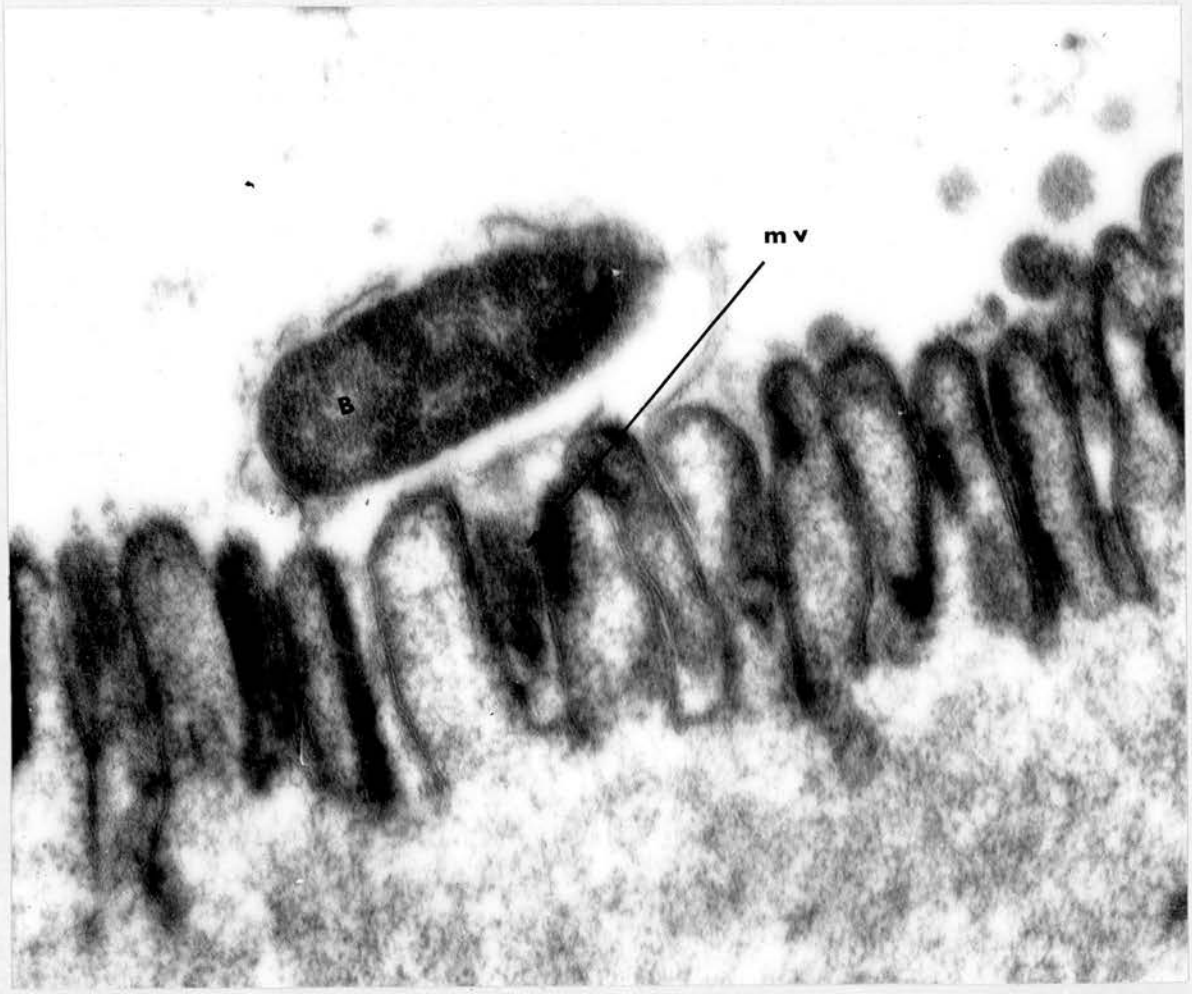

Fig. 29 Experimental Salmonellosis VI. Electron-micrograph of a section of the duodenum of an albino rat showing a bacterium (B) close to the micro-villi (MV). No changes in the host membranes and cytoplasm are seen. Magnification 100,000

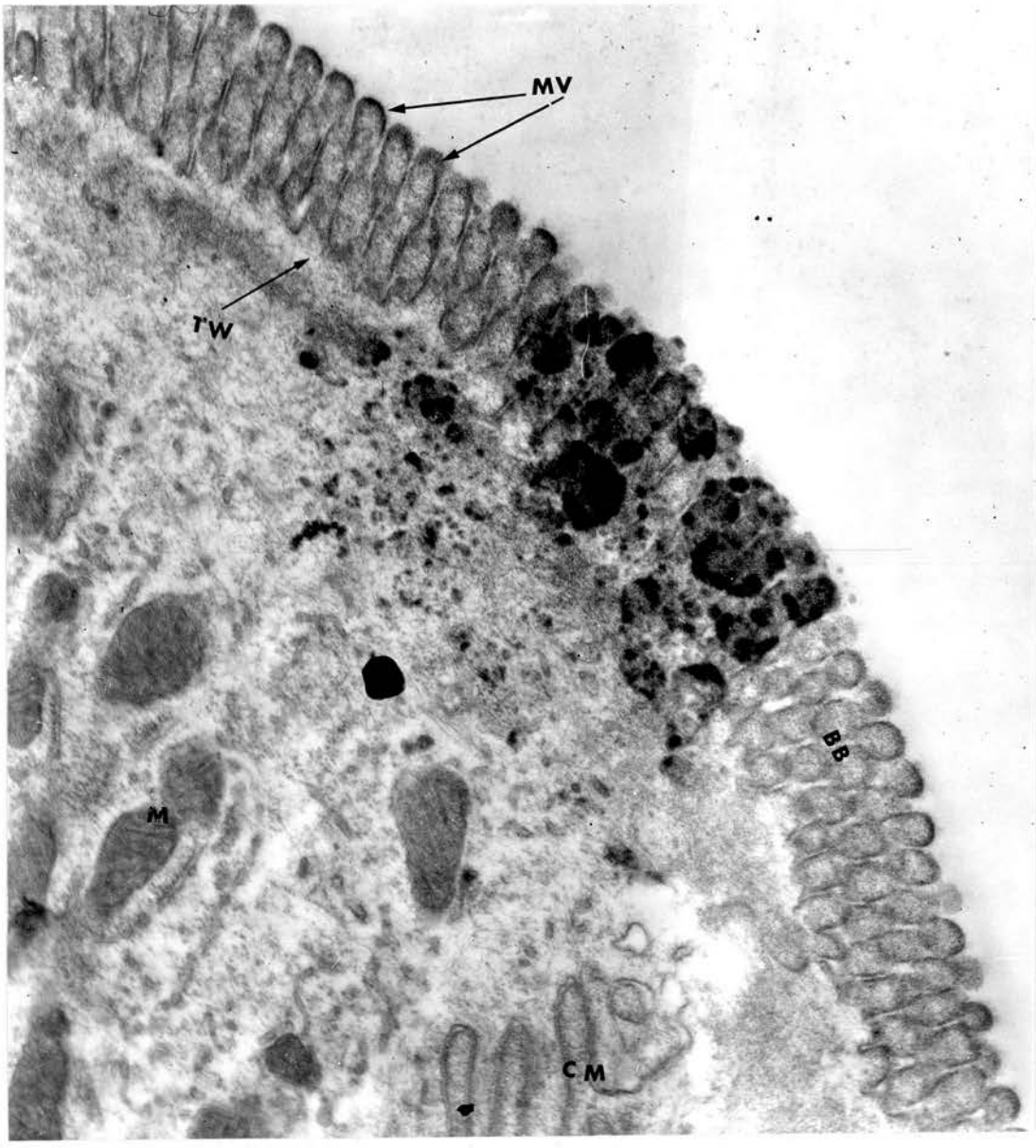

Fig. 30 Experimental Salmonellosis VI. Electron-micrograph showing unidentified debris close to the brush border(BB) of the epithelial cells. Brush border of microvilli (MV); the terminal web (TW), intercellular membrane (CM), mitochondria (M) are indicated.
Magnification 32,000.

Fig. 31 Experimental Salmonellosis VI. Electron-micrograph of a section of the duodenum of an albino rat showing bacteria (B) in the intercellular region of the epithelial cells. Also seen are mitochondria (M) and intercellular membrane (CM). The mitochondria are easily distinguished from the bacteria by the presence of well defined cristae (cri). Magnification 45,000.

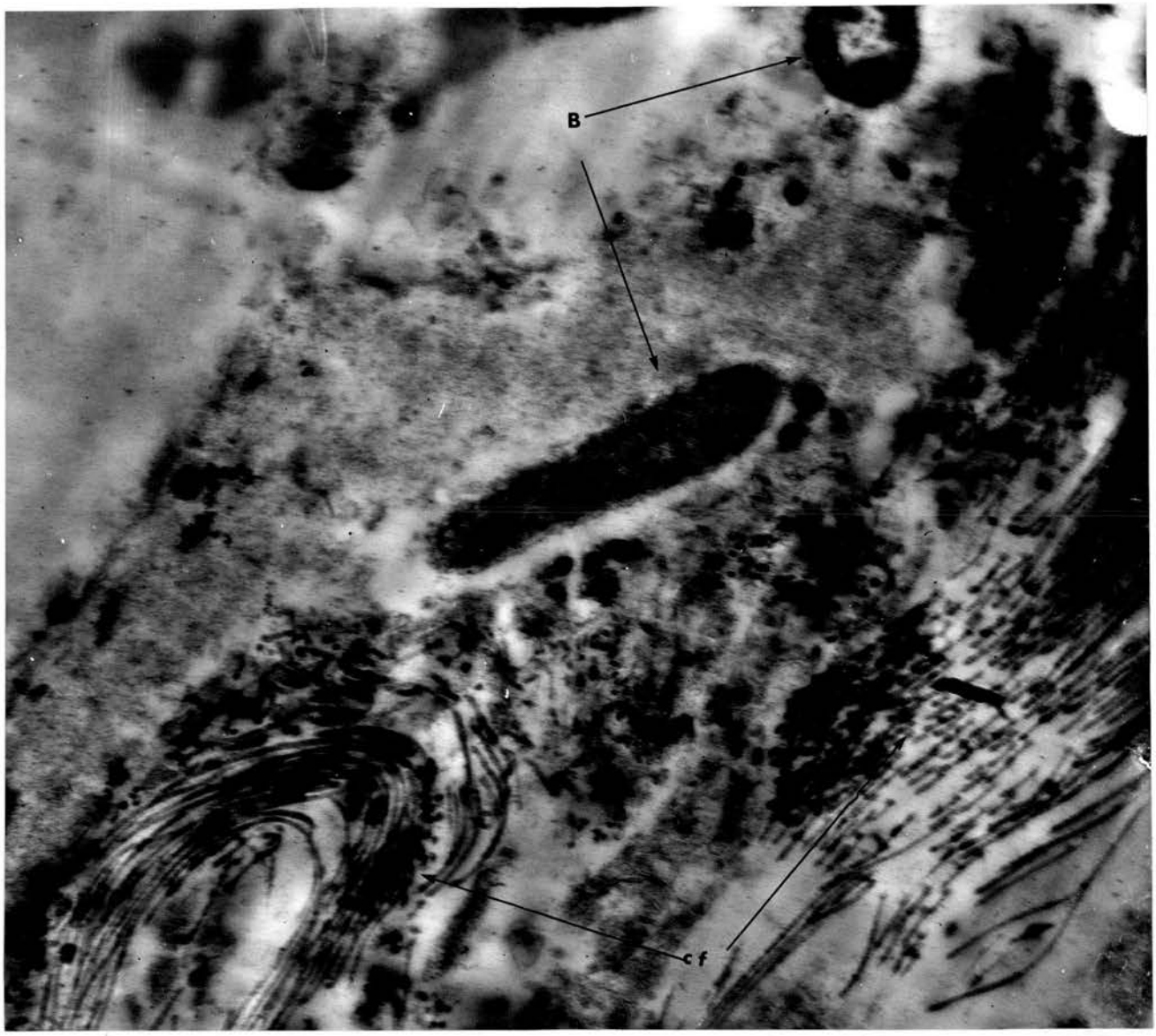

Fig. 32 Experimental Salmonellosis VI. Electron-micrograph of a section of the duodenum of an albino rat showing two bacteria (B) - one in transverse section shows fimbriae and one in longitudinal section (compare with Fig. 33) lying close to the base of the epithelial cell where numerous collagen fibres (cf) are seen. Magnification 30,000.

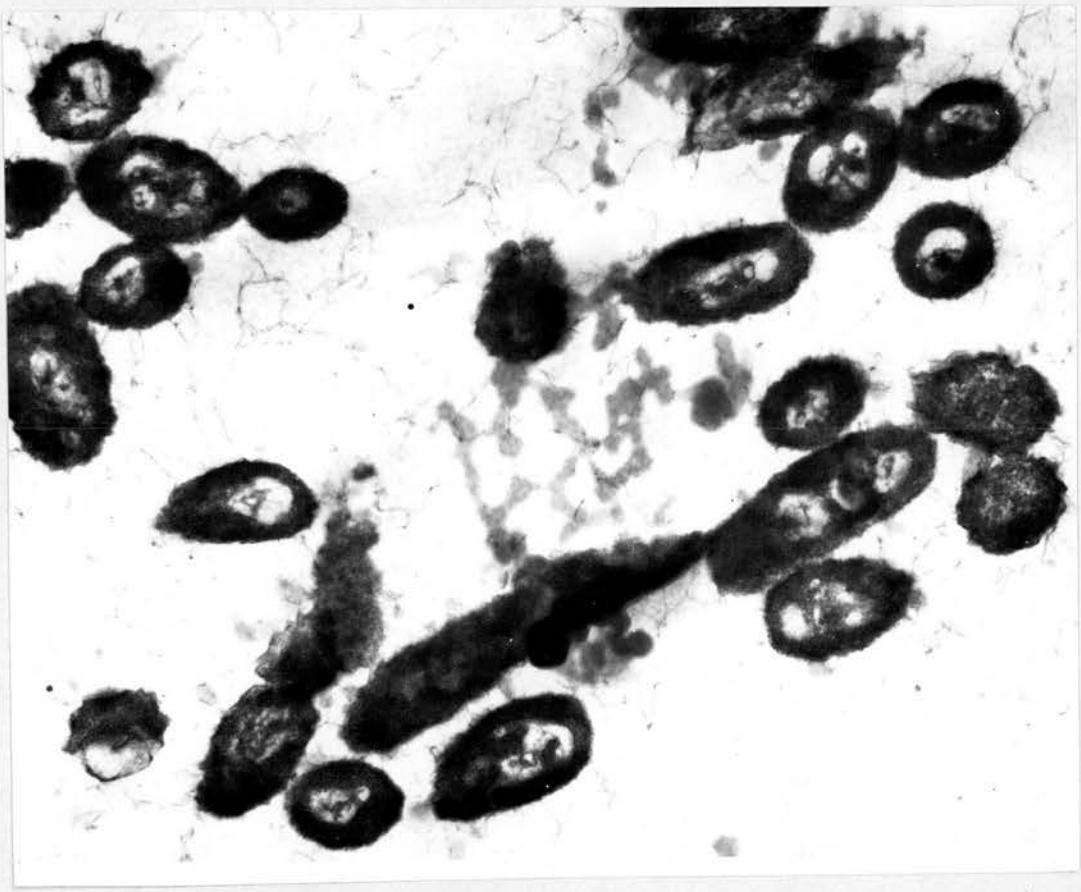

Fig. 33 Experimental Salmonellosis VI. Electron micrograph showing transverse and oblique sections of araldite embedded fimbriate/flagellate S. typhimurium organisms. Magnification 32,000.

TABLE XV

Experimental salmonellosis VII. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY AVIRULENT FIMBRIATE/FLAGELLATE S. TYPHIMURIUM 6351 NF/nfl, PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY SINGLE SUBCUTANEOUS INJECTIONS AND THEY WERE CHALLENGED ON THE 15th DAY WITH AN INTRAPERITONEAL INJECTION OF A HETEROLOGOUS STRAIN (S. TYPHIMURIUM 1566F OF PHAGE TYPE 1a/U57). SURVIVORS WERE KILLED ON THE 22nd DAY OF CHALLENGE.

S.typhimurium 6351 NF/nfl vaccine preparation	Active immunisation		Deaths prior to challenge	Intraperitoneal challenge		Mortality	Survivors	Survivors infected	Percentage Mortality	Percentage Infectivity	Remarks
	Log dose	No. of mice		Log dose	No. of mice						
Heat-killed phenol-preserved	3	36	0	3.1	18	11/18	7	7	66.1	100	No protection
				5.1	18	14/18	4	4	77.8	100	" "
	5	36	0	3.1	18	10/18	8	8	55.5	100	" "
				5.1	18	14/18	4	4	77.8	100	" "
	7	36	2	3.1	17	5/17	12	12	29.4	100	Fair protection
				5.1	17	13/17	4	4	76.5	100	No protection
Acetone-killed freeze-dried	3	36	1	3.1	17	9/17	8	8	52.9	100	" "
				5.1	18	14/18	4	4	77.8	100	" "
	5	36	0	3.1	18	12/18	6	5	66.6	93.6	No protection but one survivor was free of infection
				5.1	18	14/18	4	4	77.8	100	No protection
	7	36	0	3.1	18	10/18	8	8	55.5	100	" "
				5.1	18	14/18	4	4	77.8	100	" "
Control group of 36 unvaccinated mice			0	3.1	18	10/18	8	8	55.5	100	Control
				5.1	18	12/18	6	6	66.6	100	"

Mortality resulting from immunisation = 3/216 or 1.5 per cent.

Log dose of vaccine was adjusted after turbidimetric estimation using Brown's opacity tubes.

Log dose challenge was estimated from viable counts by the Miles and Misra method (1938). The intraperitoneal challenge doses log 3.1 and log 5.1 were equivalent to approximately 0.8×10^{2} and 0.8×10^{4} intraperitoneal LD50 doses for mice respectively.

Each group of test animals contained an equal number of male and female mice; the mice weighed 19-22 grams each and the groups were balanced by weight.

F = fimbriate
NF = non-fimbriate
nfl = non-flagellate

Experimental salmonellosis VII

Immunity Studies

Experiments were designed and carried out to compare the protective potencies for Swiss white mice of (a) killed and (b) alive attenuated vaccines prepared from the parent non-fimbriate/non-flagellate S. typhimurium strain 6351 N/nfl and its phage-mediated transduced fimbriate/flagellate derivative, strain 6354 FT/flt. Various immunising schedules were initially employed and in all of these early experiments the mice were subsequently challenged by injections of S. typhimurium administered intraperitoneally. After establishing reliable immunising schedules, these were employed in experiments in which the mice were subsequently challenged by the oral route. Throughout this study the same known virulent, heterologous, fimbriate/flagellate S. typhimurium strain 1566 F/fl was used to challenge the mice. An ample freeze-dried stock of the challenge strain was prepared beforehand in ampoules and for each experiment a fresh ampoule from this stock was used to produce the culture from which the challenge bacterial inocula were prepared.

(a) Experiments with killed vaccines

Heat-killed phenol-preserved (HP) and acetone-killed freeze-dried (AK) vaccines were prepared from common pooled washed cultures of the respective S. typhimurium strains, 6351 N/nfl and 6354 FT/flt, and stocked in bulk (see Methods). Throughout this series of experiments,

TABLE XVI

Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY VIRULENT *S. TYPHIMURIUM* 6454 FT/flt (A PHAGE-TRANSDUCED FIMBRIATE/FLAGELLATE STRAIN DERIVED FROM *S. TYPHIMURIUM* 6351 NF/nfl). THE MICE WERE IMMUNISED BY SINGLE SUBCUTANEOUS INJECTIONS AND WERE CHALLENGED ON THE 15th DAY BY INTRAPERITONEAL INJECTIONS WITH A HETEROLOGOUS STRAIN (*S. TYPHIMURIUM* 1566F PHAGE TYPE 1a/U57). SURVIVORS WERE KILLED ON THE 22nd DAY OF CHALLENGE.

S. typhimurium 6354 FT/flt vaccine preparation	Immunisation: Log dose vaccine	Immunisation: No. of mice	Deaths prior to challenge	Intraperitoneal challenge: Log dose	Intraperitoneal challenge: No. of mice	Mortality	Survivors	Survivors infected	Infectivity	Percentage Mortality	Percentage Infectivity	Remarks
Heat-killed phenol-preserved	3	24	0	3.2	12	10/12	2	2	12/12	83.3	100	No protection
				5.2	12	9/12	3	3	12/12	75	100	" "
	5	24	2	3.2	11	7/12	5	5	12/12	58.3	100	Slight protection
				5.2	11	11/11	0	0	11/11	100	100	No protection
	7	24	0	3.2	12	4/12	8	7	11/12	33.3	91.6	Fair protection. One survivor was free of infection
				5.2	12	6/12	6	6	12/12	50	100	Fair protection
Acetone-killed freeze-dried	3	24	0	3.2	12	9/12	3	3	12/12	75	100	No protection
				5.2	12	6/12	6	6	12/12	50	100	Fair protection
	5	24	2	3.2	11	11/11	0	0	11/11	100	100	No protection
				5.2	11	10/11	1	1	11/11	90.9	100	" "
	7	24	4	3.2	10	5/10	5	5	10/10	50	100	Fair protection
				5.2	10	7/10	3	3	10/10	70	100	Slight protection
Control group of 24 unvaccinated mice			2	3.2	11	8/11	3	3	11/11	72.7	100	Control
				5.2	11	10/11	1	1	11/11	90.9	100	"

Mortality resulting from immunisation = 8/144 or 2.7 per cent.

Log dose of vaccines was adjusted by Brown's opacity tubes.

Log dose challenge was estimated from viable counts by the Miles and Misra method (1938). The intraperitoneal challenge doses were approximately equivalent to 10^2 and 10^4 intraperitoneal LD50 doses for mice.

Each group of the test animals contained an equal number of male and female mice; the mice weighed 19-22 grams each and the groups were balanced by weight.

F = fimbriate
NF = non-fimbriate
nfl = non-flagellate
FT = phage-transduced fimbriate
flt = phage-transduced flagellate

appropriate fresh dilutions of the vaccines were prepared from the stocks for immediate use.

Studies of degrees of protection resulting from single subcutaneous immunising doses of killed vaccines against intraperitoneal challenge

In the first series, the vaccines were prepared from S. typhimurium 6351 N/nfl. One group of mice was immunised with heat-killed phenol-preserved (HP) and another group received acetone-killed freeze-dried (AK) vaccine. In the second series, HP and AK killed vaccines prepared from S. typhimurium strain 6354 FT/flt were similarly employed to immunise mice. Single subcutaneous doses of the killed vaccines containing log 3, log 5 and log 7 organisms were compared. On the 15th day of immunisation each group of mice was divided into two approximately equal sub-groups; one sub-group was challenged intraperitoneally with an inoculum containing approximately log 3 organisms of S. typhimurium strain 1566 F/fl, and the other sub-group received a challenge dose of log 5 organisms. These challenge doses are approximately equivalent to 100 and 10,000 intraperitoneal LD50 doses for non-immune mice respectively. The survivors were killed on the 22nd day following challenge. The results of the experiments are shown in detail in tables XV and XVI. A single subcutaneous dose of the killed vaccine prepared from either of the two S. typhimurium strains gave no protection to the mice

TABLE XVII

Experimental salmonellosis VII. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES (USED WITH AND WITHOUT FREUND'S INCOMPLETE ADJUVANT). THE VACCINES WERE PREPARED FROM A RELATIVELY AVIRULENT S. TYPHIMURIUM 6351 NF/nf1: phage type 2a/13. THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS CONTAINING LOG 7 AND LOG 8 ORGANISMS, AND THE INTERVAL BETWEEN INJECTIONS WAS 14 DAYS. THE MICE WERE CHALLENGED INTRAPERITONEALLY, 10 DAYS AFTER THE SECOND IMMUNISING DOSE, WITH S. TYPHIMURIUM 1566 F: PHAGE TYPE 1a/U57. SURVIVORS WERE KILLED ON THE 29th DAY AFTER THE CHALLENGE.

S. typhimurium 6351 NF/nf1 vaccine preparation	No. of mice immunised	Deaths prior to challenge		Intra-peritoneal challenge		Mortality	Survivors	Survivors infected	Infect-ivity	Percentage mortality	Percentage infectivity	Remarks
		1st dose	2nd dose	Log dose	No. of mice							
Heat-killed phenol preserved (HP)	40	2	0	3.5	19	8/19	11	11	19/19	42.1	100	Some protection
				5.5	19	14/19	5	5	19/19	73.6	100	No protection
HP + Freund's incomplete adjuvant	40	2	1	3.5	18	10/18	8	7	17/18	55.5	94.4	No protection. One survivors was free from infection
				5.5	19	12/19	7	7	19/19	63.1	100	Some protection
Acetone-killed and freeze-dried (AK)	40	2	0	3.5	19	14/19	5	5	19/19	73.6	100	No protection
				5.5	19	16/19	3	3	19/19	86.1	100	" "
AK + Freund's incomplete adjuvant	40	4	1	3.5	18	15/18	3	3	18/18	83.3	100	" "
				5.5	17	15/17	2	2	17/17	88.2	100	" "
Control group of 20 unvaccinated mice		1		3.5	10	6/10	4	4	10/10	60	100	Non-immune control
				5.5	9	8/9	1	1	9/9	89.1	100	" " "

The intraperitoneal challenge doses were approximately equivalent to 2×10^2 and 2×10^4 intraperitoneal ID50 doses for mice.

Each group contained an equal number of male and female mice which weighed 18 to 22 grams each. The groups were balanced by weight.

F = fimbriate NF = non-fimbriate nf1 = non-flagellate

against intraperitoneal challenge with the heterologous S. typhimurium. It is interesting that the mortality was slightly higher in the vaccinated groups of mice than in the unvaccinated control groups. In both the experiments almost all of the survivors were found to be infected.

Studies of protection afforded by (a) two subcutaneous immunising doses of killed vaccine or (b) a single dose with adjuvant, against intraperitoneal challenge

Two series of experiments were performed to test HP and AK vaccines prepared from the non-fimbriate/non-flagellate strain of S. typhimurium 6351 N/nfl and its fimbriate/flagellate derivative strain 6354 FT/flt. In each case, a subcutaneous immunising dose of log 7 organisms was followed, after an interval of 14 days, with a subcutaneous dose of log 8 organisms. In addition to the experiments (table XVII) with the killed vaccines prepared from the non-fimbriate/non-flagellate S. typhimurium strain 6351 N/nfl, tests were also performed employing similar groups of mice immunised with a single subcutaneous injection of the vaccines prepared with Freund's incomplete adjuvant and containing log 7 organisms. Ten days after the second immunising doses of the vaccines, or 24 days after the single doses of the vaccines with Freund's incomplete adjuvant, the mice were challenged intraperitoneally as described in the earlier experiments.

TABLE XVIII

Experimental salmonellosis VII

Immunity studies. Experiment VII. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY VIRULENT STRAIN OF S. TYPHIMURIUM 6354 FT/flt, PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS CONTAINING LOG 7 AND LOG 8 ORGANISMS RESPECTIVELY. THE INTERVAL BETWEEN THE INJECTIONS WAS 14 DAYS AND THE MICE WERE CHALLENGED INTRAPERITONEALLY 10 DAYS AFTER THE SECOND IMMUNISING DOSE. THE CHALLENGE STRAIN WAS S. TYPHIMURIUM 1566F: PHAGE TYPE 1a/U57. THE SURVIVORS WERE KILLED ON THE 29th DAY AFTER CHALLENGE.

S. typhimurium 6354 FT/flt vaccine preparation	No. of mice immunised	Deaths prior to challenge		Intraperitoneal challenge		Mortality	Survivors	Survivors infected	Infectivity	Percentage mortality	Percentage infectivity	Mean time to death in days	Remarks
		after 1st dose	after 2nd dose	Log dose	No. of mice								
Heat-killed phenol-preserved	40	0	0	3.0	20	6/20	14	14	20/20	30	100	19	Good protection
				5.0	20	7/20	13	13	20/20	35	100	15.8	Good protection
Acetone-killed freeze-dried	40	0	0	3.0	20	8/20	12	11	19/20	40	95	15.3	Good protection. One survivor was free of infection
				5.0	20	12/20	8	8	20/20	60	100	14.4	Fair protection
Control: group of 20 unvaccinated mice		0		3.0	10	9/10	1	1	10/10	90	100	12.8	Control
				5.0	10	10/10	0	0	0	100	100	11.1	Control

The intraperitoneal challenges were approximately equivalent to 0.6×10^2 and 0.6×10^4 intraperitoneal LD50 doses for mice.

Each group contained an equal number of male and female mice and the groups were balanced by weight. The mice weighed from 18 to 22 grams each.

FT = phage-transduced fimbriate flt = phage-transduced flagellate

The results of the experiments with the two-dose immunising schedule employing the killed vaccines prepared from S. typhimurium strain 6351 N/nfl, and the single-dose immunisation with this vaccine as Freund's incomplete adjuvant mixture, are shown in table XVII. The percentage mortalities indicate that the heat-killed phenol-preserved vaccine (used with or without the adjuvant) gave some small degree of protection whilst no such protection resulted from immunisation with the acetone-killed freeze-dried vaccine. In contrast, immunisation with either the heat-killed phenol-preserved or the acetone-killed freeze-dried vaccines prepared from the relatively virulent S. typhimurium strain 6354 FT/flt gave fairly good protection (table XVIII). Here again the heat-killed phenol-preserved vaccine gave slightly better protection than did the acetone-killed vaccine. As in the single dose immunisation schedules, most of the survivors were found to be infected when killed on the 29th day of challenge.

Studies of protection afforded by two subcutaneous immunising doses of killed vaccine against an oral challenge

Four groups of mice were immunised with four killed vaccines using the two-dose schedule described earlier, and these animals were challenged orally at two dose levels on the 10th day after the administration of the second immunising dose. The oral challenge doses of the

TABLE XIX

Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM S. TYPHIMURIUM STRAINS 6351 N/nfl AND 6354 FT/flt. THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS OF LOG 7 AND LOG 8 ORGANISMS RESPECTIVELY. THE INTERVAL BETWEEN THE INJECTIONS WAS 14 DAYS AND THE MICE WERE CHALLENGED ORALLY, 10 DAYS AFTER THE SECOND IMMUNISING DOSE WITH S. TYPHIMURIUM 1566 F/fl. SURVIVORS WERE KILLED ON THE 23rd DAY FOLLOWING CHALLENGE.

Vaccine preparation	No. of mice immun-ised	Deaths prior to challenge		Oral challenge		Mortality	Survivors	No. of survivors infected	Infectivity	Percentage		Remarks
		after 1st dose	after 2nd dose	Log dose	No. of mice					Mortality	Infectivity	
Heat-killed phenol-preserved (HP) strain 6351N/nfl	24	0	0	6.1	12	2/12	10	9	11/12	16.6	91.6	Good protection: one survivor free of injection
				8.1	12	2/12	10	10	12/12	16.6	100	Good protection
Acetone-killed freeze-dried (Ak) strain 6351N/nfl	24	1	0	6.1	11	0/12	12	11	11/12	0	91.6	Good protection: one survivor free of injection
				8.1	12	3/12	9	9	12/12	25	100	Good protection
Heat-killed phenol-preserved (HP) strain 6354 FT/flt	24	2	0	6.1	11	1/11	10	8	9/11	9.1	81.9	Good protection: two survivors free of injection
				8.1	11	4/11	7	6	10/11	36.4	90.9	Good protection: one survivor free of infection
Acetone-killed freeze-dried (Ak) strain 6354FT/flt	24	1	3	6.1	11	1/11	10	8	9/11	9.1	81.9	Good protection - two survivors free of infection
				8.1	9	2/9	7	5	7/9	22.2	77.7	Good protection - two survivors free of injection
Control: group of 24 unvaccinated mice		0	0	6.1	12	5/12	7	7	12/12	41.7	100	Non-immune controls
				8.1	12	9/12	3	3	12/12	75	100	

Log dose of vacinne was adjusted after turbidimeteric estimation using Brown's standard opacity tubes. Log dose challenge was estimated from viable counts by Miles and Misra Method. Each group of test animals contained an equal number of male and female mice and each animal weighed 16-22 grams. The groups were balanced by weight.

N = non-fimbriate F = fimbriate FT = phage-transduced fimbriate nfl = non-flagellate
flt = phage-transduced flagellate fl = flagellate

S. typhimurium strain 1566 F/fl contained log 6.1 and log 8.1 organisms, equivalent to 30 and 300 oral LD50 doses for mice. The detailed results are shown in table XIX. The percentage mortalities indicate that fairly good protection of mice against oral challenge results from immunisation with heat-killed phenol-preserved or acetone-killed freeze-dried vaccines prepared from the non-fimbriate/flagellate S. typhimurium strain 6351 N/nfl or the phage-transduced fimbriate/flagellate S. typhimurium strain 6354 FT/flt. About 80 to 100 per cent. of the survivors were found to be infected on the 23rd day after the challenge.

(b) Experiments with alive attenuated vaccines

These vaccines consisted of single step streptomycin-resistant mutants prepared from the parent non-fimbriate/non-flagellate S. typhimurium strain 6351 N/nfl/SR and its phage-mediated fimbriate/flagellate derivative strain 6354 FT/flt/SR. Three experiments were carried out. The first experiment was designed to find out whether the subcutaneous or the oral route of immunisation gave better protection and to determine a suitable immunising dose. In the second and third experiments the protective potencies of the two attenuated vaccines were compared. In the first two experiments the immunised mice were challenged intraperitoneally and in the third experiment they were challenged orally. The challenge strain employed in these experiments was the same as that used in

TABLE XX (Contd.)

Vaccine preparation	Active immunisation			Deaths prior to challenge	Intraperitoneal challenge		Mortality	Survivors	Survivors infected	Percentage mortality	Percentage infectivity	Remarks
	Route	Log dose	No. of mice		Log dose	No. of mice						
Live attenuated vaccine 6351 N/nfl/SR	Oral	0.9	24*	1	3.2	11	0/11	11	11	0	100	Good protection
					5.2	12	2/12	10	10	16.5	100	Fairly good protection
		1.9	24*	0	3.2	12	0/12	12	11	0	91.6	Good protection: one survivor free from infection
					5.2	12	0/12	12	12	0	100	Good protection
		2.9	24*	0	3.2	12	0/12	12	11	0	91.6	Good protection
					5.2	12	1/12	11	10	8.2	91.6	Good protection: two survivors free from infection
Control: a group of 24 unvaccinated mice				1	3.2	11	9/11	2	2	81.9	100	Control
					5.2	12	11/12	1	1	91.6	100	Control

Mortality resulting from immunisation = 14/216 or 6.5 per cent.

Log dose of vaccine and challenge were estimated from viable counts by the Miles and Misra method (1938). The intraperitoneal challenge doses were approximately equivalent to 10^2 and 10^4 intraperitoneal LD50 doses for mice.

Mice were distributed and balanced as in the previous experiment.

* The mice in these groups were treated with streptomycin orally on the day prior to vaccination.

F = fimbriate NF = non-fimbriate nfl = non-flagellate

TABLE XX

Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCY FOR SWISS WHITE MICE OF LIVE ATTENUATED VACCINE (A SINGLE STEP STREPTOMYCIN-RESISTANT MUTANT) PREPARED FROM A RELATIVELY AVIRULENT S. TYPHIMURIUM 6351 NF/nf1 PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY THE SUBCUTANEOUS AND ORAL ROUTES WITH SINGLE DOSES OF THE VACCINE. ONE GROUP OF THE ORALLY IMMUNISED MICE WERE STREPTOMYCIN-TREATED. THE MICE WERE CHALLENGED ON THE 15th DAY WITH AN INTRAPERITONEAL INJECTION OF A VIRULENT S. TYPHIMURIUM 1566F OF PHAGE TYPE 1a/U57. SURVIVORS WERE KILLED ON THE 22nd DAY OF CHALLENGE.

Vaccine preparation	Active immunisation			Deaths prior to challenge	Intraperitoneal challenge		Mortality	Survivors	Survivors infected	Percentage mortality	Percentage infectivity	Remarks
	Route	Log dose	No. of mice		Log dose	No. of mice						
Live attenuated vaccine 6351N/ nf1/SR	Subcutaneous	0.9	24	0	3.2	12	2/12	10	9	16.5	91.6	Fairly good protection. One survivor free of infection
					5.2	12	3/12	9	9	25	100	Fair protection
		1.9	24	2	3.2	11	1/11	10	10	9.1	100	Good protection
					5.2	11	2/11	9	9	18.2	100	Fairly good-protection
		2.9	24	3	3.2	10	0/11	10	9	0	90	Good protection: one survivor free of infection
					5.2	11	0/11	11	11	0	100	Good protection
-do-	Oral	0.9	24	2	3.2	11	5/12	7	6	41.6	91.6	Fair protection: one survivorfree of infection
					5.2	11	8/12	4	4	66.6	100	Slight protection
		1.9	24	2	3.2	11	9/11	2	2	81.9	100	No protection
					5.2	11	7/11	4	4	63.7	100	Slight protection
		2.9	24	3	3.2	10	3/10	7	7	30	100	Fair protection
					5.2	11	10/11	1	1	91	100	No protection

Contd.

the experiments with the killed vaccines. Appropriate control mice were included in each experiment.

The influence of mode of administration and dose on degree of protection afforded by live attenuated vaccine

This experiment was carried out with attenuated vaccine 6351 N/nfl/SR. The vaccine was administered subcutaneously to 3 groups of mice in three strengths of approximately log 1, log 2 and log 3 organisms. The same graded doses of the attenuated vaccine were given orally to another 3 groups of mice and this experiment was duplicated using mice that had been treated orally with streptomycin on the previous day. Streptomycin clears the gut of the normal commensal flora of the intestine and this helps the attenuated S. typhimurium organisms to establish themselves. On the 15th day of immunisation all of the mice were challenged intraperitoneally as described in the previous experiments. The challenge dose of S. typhimurium 1566 F/fl was employed in two strengths of log 3.2 and log 5.2 organisms. The survivors were killed on the 22nd day of challenge. The details of the experiment and the results obtained are shown in table XX. A single dose of log 3 organisms gave the best protection in all three lots of the immunised mice. Immunisation by the subcutaneous route, and the oral route in the case of streptomycin-treated mice resulted in almost complete protection, the percentage mortalities ranging from 0 to 8.2 whereas 81.9 and 91.6 per cent. of the control

TABLE XXI

Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCY FOR SWISS WHITE MICE OF ALIVE ATTENUATED VACCINES (SINGLE STEP STREPTOMYCIN-RESISTANT MUTANTS) PREPARED FROM S. TYPHIMURIUM STRAINS 6351 NF/nfl AND 6354 FT/flt BOTH OF PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY SINGLE SUBCUTANEOUS INJECTIONS. THE VACCINES WERE EMPLOYED IN THREE STRENGTHS. THE MICE WERE CHALLENGED ON THE 15th DAY OF IMMUNISATION BY INTRAPERITONEAL INJECTION OF S. TYPHIMURIUM 1566F OF PHAGE TYPE 1a/U57. SURVIVORS WERE KILLED ON THE 28th DAY OF CHALLENGE.

Attenuated S. typhimurium vaccine preparation	Immunisation: Log dose	Immunisation: No. of mice	Deaths prior to challenge	Intraperitoneal challenge: Log dose	Intraperitoneal challenge: No. of mice	Ward life survivors	Survivors/Surv. infected	Infectivity	Percentage mortality	Percentage infectivity	Remarks
6351N/nfl/SR	1.1	24	0	5.3	9	4/9	5	5	9/9	44.4	100	Fair degree of protection
				7.3	9	9/9	0	0	9/9	100	100	No protection
	2.1	24	1	5.3	9	0/9	9	9	9/9	0	100	Good protection
				7.3	9	7/9	2	2	9/9	77.7	100	Slight "
	3.1	24	0	5.3	9	0/9	9	9	9/9	0	100	Good "
				7.3	9	7/9	2	2	9/9	77.7	100	Slight "
6354FT/flt/SR*	1.0	24	0	5.3	9	9/9	0	0	9/9	100	100	No "
				7.3	9	9/9	0	0	9/9	100	100	" "
	2.0	24	0	5.3	9	5/9	4	4	9/9	55.5	100	Fair "
				7.3	9	9/9	0	0	9/9	100	100	No "
	3.0	24	0	5.3	9	8/9	1	1	9/9	88.8	100	" "
				7.3	9	9/9	0	0	9/9	100	100	" "
Control: group of 24 unvaccinated mice			0	5.3	12	11/12	1	1	12/12	91.6	100	Control
				7.3	12	12/12	0	0	12/12	100	100	
Strain 6351 NF/nfl/SR immunised cont: not challenged	1.1	6		0	0	0/6	6	1	1/6	0	16.6	Control
	2.1	5		0	0	0/5	5	0	0/5	0	0	
	3.1	6		0	0	0/6	6	0	0/6	0	0	
Strain 6354 FT/flt/SR immunised control: not challenged	1.0	6		0	0	0/6	6	0	0/6	0	0	Control
	2.0	6		0	0	0/6	6	0	0/6	0	0	
	3.0	6		0	0	0/6	6	0	0/6	0	0	

Mortality resulting from immunisation = 1/144 or 0.7 per cent.
Log doses of the vaccines and challenge strains were estimated by the Miles & Misra method. The intraperitoneal challenge doses were approximately equivalent to 1.2×10^4 and 1.2×10^6 intraperitoneal LD50 doses for mice.
Each group of test animals contained an equal number of male and female mice; the mice weighed 16 to 20 grams each and the groups were balanced by weight.

* Strain 6354FT/flt/SR was found to be streptomycin dependent for growth.

NF = non-fimbriate nfl = non-flagellate SR = streptomycin-resistant
FT = phage-transduced fimbriate flt = phage-transduced flagellate

unvaccinated mice died. As in the immunity experiments with the killed vaccines, 90 to 100 per cent. of the survivors were found infected on the 22nd day after the intraperitoneal challenge.

Comparison of the protective potencies of attenuated vaccines

The vaccines prepared from the parent non-fimbriate/non-flagellate S. typhimurium 6351 N/nfl and its fimbriate/flagellate derivative 6354 FT/flt were labelled 6351 N/nfl/SR and 6354 FT/flt/SR. Two experiments were carried out as follows. In the first experiment (table XXI) single subcutaneous doses of three different concentrations (containing approximately log 1, log 2 and log 3 organisms) of the two vaccines were used to immunise groups of Swiss white mice. On the 15th day of immunisation the mice were challenged intraperitoneally at two dose levels as described in previous experiments; the challenge doses contained log 5.3 and log 7.3 organisms of S. typhimurium 1566 F/fl. The survivors were killed on the 28th day of challenge. In the other experiment (table XXII) two groups of mice were immunised with single subcutaneous doses (containing log 3 organisms) of the two vaccines. In this experiment the mice were challenged orally on the 15th day of immunisation. As in all the other experiments, the mice were challenged at two dose levels with S. typhimurium 1566 F/fl (log 6.1 and log 8.1 organisms). The survivors were killed on the 23rd day following challenge. Appropriate non-immune and immunised mice were included as

TABLE XXII

Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF ALIVE ATTENUATED VACCINES (SINGLE STEP STREPTOMYCIN-RESISTANT MUTANTS) PREPARED FROM S. TYPHIMURIUM STRAINS 6351N/nf1 and 6354FT/f1t. THE MICE WERE IMMUNISED WITH SINGLE SUBCUTANEOUS INJECTIONS. THEY WERE CHALLENGED ORALLY ON THE 15th DAY OF IMMUNISATION WITH S. TYPHIMURIUM STRAIN 1566F/f1t. THE SURVIVORS WERE KILLED ON THE 23rd DAY FOLLOWING THE CHALLENGE.

Attenuated S.typhimurium vaccine preparation	Immunisation No. of mice	Deaths prior to challenge	Oral challenge Log dose	Oral challenge No. of mice	Mortality	Survivors	No. of survivors infected	Infectivity	Percentage Mortality	Percentage Infectivity	REMARKS
6351N/nf1/SR	24	1	6.1	11	2/11	9	6	8/11	18.2	72.7	Good protection: 3 survivors free of infection
			8.1	12	2/12	10	5	7/12	16.6	58.3	Good protection: 5 survivors free of infection
6354FT/f1t/SR	24	0	6.1	12	1/12	11	10	11/12	8.3	91.6	Good protection: 1 survivor free of infection
			8.1	12	5/12	7	7	12/12	41.7	100	Fair protection
Immunised controls not challenged: Strain 6351N/nf1/SR	12	0			0/12	12	3	3/12	0	24.9	Immunised control: not challenged
Immunised controls not challenged: Strain 6354FT/f1t/SR	12	0			0/12	12	1	1/12	0	8.3	Immunised control: not challenged
Control: group of 24 unvaccinated mice		1	6.1	12	5/12	7	7	12/12	41.7	100	Non-immune control challenged
			8.1	11	8/11	3	3	11/11	72.7	100	Non-immune control challenged

Each group contained an equal number of male and female mice which weighed 16-22 grams each. The groups were balanced by weight.

Log doses of the vaccines and the challenges were estimated from viable counts by Miles & Misra method.

N = non-fimbriate FT = phage-transduced fimbriate F = fimbriate nf1 = non-flagellate

f1t = phage-transduced flagellate f1 = flagellate SR = streptomycin-resistant

controls in each experiment. The results of the first experiment (table XXI) confirm the findings of the previous experiment (table XX) that the immunisation of mice with a single subcutaneous dose of attenuated vaccine 6351 N/nfl/SR containing log 3.1 organisms, conferred good protection against intraperitoneal challenge with log 5.3 organisms of the challenge strain, but there was no significant protection when the higher challenge dose of log 7.3 organisms was employed. Immunisation with attenuated vaccine 6354 FT/flt/SR resulted in little or no protection, probably because this attenuated strain was found to be streptomycin dependent for its growth. All survivors killed on the 28th day of challenge were found to be infected. There were no deaths amongst the immunised but unchallenged control groups of mice.

The results of the second experiment (table XXII) show that immunisation with both attenuated vaccines conferred equally good degrees of protection of the mice against oral challenge with log 6.1 and log 8.1 organisms of S. typhimurium 1566 F/fl. Here again there were no deaths amongst the immunised but unchallenged control groups of mice.

Mortality attributable to immunisation procedures

In these experiments, the mortality of mice as a result of immunisation with the killed vaccines ranged from 0 to 6.25 per cent.; with the attenuated vaccines, mortality ranged from 0.7 to 6.5 per cent.

O-antibody response in mice to immunisation with killed and

TABLE XXIII

Experimental salmonellosis VII. IMMUNITY STUDIES. SOMATIC (O) ANTIBODY RESPONSES OF WHITE MICE TO IMMUNISATION WITH HEAT-KILLED AND ACETONE-KILLED VACCINES (WITH AND WITHOUT FREUND'S INCOMPLETE ADJUVANT) AND ATTENUATED VACCINES PREPARED FROM S. TYPHIMURIUM STRAINS 6351NF/nfl AND 6354FT/flt. THE MICE WERE IMMUNISED BY THE SUB-CUTANEOUS ROUTE. DOSAGE:- (i) HEAT-KILLED (HP) OR ACETONE-KILLED (AK) VACCINE - TWO INJECTIONS CONTAINING LOG 7 AND LOG 8 ORGANISMS; INTERVAL BETWEEN INJECTIONS WAS 14 DAYS.
(ii) HP OR AK VACCINE PLUS ADJUVANT - A SINGLE INJECTION CONTAINING LOG 7 ORGANISMS.
(iii) ATTENUATED VACCINES - SINGLE INJECTION CONTAINING LOG 3 ORGANISMS.

* SERUM SAMPLES COLLECTED FROM 12 NON-IMMUNE MICE AT THE COMMENCEMENT OF THE EXPERIMENT WERE ALL NEGATIVE FOR THE O ANTIBODIES.

S.typhimurium vaccine preparation	Tube agglutination test: reciprocals of O antibody titres on day *						
	7	14	21	28	35	42	49
Killed vaccines							
HP vaccine strain 6351NF/nfl	<10	< 10	< 10	< 10	40	> 320	-
	<10	< 10	< 10	< 10	> 320	80	320
	<10	< 10	< 10	< 10	> 320	80	-
AK vaccine strain 6351NF/nfl	<10	< 10	< 10	160	10	10	-
	<10	< 10	< 10	160	< 10	< 10	320
	<10	< 10	< 10	< 10	< 10	< 10	> 320
Freund's incomplete adjuvant + HP vaccine strain 6351NF/nfl	<10	< 10	< 10	320	40	160	-
	<10	< 10	< 10	160	20	40	320
	<10	< 10	< 10	160	> 320	40	-
Freund's incomplete adjuvant + AK vaccine strain 6351NF/nfl	<10	< 10	< 10	160	40	40	80
	<10	< 10	< 10	20	10	40	320
	<10	< 10	< 10	20	< 10	< 10	
HP vaccine strain 6354FT/flt	<10	40	40	> 320	640	> 5120	-
	20	80	20	> 320	320	640	-
	<10	40	> 320	> 320	-	-	-
AK vaccine strain 6354FT/flt	<10	20	160	> 320	> 5120	> 5120	-
	<10	40	80	> 320	> 5120	> 5120	-
	<10	40	80	160	-	-	-
Attenuated live vaccines							
Strain 6351NF/nfl/SR	80	> 320	40	160	< 10	1280	160
	80	80	160	160	< 10	320	160
	40	20	40	80	< 10	160	80
Strain 6354FT/flt/SR	<10	20	10	320	2560	> 5120	160
	80	80	10	320	> 5120	> 5120	80
	80	80	40	320	> 5120	> 5120	80

At weekly intervals serum from 2 or 3 mice from each of the immunised groups was collected and titrated for O antibodies.

HP = heat-killed phenol-preserved vaccine NF = non-fimbriate nfl = non-flagellate < = less than > = more than - = not tested AK = acetone-killed freeze-dried vaccine FT = phage-transduced fimbriate flt = phage-transduced flagellate SR = streptomycin-resistant

attenuated S. typhimurium vaccines

The immunisation schedules adopted in this experiment were those which in the previous protection experiments had given the best protection results. The immunising schedules used were as follows:-

(i) Killed vaccines

Both the heat-killed phenol-preserved (HP) and the acetone-killed freeze-dried (AK) vaccines were administered in two subcutaneous injections containing log 7 and log 8 organisms respectively with an interval of 14 days between the injections.

(ii) The killed vaccines used as Freund's incomplete adjuvant mixtures were administered as a single subcutaneous injection containing log 7 organisms.

(iii) The attenuated vaccines were administered as a single subcutaneous injection containing log 3 organisms.

Blood samples were collected on day 0 from 12 non-immune control mice. Thereafter weekly samples were collected from three mice from each immunised group of animals, and this was continued for 7 weeks.

The results of the O-antibody responses are shown in table XXIII. A single injection of the killed vaccines produced no detectable antibody response during the 14 days of observation. Following the second injection of these vaccines there was an observable antibody response; this was decidedly better in mice immunised with the heat-killed and acetone-killed vaccines prepared from the

relatively virulent S. typhimurium strain 6354 FT/flt. The antibody responses produced by these killed vaccines were practically as good as those resulting from immunisation with the attenuated vaccines, with the difference that immunisation with the latter vaccines produced an earlier antibody response demonstrable in the first and second weeks following immunisation. The addition of Freund's incomplete adjuvant to the killed vaccines did not enhance the O-antibody responses of mice studied during the 7 weeks of this experiment.

Mean time to death of mice immunised by killed or attenuated S. typhimurium vaccine and thereafter challenged by the intraperitoneal or oral route

The mean times to death of the mice dying in the various vaccinated groups following intraperitoneal or oral challenge doses of S. typhimurium are noted in the relevant appendices (no. 29 - 38) and recorded in graph form in figs. 34 - 35. Deaths in those groups that received a protective vaccine are fewer in number and tend to occur in the middle or latter part of the challenge period, whereas deaths in groups of mice receiving a less potent vaccine or no vaccine are more numerous, begin to occur earlier and continue to occur later in the challenge period. Minor differences in degrees of protection afforded by different vaccines may therefore be demonstrated in this way under circumstances in which direct comparison of percentage mortalities occurring in the

various groups may show no apparent difference. In the present study, the evidence presented in figs. 34 - 38 (based on the mean times to death of mice dying in the various vaccinated and control groups) confirms the conclusions drawn from the percentage mortality figures.

It should be noted, however, that this type of graph may give a misleading impression in circumstances in which protective vaccination results in cessation of deaths early in the challenge period - see fig. 36. In this case the graph recording the mean time to death of protected mice is continued horizontally because the value remains constant in the absence of further deaths, although it would be more proper to extend the line vertically to infinity. The slopes of the lines would then be comparable and would give a truer indication of the protective value of each vaccine in comparison with the control.

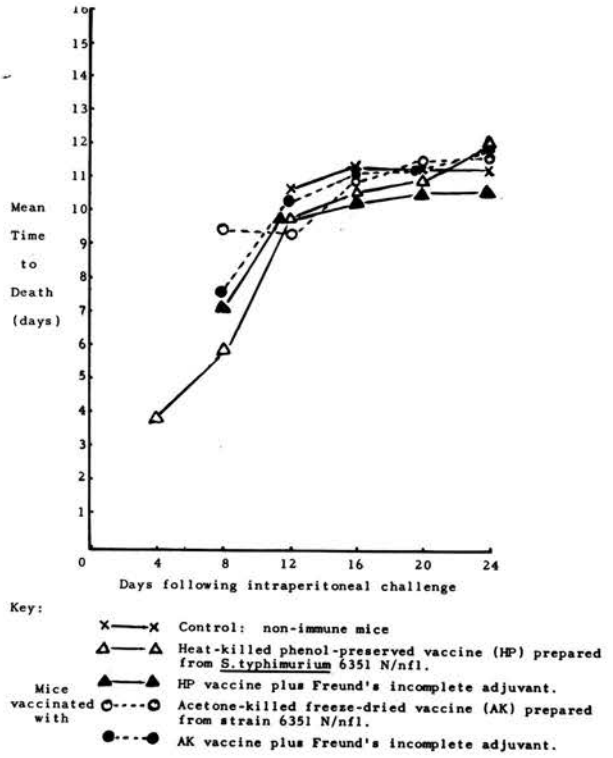

Fig. 34. Experimental salmonellosis VII

Studies of protection afforded by (a) two subcutaneous immunising doses of killed vaccines, or (b) a single dose with adjuvant, against a heterologous intraperitoneal challenge. In the figure, the mean time to death is plotted at 4-day intervals following challenge. The mean times to death of the immunised and non-immune control mice are seen to run very nearly in parallel. Immunisation with these vaccines (prepared from a non-fimbriate/non-flagellate strain of S. typhimurium 6351 N/nfl) gave little or no protection to the mice.

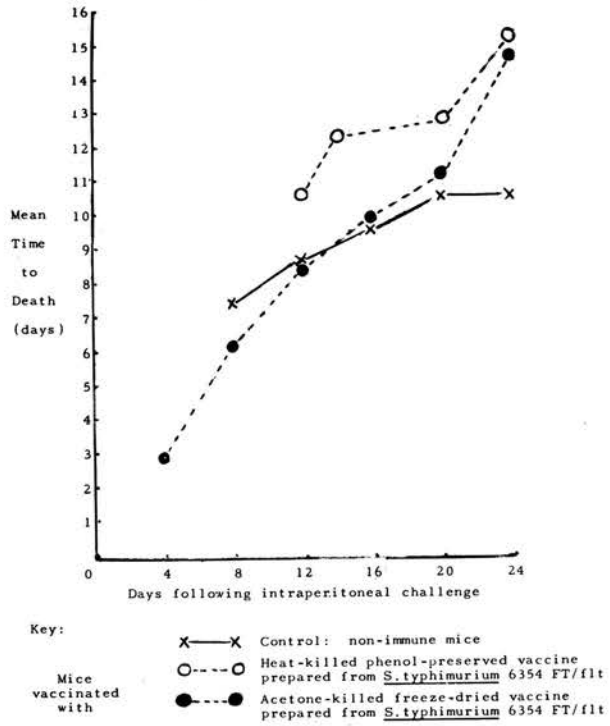

Fig. 35. Experimental salmonellosis VII

Studies of protection afforded by two subcutaneous immunising doses of killed vaccines against a heterologous intraperitoneal challenge. In the figure the mean time to death is plotted at 4-day intervals following challenge. The mean times to death of the immunised mice are greater than those of the non-immune control mice. Percentage mortality figures of mice challenged after immunisation with these vaccines (prepared from a fimbriate/flagellate strain of S. typhimurium 6354 FT/flt) indicated that a fair degree of protection was afforded.

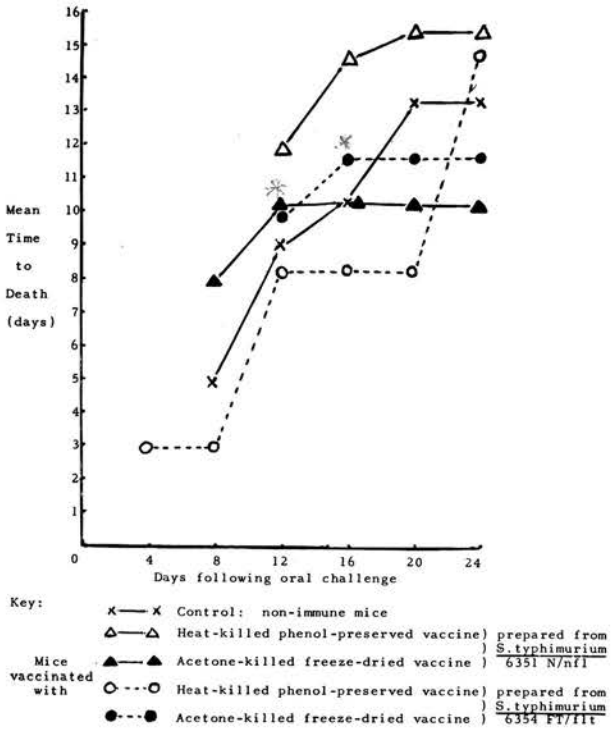

Fig. 36. **Experimental salmonellosis VII**

Studies of protection afforded by two subcutaneous immunising doses of killed vaccine against oral heterologous challenge. In the figure the mean times to death is plotted at 4-day intervals following challenge. The mean times to death of mice immunised with (a) heat-killed and acetone-killed vaccines prepared from non-fimbriate/non-flagellate S. typhimurium 6351 N/nfl and (b) acetone-killed vaccine prepared from fimbriate/flagellate S. typhimurium 6354 FT/flt were greater than the corresponding times to death of the non-immune control mice. Mice immunised with these vaccines were fairly well protected.
* No further deaths were recorded thereafter; the subsequently horizontal plotting of these lines is therefore misleading (see text p.199).

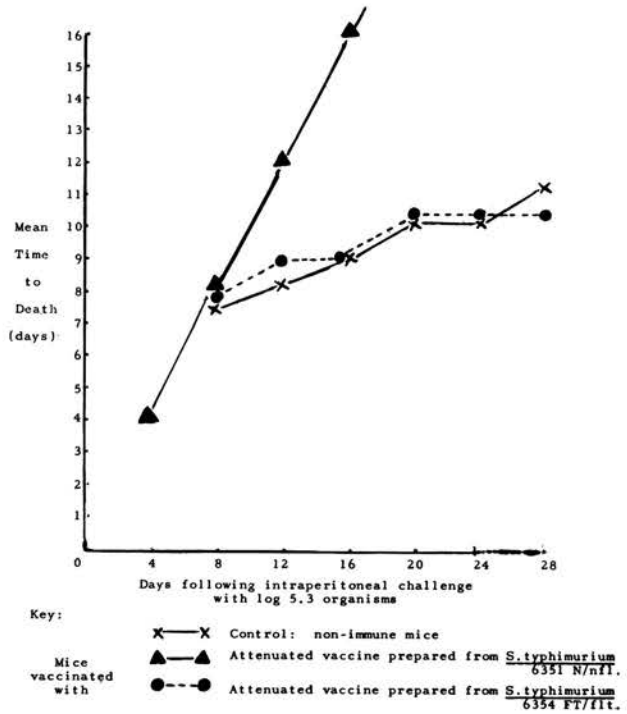

Fig. 37. Experimental salmonellosis VII

Studies of protection afforded by single subcutaneous immunising doses of attenuated vaccines against a heterologous intraperitoneal challenge. In the figure the mean time to death is plotted at 4-day intervals following challenge. The mean times to death of mice immunised with attenuated S. typhimurium 6351 N/nfl vaccine are much greater than those of mice immunised with attenuated S. typhimurium 6354 FT/flt vaccine or with unvaccinated control. This also corroborates the results of protection obtained from the percentage mortalities in the groups of mice.

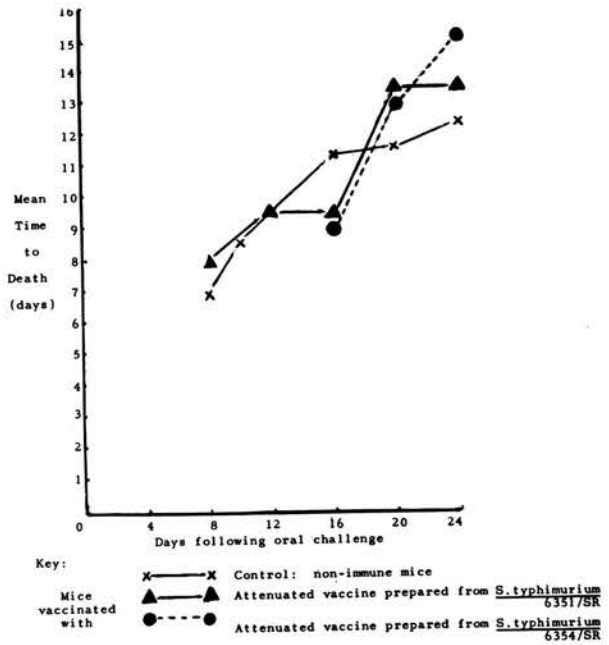

Fig. 38. Experimental salmonellosis VII

Studies of protection afforded by single subcutaneous doses of attenuated vaccines against heterologous oral challenge. In the figure the mean time to death is plotted at 4-day intervals following challenge. The mean times to death in the immunised groups of mice are relatively greater.

DISCUSSION

DISCUSSION

Role of fimbriae and flagella of S. typhimurium in virulence for mice

When the parent non-fimbriate/flagellate S. typhimurium strains representing various S. typhimurium phage types were compared with the fimbriate/flagellate derivatives with regard to their virulence for mice by intraperitoneal or oral routes of infection, no obvious differences were observed. The results of the experiments involving intraperitoneal infection are in agreement with those obtained by Campbell (1961), but the results of the oral infection experiments do not agree. In his series of experiments with phage-transduced fimbriate strains and non-fimbriate parent strains, Campbell found that fimbriate S. typhimurium strains were significantly more virulent than the non-fimbriate parent strains when administered orally to mice. However, in the present study, it appears that although there was no significant difference in virulence by the oral route of infection between the parent non-fimbriate/flagellate and the fimbriate/flagellate derivatives, the parent non-fimbriate/non-flagellate S. typhimurium strains were less virulent than their fimbriate/non-flagellate, non-fimbriate/flagellate and fimbriate/flagellate derivatives. In the latter group the fimbriate/flagellate derivatives were the most virulent; there was no significant difference in virulence between the fimbriate/non-flagellate and non-fimbriate/flagellate

derivatives. This point was rather clearly demonstrated in experiments with strain 6351 and its derivatives but not so with strain SW 573 and derivatives, presumably because the latter strain was rather weakly virulent for mice. It is unfortunate that only two non-fimbriate/non-flagellate strains of S. typhimurium were studied. However, the available results indicate that both fimbriae and flagella may play some small part in enhancing the virulence of S. typhimurium strains. The precise way in which this is achieved is not clearly understood. Probably the property of adhesion of fimbriate strains to mucous cells and blood cells allows "toxic" damage to be inflicted on the host cells. This hypothesis appears to be supported by the results obtained from the rabbit gut loop experiments with S. typhimurium and E. coli strains and from the experiments investigating the effect of fimbriae of S. typhimurium and Shigella flexneri on human leucocytes (see below). The significance and possible role of flagella in pathogenesis is also obscure. It has been suggested that flagella may inflict damage upon the host cells by their continuous lashing and screwing movements, but this appears to be somewhat speculative.

The effect of fimbriae of S. typhimurium and enteropathogenic serotypes of E. coli strains on ligated loops of the rabbit gut

The application of this technique has given some interesting results in reported studies of the pathogenicity

of strains of S. typhimurium and enteropathogenic E. coli. As would be expected in any experiment with living animals, the results showed variations from rabbit to rabbit (Taylor, Maltby and Payne, 1958; McNaught and Roberts, 1958). In the present investigation, a series of experiments involving at least 2 or 3 loop tests per strain carried out in rabbits with twenty-one S. typhimurium strains and thirty-seven enteropathogenic E. coli strains belonging to six serotypes; no striking differences in the production of gut dilatation were observed in tests comparing non-fimbriate/non-flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate and fimbriate/flagellate strains. The reactivity of different parts of the small intestine varied in these tests; loops nearest the stomach were more sensitive. The E. coli test strains included Taylor's positive and negative loop test controls (both are fimbriate strains), and these produced the anticipated results, but the results of this investigation showed no correlation between the ability of the specific serological types of E. coli to produce positive effects in gut loops and their recent association with gastro-enteritis in infants; this contrasts with the experience of De, Bhattacharya, and Sarkar (1954), McNaught and Roberts (1958) and Taylor, Maltby and Payne (1958). The degree of reaction produced in the experimental animals by the positive strains did not appear to be related to the severity of gastro-enteritis produced by

these strains in infants. This may be attributed to the many variable factors influencing host resistance to infection. Although the results of these investigations demonstrate that some fimbriate/flagellate, fimbriate/non-flagellate, non-fimbriate/flagellate and non-fimbriate/non-flagellate strains of S. typhimurium and E. coli can produce gut dilatation in rabbits, results have not been obtained with sufficient frequency and consistency in experiments with E. coli strains to accept this method as reliable for estimating their potential enteropathogenicity for human hosts. The differences in the present observations from those of the previous workers may be due to differences in the criteria accepted for a positive gut reaction. In the present study, a gut loop dilatation test was judged positive only if definite evidence of dilatation was apparent on naked eye examination. Taylor et al. (1958) and McNaught and Roberts (1958) included loops showing histological evidence of inflammation as weakly positive. However, it was observed that the fimbriate strains of S. typhimurium and E. coli gave a slightly higher percentage of positive loops than the non-fimbriate strains. The above results and the results of the present investigations to determine the minimal dose of S. typhimurium organisms necessary to produce a positive loop test suggest that the presence of fimbriae and flagella may slightly enhance the pathogenicity of a strain. The actual dose of organisms necessary to produce a positive dilatation was large and ranged from almost a

million to 1000 million organisms. This latter observation is in agreement with the reports of McNaught and Roberts (1958), and Taylor and Wilkins (1961). The findings seem to support the conclusions of the earlier comparative virulence studies in mice, that non-fimbriate/non-flagellate strains are slightly less virulent than their fimbriate/flagellate counterparts, but it should be borne in mind that the phage lysate used for transduction of the fimbriate state to the non-fimbriate parent S. typhimurium strains may perhaps have transduced additional characters that could contribute to virulence. These results have not been subjected to statistical analysis, but the observed differences are so small that it is unlikely they will be significant.

Comparative studies in rats:

Oral and intraperitoneal infection of albino rats with S. typhimurium

The experimental observations of oral and intraperitoneal infection of albino rats with S. typhimurium suggest that rats suffer a disease similar to mouse typhoid but they required a much larger infecting dose of the organisms, and even then the disease produced in the rats was rather mild and was soon followed by recovery. No apparent difference was observed in the course of the disease in rats resulting from infection with either fimbriate or non-fimbriate S. typhimurium strains.

Studies of the bacterial flora of the small intestine and

the passage of organisms through the gastro-intestinal tract of rats

It was confirmed that the proximal small intestine of healthy normal rats contains only a few transient organisms. Similar findings have been reported in healthy persons by various workers, even when free acid is absent from the stomach (Cregan and Hayward, 1953; Cregan, Dunlop and Hayward, 1953; Martini et al., 1957 and Haenal and Müller-Beuthow, 1958). The small intestine apparently possesses some antibacterial mechanism causing organisms that escape destruction in the stomach to be either destroyed or removed from the lumen before they can multiply. Dixon (1960) investigated this point. The test organisms were inoculated directly into subcutaneously displaced loops of small intestine of non-anaesthetised rats together with an inert unabsorbable radio-active marker. An isotope of chromium Cr^{51} tagged to red cells by the method of Gray and Stirling (1950) was used; this is virtually unabsorbed from the intestine (Owen, Bollman and Grindlay, 1954; and Jone, 1958). Dixon found no conclusive evidence of bactericidal action within the lumen of the small intestine against E. coli, Chr. prodigiosum, Ps. aeruginosa and Staph. saprophyticus during a period of one hour. Should such destructive action occur after one and a half or more hours in the small intestine, it could account for the disappearance of only a small proportion of organisms as his experiments indicated that

most of the inoculated material did not remain for more than that length of time in the small gut of the rat. Van Liere et al. (1944) observed that a suspension of charcoal administered to unanaesthetised dogs by stomach tube was propelled through more than half the length of the small intestine in 15 minutes. Dack and Petran's (1934) experiments on monkeys and dogs suggested mechanical cleansing of the lumen by peristaltic action of the small intestine. Florey (1933) suggested that mucus played a physical role in aiding the elimination of bacteria; particles adhered to mucous strands and were then apparently rolled up and propelled into the colon. Morson (1959) observed disintegrating tablets of sulphonamide wrapped up in a neat parcel of mucus in the small intestine. Dixon (1960) concluded that the lumen of the small gut contained very few viable organisms because the mechanism that removed the chyme is so efficient that solid material and mucus harbouring bacteria are rapidly and completely removed. Mucus thus appears to be useful in the removal of particulate matter from the small intestine. However, it is likely that if stasis occurs in the small gut then bacteria can survive and multiply. Thus it will be seen that, in the present study, the finding of only a few organisms in the small intestine and the recovery of both fimbriate and non-fimbriate strains of S. typhimurium from as far down the gastro-intestinal tract as the caecum within a period of 30 minutes

after oral administration is in agreement with the results and views of Dixon.

Evidence of penetration of intestinal wall

The isolation of S. typhimurium from the heart blood, liver, spleen and mesenteric glands of animals inoculated in the present investigation provides ample proof of their successful penetration of the mucosal lining of the gastro-intestinal tract of the rat. The observations suggest that penetration can take place from any part of the gastro-intestinal tract but it seemed to occur most commonly from the proximal part of the small intestine - the duodenum and jejunum. The following factors may account for this:- (i) test meal studies by Atkinson (1960), in which isotopes were included, indicated that the absorption of fat, carbohydrates and protein normally starts in the duodenum and is almost completed in the proximal jejunum; (ii) the state of restless activity of the jejunum as shown radiologically by Shanks (1938) may impose some degree of physiological stress and increase the vulnerability of this segment to microbial attack; and (iii) the generally empty state of the small gut containing very few commensal organisms.

The speed with which the bacteria appeared in the blood stream, and the fact that in three experiments the inoculated S. typhimurium strain was recovered from the liver only, the heart blood and spleen cultures being negative indicate that organisms were taken from the

intestinal lumen to liver via the portal circulation, This supports the conclusion of Gerichter (1960) that the organisms can enter the blood directly by way of the capillaries. However, it would appear that this is not the only route. The organisms also appear to gain entrance into the tissues and blood stream via the lymphatic system. In almost all the experiments, cultures of the mesenteric lymph glands were consistently positive for the test strain.

The passage of the test strain through the gastro-intestinal tract and the penetration of the intestinal mucosal lining of rats do not appear to be affected by the presence or absence of fimbriae. The mode of penetration of the intestinal mucosal lining was also studied by electron microscopic examination of sections embedded in araldite, but these studies did not elucidate the matter further (see below).

The effect of fimbriae on human polymorphonuclear cells

The present series of in vitro experiments with permanently fimbriate and non-fimbriate S. typhimurium and Shigella flexneri strains, and the phage-transduced fimbriate and parent non-fimbriate strains of S. typhi-murium, seem to suggest that the attachment of the fimbriate strains to the leucocytes resulted in the early death of the leucocytes. Whether the death of the leucocytes was the result of biochemical interference with their metabolism or the result of a mechanical injury by

the fimbriae to the cell wall is largely a matter of conjecture. It may be argued that the attachment of many fimbriae to a leucocyte may prevent visualisation of movement of its granules, but this does not seem to be a valid objection to the present evidence because the nuclei of the dead leucocytes remained clearly visible, and the special affinity of the nucleus of a dead leucocyte for the methylene blue stain was easily observed.

Electron microscopic studies

These studies together with the haemagglutination and motility tests confirmed the presence or absence of fimbriae or flagella on the test strains of S. typhimurium, E. coli and Sh. flexneri, and helped to divide them into the following sub-groups:- non-fimbriate/non-flagellate strains; non-fimbriate/flagellate strains; fimbriate/non-flagellate strains and fimbriate/flagellate strains. In the course of these studies it was also demonstrated that fimbriate organisms adhere to human leucocytes, red cells and platelets just as readily as they adhere to guinea-pig red blood cells and epithelial cells taken from the guinea-pig or human colon.

The electron microscopic examination of sections of S. typhimurium confirmed reports of recent studies on the fine structure of the bacterial cells, flagella and fimbriae. Many different bacteria have now been studied and the main characteristics of the fine structure are rather well established. The fine structure of S. typhimurium is like that of most other Gram-negative bacteria

(such as E. coli) and differs from that of Gram-positive bacteria in two ways:- (i) the cell-wall of Gram-negative bacteria is very thin, with a total thickness of about 75Å (Kellenberger and Ryter, 1958); in contrast, the cell walls of Gram-positive bacteria vary from 150Å to 350Å in thickness (Glauert, 1962). (ii) small membranous bodies which are called the peripheral bodies (Chapman and Hillier, 1953) or mesosomes (Fitz-James, 1960) are not typically found in Gram-negative bacteria; these are often present in Gram-positive bacteria and are situated near the periphery of the cell.

The flagella of S. typhimurium are about 120Å in diameter and are attached to a cell by a "hook" (Glauert, 1964); they are composed of globular molecules of flagellin arranged in five parallel rows to form a hollow tube (Kerridge, Horne and Glauert, 1962) and this in thin transverse section presents a five-fold symmetry. In contrast, all vibratile cilia and flagella contain eleven longitudinal fibres, nine of which form a cylinder around a central pair (Grimstone, 1962) and it is remarkable that, although hundreds of thousands of cilia and flagella have been studied in the electron microscope, not only has no exception to the basic "nine-and-two" arrangement been found, but also, for a given type of cell, the dimensions and spacing of all structures seem to be invariably constant. According to Kerridge (1964) the synthesis and functioning of the flagella in Salmonella spp. are

controlled by a number of genetic loci, and no evidence has been found to suggest that the formation of the flagellin molecules differs fundamentally from the synthesis of other cell proteins, but it is recognised that the flagellin from certain salmonella strains is unusual in that it contains the amino acid ε-N-methyl-lysine (Ambler and Rees, 1959; Stocker, McDonough and Ambler, 1961).

Fimbriae, which have diameters of about 50Å, are much shorter and thinner than flagella. Sections of fimbriae do not show any subunits (Kerridge, Horne and Glauert, 1962) and in the present work some examples appeared as long hollow cylinders.

The ultrastructure of the normal duodenal villus of the albino rat appeared to be similar to that of the human villus which has been described by Curran and Creamer (1963). Similar observations were recorded by Palay and Karlin (1959) who also drew attention to the importance of pinocytosis as a mechanism by which epithelial cells take up soluble substances from the gut lumen. Hampton (1962) carried out observations on the interaction between the epithelial cells and bacterial cells commonly seen in the lumen of the lower ileum of the mouse in an attempt to learn something of the likelihood of hydrolysis and of absorption by means other than pinocytosis at the surface membrane. During the course of the present work electron-micrographs were obtained showing unidentified

cellular debris and bacteria adhering to the microvilli; bacteria which appeared to be fimbriate were seen lying in the intercellular region of the epithelial cells and deep in the intestinal tissue. These appearances are very similar to the electron micrographic evidence published by Hampton who suggested a possible sequence of events leading to the attachment of an unidentified organism to the cell. However, the results of the present experimental study were somewhat disappointing. It was hoped to identify the test S. typhimurium strain in the tissues by the recognition of its fimbriae, but unfortunately this was not possible because it was found very difficult to identify fimbriae in ultrathin tissue sections. Perhaps more encouraging results would be obtained if the experiments were carried out using test strains suitably labelled with isotopes or by the use of fluorescent staining techniques.

Studies of immunisation of mice:

Mortalities attributable to immunisation procedures

In the present series of experiments the overall fatality rates following immunisation and prior to challenge, and therefore possibly attributable to immunisation procedures, were very nearly the same for mice receiving the killed or the attenuated S. typhimurium vaccines. Fatality rates with the killed vaccines ranged from 0 to 6.25 per cent., and with the attenuated vaccines they ranged from 0.7 to 6.5 per cent., prior to challenge.

Greenwood, Topley and Wilson (1931) reported that killed vaccines are liable to cause up to 7 per cent. deaths when used in effective dosage, and Hobson (1957) reported 4 per cent. deaths in his group of mice vaccinated with killed vaccine. In Hobson's (1957) series of immunisation experiments employing live attenuated S. typhimurium strain S2/R, the fatality rate resulting from primary infection of mice with the live vaccine varied from 0 - 8 per cent. The case mortality of vaccinated mice prior to challenge in the present series of experiments was very similar to that noted by previous workers and was never excessive. As the groups of mice surviving to the time of challenge thus represented virtually all of the original groups, the enhanced resistance demonstrated in some of the immunised mice in the present series of mouse-protection experiments using intraperitoneal and oral challenge with doses of a heterologous S. typhimurium organism seems unlikely to be due to the effects of natural selection. The enhanced resistance has therefore been attributed to characteristics acquired from immunisation with effective dosage of the killed or attenuated S. typhimurium vaccines employed.

Protection of mice resulting from immunisation with killed vaccines

Immunisation with a single subcutaneous dose of either the heat-killed phenol-preserved (HP) or acetone-killed freeze-dried (AK) S. typhimurium vaccines prepared from

either the parent non-fimbriate/non-flagellate strain or its phage-transduced fimbriate/flagellate strain, gave no protection to mice. This is not surprising and is a common experience shared with other workers. The lack of protection is attributable to inadequate antigenic stimulus and this was confirmed by studies of the O-antibody response. The two-dose subcutaneous immunisation of mice with the HP and AK vaccines prepared from the fimbriate/flagellate strain of S. typhimurium 6354 FT/flt gave fairly good protection against intraperitoneal challenge. The protection afforded was better than that produced in mice similarly immunised with vaccines prepared from the parent non-fimbriate/non-flagellate S. typhimurium 6351 N/nfl, and challenged by the same route. It would appear that for the production of suitable killed S. typhimurium vaccines from cultures grown in vitro it is advantageous to select a fimbriate/flagellate strain. However, on oral challenge of similar immunised groups of mice, the results indicated that both HP and AK vaccines prepared from either strain of S. typhimurium were equally protective. The strain 6354 FT/flt is relatively more virulent for mice than the strain 6351 N/nfl. These findings appear to support the work of Auzins (cited by Jenkin and Rowley, 1963) who reported that a suitably prepared killed vaccine prepared from a suitably selected strain of S. typhimurium given in adequate amounts would protect mice against S. typhimurium challenge.

Auzins maintained that the protection of mice with a killed vaccine depended upon the preservation of a heat-labile antigen that is associated with virulent strains of S. typhimurium.

Protection of mice resulting from immunisation with attenuated vaccines

With the exception of one experiment in which the mice were immunised with an attenuated strain of S. typhimurium (6354 FT/flt/SR) which had become streptomycin dependent for its growth, immunisation of mice with either the non-fimbriate/non-flagellate or fimbriate/flagellate attenuated strains of S. typhimurium gave far better protection than immunisation with the killed vaccine. This is probably due to the fact that the attenuated S. typhimurium organisms are able to establish themselves temporarily in the host tissues and proliferate. Thus the total amount of bacterial antigens in the tissues would be much greater than that given in the form of a killed vaccine. It is also possible that the in vivo proliferation of the attenuated S. typhimurium organisms results not only in the production of better antigens but also of protective antigens which are not present in significant amount in killed vaccines. This may be because certain protective antigens occur as a result of some unknown in vivo host-parasite relationship, but Auzins' concept of denaturation of a labile protective factor or factors during the preparation of killed vaccine may account for the failure

of killed vaccines used in previous studies. There may of course be many mechanisms involved in the production of a solid immunity against this type of infection. Both of the attenuated vaccines and the killed vaccines prepared from the fimbriate/flagellate strain of S. typhimurium 6354 FT/flt provoked good O-antibody responses in mice and these vaccines were the ones which gave good protection. Perhaps killed vaccines prepared from S. typhimurium organisms grown in vivo might give better protection than those prepared from organisms grown in vitro. Olitzki and Godinger (1963) carried out comparative studies on S. typhi grown in vivo and in vitro and they demonstrated (i) that S. typhi Ty2 grown in vivo was more virulent for mice than its corresponding culture grown in vitro; and (ii) S. typhi, strain Ty2, grown in vivo and employed as acetone-dried vaccine possessed a higher immunising potency than the descendants of the same parent strain grown in vitro and employed as an acetone-dried vaccine.

Recent studies on immunity in mouse-typhoid (Jenkin, Rowley and Auzins, 1964; Turner, Jenkin and Rowley, 1964; Rowley, Turner and Jenkin, 1964) contest the concept of antibacterial "cellular" immunity. It is claimed that immunity to mouse-typhoid was passively transferred to non-immune mice by inoculating phagocytic cells harvested from the peritoneal cavity of immune mice. This is thought to be due to the presence of cell-bound antibody which was shown to be a 19S macroglobulin. These workers believe

that "cellular immunity" is dependent on antibody for its expression just as clearly as classical humoral (antibody-mediated) immunity is in its turn dependent upon phagocytic cells. This concept is supported by the earlier work of Ushiba et al. (1959) who were able to transfer antibacterial immunity against S. enteritidis to the peritoneal cavities of normal mice by transferring washed peritoneal macrophages from immunised mice. Indeed one on the pillars of the concept of "cellular" immunity to tuberculosis has been broken by the recent work of Larson et al. (1963) in which cell-walls of Myco. tuberculosis prepared under special conditions were found to be as effective as BCG vaccine in provoking active immunity to tuberculosis in mice. Moreover, results of recent studies on the W and V antigens of Pasteurella pestis suggest a similar type of immune reaction to that provoked by the preparation of Myco. tuberculosis cell walls (Lawton, Erdman and Surgulla, 1963). Other examples of labile antigens that have hitherto been destroyed in the preparation of killed bacterial vaccines are cited by Keppie et al. (1963). In the light of these findings, the concepts of cell-bound antibody and "cellular immunity" certainly call for further investigation; pursuance of such a line of research may be richly rewarding.

Correlation of the results of the present immunisation studies in mice with the present state of knowledge of human immunity against typhoid

The present work was designed to investigate possible

mechanisms of invasion and pathogenicity, and to evaluate immunising procedures, using S. typhimurium infection in mice as a model. It was hoped that these studies may elucidate some of the mechanisms involved in enteric infections in man. The results of the mouse immunisation studies certainly seem to parallel human experience in typhoid immunisation. Although one cannot be satisfied with the present state of knowledge of immunity in typhoid, particularly with regard to the interpretation of various laboratory tests, an impressive amount of literature on typhoid vaccine trials has become available in the last decade. The first controlled field trial of immunisation against typhoid was organised by the World Health Organisation and was carried out in 1952 in Yugoslavia (Yugoslav Typhoid Commission, 1962). This trial provided definite proof that doses of heat-phenol inactivated vaccines gave good protection while the alcohol inactivated vaccine gave little or insignificant protection in man. In this trial it was found impossible to establish correlation between the results of laboratory tests and the effectiveness of those liquid (unstable) vaccines in man. Unfortunately these difficulties still persist. However, subsequent to this trial the World Health Organisation has sponsored co-operative field and laboratory assays of two stable dried typhoid vaccines viz. heat-phenol inactivated (code "L") and acetone inactivated (code"K"). The objects were to test their effectiveness in man so as to establish them

as reference typhoid vaccine preparations and also to find out a suitable laboratory test to evaluate such vaccines. The currently available animal tests are unreliable means of measuring the potency of vaccines and require to be supported by evidence of protection in man. The subsequent W.H.O. typhoid vaccine trials were carried out in Yugoslavia, British Guiana, U.S.S.R., and Poland. According to Cvjetanovic (1964) the heat-phenol inactivated dried typhoid vaccine proved effective to more or less the same degree as a similar vaccine used in the first trial in Yugoslavia, while the acetone inactivated vaccine proved to be significantly superior to the heat-phenol inactivated vaccines. In the present studies, mice immunised with heat-killed phenol-preserved or acetone-killed dried vaccine from a relatively virulent S. typhimurium strain were found to be almost equally well protected against oral challenge but immunisation with live attenuated vaccines prepared from a relatively avirulent strain gave much better protection in mice particularly against intraperitoneal challenge. The differences in protective potency of the acetone-killed vaccines studied in the field trials and in the present experimental series may be due to species differences in immune responses of man and mouse, differences in the modes of preparation of the vaccines, or chance differences involving unknown factors. In view of the possible factors and because the killed vaccines used in the present studies were prepared from relatively avirulent S. typhi-

murium strains, it would be of interest to repeat these comparative immunisation studies with vaccines prepared from virulent S. typhimurium strains and inactivated in such a way as to preserve any labile antigens.

In the W.H.O. typhoid vaccine trials the only serological indicator of superiority of the acetone inactivated vaccine was the higher H agglutinin titre in man. O and Vi antibodies seem to be of no use in differentiating the effectiveness of typhoid vaccines. However, in the present studies a good correlation between the degree of protection of mice and O antibody responses in mice was observed. The differences in antibody responses in man and mouse may illustrate that different immune mechanisms are involved in the two species, but it seems more likely that much of the confusion in the literature regarding so-called protective antigens of salmonella organisms arises because the elusive protective antigen or antigens have yet to be properly identified.

Alternatively, in view of the excellent protection of mice immunised with live attenuated S. typhimurium vaccines, it may perhaps be worth while to give consideration to the development of a live attenuated S. typhi vaccine for use in man.

S U M M A R Y

SUMMARY

This investigation of pathogenesis and control of experimental salmonella infections includes a comprehensive review of the literature and involved some 3,940 animal experiments relating to virulence and immunity studies of S. typhimurium infections in mice and rats. Results of electron microscopic studies, and in vitro experiments with human leucocytes exposed to the effect of fimbriae of S. typhimurium and Shigella flexneri are also reported. The results of these investigations may be briefly summarised as follows:-

1. The test strains were isolated from diverse sources and belonged to six different phage-types. They are fairly representative of the known S. typhimurium strains. No apparent differences in virulence for mice, by either the intraperitoneal or oral routes of infection, were observed in the course of comparative virulence experiments with the parent non-fimbriate/flagellate S. typhimurium strains and their fimbriate/flagellate derivatives obtained by phage transduction or by the use of a mutagenic agent ($MnCl_2$). However, the parent non-fimbriate/non-flagellate S. typhimurium strains were somewhat less virulent than their fimbriate/flagellate, or non-fimbriate/flagellate or fimbriate/non-flagellate derivatives. Of these derivative strains, the fimbriate/flagellate derivative was demonstrably more virulent than the others. It seems that fimbriae and flagella may play some small role in the pathogenesis of the disease in mice, but the

mechanisms involved are unknown.

2. In rabbit gut loop tests carried out with twenty-one S. typhimurium strains and thirty-seven enteropathogenic Escherichia coli, a positive reaction was obtainable with non-fimbriate/non-flagellate or fimbriate/non-flagellate or non-fimbriate/flagellate or fimbriate/flagellate strains, but other strains in each of these categories gave negative results. The E. coli strains tested included Taylor's positive and negative test controls and they produced the anticipated results; both of these strains are fimbriate. In comparative studies of fimbriate and non-fimbriate strains of S. typhimurium and E. coli a slightly higher proportion of fimbriate test strains produced positive loop tests. The reactivity of different parts of the small intestine varied with tests; loops nearest the stomach were more sensitive.

The minimal dose of organisms required to produce a positive gut loop test was rather large. The minimal log dose of unpassaged S. typhimurium producing a positive gut loop test ranged from 5 to 9; the minimal log dose of enteropathogenic E. coli was about 9. After passage through the rabbit gut, the minimal log dose of S. typhimurium producing a positive loop test ranged from 4 to 9. It was observed that the minimal log dose producing a positive loop was less (by log 1 to 3) for the fimbriate/flagellate S. typhimurium, than the parent non-fimbriate/flagellate strains. However, with E. coli strains, the pres-

ence of the absence of fimbriae or flagella, or prior passage of the organisms through the rabbit gut, did not apparently influence the minimal log dose of the organisms required to produce a positive gut dilation effect.

3. Oral or intraperitoneal infection of albino rats with fimbriate or non-fimbriate S. typhimurium produced a disease that was comparable to mouse-typhoid, but a much larger infecting dose of the organisms, was required in rats; the resulting disease was rather mild and was soon followed by recovery.

4. The proximal small intestine of healthy albino rats was found to be empty and contained only a few transient organisms. S. typhimurium (fimbriate and non-fimbriate) organisms administered orally reached the stomach rapidly and they could be passed down as far as the caecum within 30 minutes.

Within a matter of minutes the S. typhimurium organisms were able to penetrate the mucosa of the intestinal tract and appeared in the blood stream and organs (liver, spleen and mesenteric glands) of the rats. The bacteria appeared to reach the blood stream and the organs (i) by direct entry into the capillaries and thence by way of the portal system to the general circulation, and (ii) via the lymphatic system. The ability to pass down the gastro-intestinal tract, to penetrate the intestinal wall, and to enter the blood stream, was apparently not related to the presence or absence of fimbriae.

6. The haemagglutinating property of fimbriate organisms was not affected by exposure to temperatures up to 60°C. for 1½ hr. in the water-bath.

The results of *in vitro* experiments suggest that fimbriate *S. typhimurium* and *Sh. flexneri* strains produce a more rapid leucocidal effect following adhesion to human leucocytes, than do non-fimbriate strains.

7. *Electron-microscopic studies*: (i) Fimbriae and flagella of representative test strains of *S. typhimurium* and enteropathogenic *E. coli* were demonstrated by the examination of shadowed and negative-stained preparations in the electron-microscope. (ii) Electron-micrographs of fimbriate *S. typhimurium* organisms adhering to human leucocytes, red corpuscles and platelets were procured. (iii) Examination of araldite-embedded ultra-thin sections of *S. typhimurium* confirmed the reports of Glauert (1962) concerning the ultra-structure of Gram-negative organisms. (iv) Electron microscopic studies on the mode of penetration of the gastro-intestinal mucosa of *albino* rats by *S. typhimurium* organisms yielded no new information.

8. Subcutaneous immunisation of mice with effective dosage of heat-killed phenol-preserved and acetone-killed freeze-dried vaccines prepared from a virulent strain of *S. typhimurium* afforded fairly good protection against intraperitoneal or oral challenge with a heterologous strain of *S. typhimurium*, but immunisation with attenuated *S. typhimurium* vaccine administered subcutaneously or orally to streptomycin-treated mice afforded much better protec-

tion. The oral immunisation of streptomycin-treated mice with a single dose of attenuated vaccine resulted in as good a protection against intraperitoneal challenge of these mice as those immunised by a single subcutaneous dose containing an equivalent number of organisms of the vaccine. Although the fimbrial and flagellar antigens of S. typhimurium do not appear to play a major role in conferring immunity, it is recommended that a virulent fimbriate/flagellate strain of S. typhimurium should be selected for the preparation of killed vaccines, because such a strain would be antigenically more complete than a non-fimbriate/non-flagellate strain. Further it is recommended that in the preparation of killed vaccines every care should be taken to preserve all known and unknown antigens, including Auzin's heat-labile antigen.

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A P P E N D I C E S

EXPERIMENT I COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE AND PARENT NON-FIMBRIATE S.TYPHIMURIUM 1289 FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. typhimurium strain 1289 FT/f1.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day												Survivors Killed	Mortality	Survivors	No. of survivors infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			4	6	7	8	9	10	11	12	13	14	17	20	25						
ORAL	8.53	12				2	2		3	1	1			1		10/12	2	1	11/12	7.2	7.0
	7.53	12		1		1			2		1		1			6/12	6	2	8/12		
	6.53	12							2				2			4/12	8	2	6/12		
	5.53	12											1			1/12	11	0	1/12		
	4.53	12	1													1/12	11	0	1/12		
INTRAPERITONEAL	3.53	4=		1	2			1								4/4	0	0	4/4	1.9	1.7
	2.53	8	1	2	4			1								8/8	0	0	8/8		
	1.53	8				1					1					2/8	6	1	3/8		

= A batch of four mice were not inoculated (through oversight.)

Times to death at oral LD50

At log dose 7.53 = 6, 8, 11, 11, 13, 17)
At log dose 6.53 = 11, 11, 17, 17) Mean 12.2 days

FT = phage-transduced-fimbriate

f1 = flagellate

EXPERIMENT I COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE AND PARENT NON-FIMBRIATE S. TYPHIMURIUM 1289 FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (b) Results obtained with S. typhimurium strain 1289N/f1.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																			Mortality	Survivors	No. surs. infected	Infectivity	Log dose LD_{50}	Log dose LD_{50}
			1	3	4	5	6	7	8	9	10	11	12	13	14	16	17	18	19	22	23	25						
ORAL	9.57	12		1			1	1	1	2		2		1	2		1					SURVIVORS KILLED	12/12	0	0	12/12	6.6	6.3
	8.57	12		2			1		3		1	1	1	2									11/12	1	1	12/12		
	7.57	12	1		1		1		1	1		2	1							1			9/12	3	0	9/12		
	6.57	12										1	2	1		1		1	1				7/12	5	2	9/12		
	5.57	12												1							1		2/12	10	0	2/12		
	4.57	12																					0/12	12	0	0/12		
INTRAPERITONEAL	3.57	8				1	5			1	1												8/8	0	0	8/8	2.1	1.9
	2.57	8				1	1	3	1		1												7/8	1	1	8/8		
	1.57	8			1																		1/8	7	1	2/8		

Times to death at oral LD50

At log dose 6.57 = 11, 12, 12, 13, 16, 18, 19 Mean 14.4 days

N= non-fimbriate
f1 = flagellate

EXPERIMENT II COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE S. TYPHIMURIUM 1294 and NON-FIMBRIATE PARENT STRAIN FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.

(a) Results obtained with S. typhimurium strain 1294 FT/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day													Mortality	Survivors	No. of survivors infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			4	6	7	8	9	10	11	12	13	14	17	21	22						
ORAL	8.2	12		1	1	1	1	1		1					SURVIVORS KILLED	6/12	6	6	12/12	7.8	5.7
	7.2	12				2		1								3/12	9	8	11/12		
	6.2	12														0/12	12	8	8/12		
	5.2	12								1						1/12	11	2	3/12		
	4.2	12									1					1/12	11	1	2/12		
INTRA PERITONEAL	3.2	12	1	3	2	2	1						1	1		11/12	1	1	12/12		
	2.2	12		1	2			3			1		1			8/12	4	4	12/12		
	1.2	12					2	1	1		2	1				7/12	5	4	11/12	<1.2	<1.2

Times to death at oral LD50

At log dose 8.2 = 6, 7, 8, 9, 10, 12
At log dose 7.2 = 8, 8, 10
} Mean 8.6

FT = phage-transduced fimbriate
fl = flagellate

< = less than

EXPERIMENT II. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE AND NON-FIMBRIATE PARENT STRAIN OF S. TYPHIMURIUM 1294 BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION OF LAC GREY MICE.
(b) Results obtained with S. typhimurium strain 1294 N/f1.

Route of infection	Log dose organisms	No. of Mice infected	Number of deaths on day																Mortality	Survivors	No. of survivors infected	Infectivity	Log dose	
			1	4	5	6	7	8	9	10	11	12	13	14	15	17	21	22					LD_{50}	ID_{50}
ORAL	8.7	12						3		1		1			1			SURVIVORS KILLED	6/12	6	6	12/12	7.4	5.4
	7.7	12							1		1		1	1	1				5/12	7	5	10/12		
	6.7	12						1	1	1						1	1		5/12	7	6	11/12		
	5.7	12					1			1			1	1		1			5/12	7	4	9/12		
	4.7	12										1		1					2/12	10	0	2/12		
	3.7	12																	0/12	12	1	1/12		
INTRA PERITONEAL	2.7	12		1	3	4		3											11/12	1	1	12/12	1.0	<0.7
	1.7	12	1			3	2	2				1	1				1		11/12	1	1	12/12		
	0.7	12					1	2			1	1							5/12	7	4	9/12		

Times to death at oral LD50

At log dose 6.7 = 8, 9, 10, 17, 21
At log dose 7.7 = 9, 11, 13, 14, 15 } Mean 12.7 days

N = non-fimbriate
f1 = flagellate

<= less than

EXPERIMENT III. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE S. TYPHIMURIUM 1287 AND NON-FIMBRIATE PARENT STRAIN IN LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.

(a) Results obtained with S. typhimurium strain 1287 FT/fl.

Route of infection	Log dose organisms	No. of Mice infected	Number of deaths on day															Mortality	Survivors	No. of survivors infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			4	5	6	7	8	9	10	12	13	15	16	17	18	20	22						
ORAL	8.2	12				1	3		1		1		1	1		2	SURVIVORS KILLED	10/12	2	2	12/12	6.4	5.4
	7.2	12		1	2			1		2	1		1		1			9/12	3	2	11/12		
	6.2	12	1+				1	1		1		2						5/11	6	2	7/11		
	5.2	12											1		1			2/12	10	5	7/12		
	4.2	12								1								1/12	11	0	1/12		
INTRA PERITONEAL	3.2	12	1	2	4	2	2	1										12/12	0	0	12/12		
	2.2	12	1	6	1		1		1									12/12	0	0	12/12		
	1.2	12	1	1	3		1	1				2						9/12	3	1	10/12	<1.2	<1.2

+ Accidental death (head crushed)

FT = phage-transduced fimbriate
fl = flagellate

Time to death at oral LD50

At log dose 7.2 = 5, 6, 6, 9, 12, 12, 13, 16, 18
At log dose 6.2 = 8, 9, 12, 15, 15
} Mean 9.2 days

<= less than

EXPERIMENT III. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE S. TYPHIMURIUM 1287 and NON-FIMBRIATE PARENT STRAIN FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.
(b) Results obtained with S. typhimurium strain 1287 N/f1.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																		Mortality	Survivors	No. of survivors infected	Infectivity	Log dose	
			4	5	6	7	8	9	10	11	12	13	14	16	17	18	20	21	22						LD_{50}	ID_{50}
ORAL	8.7	12				4	2	1	2	1	1						1		SURVIVORS KILLED		12/12	0	0	12/12	6.1	5.6
	7.7	12			2	1	2	2	1	1	1	1			1						12/12	0	0	12/12		
	6.7	12					1		1		1			1			2	1			7/12	5	4	11/12		
	5.7	12				1					2		1								4/12	8	2	6/12		
	4.7	12				1						1									2/12	10	0	2/12		
	3.7	12									1										1/12	11	0	1/12		
INTRA PERITONEAL	2.7	12	2	2	3		2	1			1			1							12/12	0	0	12/12		
	1.7	12	3	2	2	2	1	1					1								12/12	0	0	12/12		
	0.7	12	1	1	1	2	1		2						1	1					10/12	2	2	12/12	<0.7	<0.7

Time to death at oral LD50

At log dose 6.7 = 8, 10, 12, 16, 20, 20, 21
At log dose 5.7 = 7, 12, 12, 14
Mean 13.1 days

N = non-fimbriate
f1 = flagellate
<= less than

Experimental Salmonellosis I. Appendix 7.

EXPERIMENT IV. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED STREPTOMYCIN-RESISTANT FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM 1289 BY ORAL INFECTION OF STREPTOMYCIN-TREATED LAC GREY MICE.

Test strains	Log dose organisms	No. of mice infected	Streptomycin Treatment 50 mgm. per mouse	Number of deaths on day												Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
				9	10	11	12	13	15	16	19	20	21	22	22					LD_{50}	LD_{50}
Non-fimbriate S. typhimurium 1289 N/fl/SR viable count 1.2×10^8 orgs. per ml.	4.08	6	NO												KILLED	0/6	6	1	1/6	CONTROL	
	4.08	6	YES	1	1			1					1			4/6	2	2	6/6	3.5	1.2
	3.08	6	YES			1										1/6	5	5	6/6		
	2.08	6	YES													0/6	6	5	5/6		
	1.08	6	YES													0/6	6	2	2/6		
	0.08	6	YES											1		1/6	5	1	2/6		
Fimbriate S. typhimurium 1289FT/fl/SR viable count 1.5×10^8 orgs. per ml.	4.17	6	NO												SURVIVORS	0/6	6	0	0/6	CONTROL	
	4.17	6	YES					1			1	1				3/6	3	3	6/6	2.9	1.3
	3.17	6	YES					1	1	1			1			4/6	2	2	6/6		
	2.17	6	YES				1									1/6	5	3	4/6		
	1.17	6	YES					1						1		2/6	4	1	3/6		
	0.17	6	YES													0/6	6	1	1/6		

Times to death at LD50 with non-fimbriate strain = 9, 10, 11, 13, 21 Mean = 12.8 days N = non-fimbriate
Times to death at LD50 with fimbriate strain = 13, 15, 16, 21 Mean = 16.2 days fl = flagellate

FT = phage-transduced fimbriate SR = streptomycin-resistant

EXPERIMENT V. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED STREPTOMYCIN-RESISTANT FIMBRIATE AND NON-FIMBRIATE S. TYPHIMURIUM 1294 BY ORAL INFECTION OF STREPTOMYCIN-TREATED LAC GREY MICE.

Test strains	Log dose organisms	No. of mice infected	Streptomycin Treatment 50 mgm. per mouse	Number of deaths on day										Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
				8	11	12	13	18	19	20	22	24						LD_{50}	ID_{50}
Non-fimbriate S. typhimurium 1294 N/fl/SR Viable Count 3.1×10^8 orgs. per ml.	4.5	6	NO										KILLED	0/6	6	0	0/6	CONTROL	
	4.5	6	YES					1					KILLED	1/6	5	5	6/6	>4.5	
	3.5	6	YES	1									KILLED	1/6	5	4	5/6		
	2.5	6	YES				1						KILLED	1/6	5	3	4/6		
	1.5	6	YES		1								KILLED	1/6	6	4	4/6		
	0.5	6	YES										KILLED	0/6	6	0	0/6		0.85
Fimbriate S. typhimurium 1294FT/fl/SR viable count 3.75×10^8 orgs. per ml.	4.7	6	NO										SURVIVORS	0/6	6	0	0/6	CONTROL	
	4.7	6	YES							1			SURVIVORS	1/6	5	4	5/6	4.7	
	3.7	6	YES						1				SURVIVORS	1/6	5	5	6/6		
	2.7	6	YES			1					1		SURVIVORS	2/6	4	1	3/6		
	1.7	6	YES										SURVIVORS	0/6	6	5	5/6		1.3
	0.7	6	YES			1							SURVIVORS	1/6	5	2	3/6		

Time to death at LD_{50} for the non-fimbriate strain > 24 days.
Time to death at LD_{50} for the fimbriate strain is 20 days.

N = non-fimbriate FT = phage-transduced fimbriate
fl = flagellate SR = streptomycin-resistant
> = more than

EXPERIMENT VI. COMPARISON OF VIRULENCE OF FIMBRIATE AND NON-FIMBRIATE AND NON-FIMBRIATE RHAMNOSE-POSITIVE *S. TYPHIMURIUM* 1566 FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.

(a) Results obtained with *S. typhimurium* strain 1566 F/f1.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																				Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	21	22	23	24						
ORAL	8.2	12				1	1	7	2	1												SURVIVORS KILLED	12/12	0	0	12/12	4.6	4.5
	7.2	12				1	4	3	1		2	1											12/12	0	0	12/12		
	6.2	12						2	3	1		1		1				1					9/12	3	0	9/12		
	5.2	12					2		1				2		1			1		1			8/12	4	1	9/12		
	4.2	12					1		1	1		1	1				1	1					7/12	5	0	7/12		
INTRA PERITONEAL	3.2	8	1	2	3	2																	8/8	0	0	8/8	<1.2	<1.2
	2.2	8	1	1	2	3	1																8/8	0	0	8/8		
	1.2	8	1			1		2								3					1		8/8	0	0	8/8		

Times to death at oral LD50

At log dose 5.2 = 7, 7, 9, 14, 14, 16, 19, 22) Mean 13.8 days.
At log dose 4.2 = 7, 9, 10, 12, 14 18, 19)

F = Fimbriate f1 = flagellate < = less than

EXPERIMENT VI. COMPARISON OF VIRULENCE OF FIMBRIATE AND NON-FIMBRIATE RHAMNOSE POSITIVE S. TYPHIMURIUM 1566 FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.

(b) Results obtained with S. Typhimurium strain 1566 N/fl.

Route of infection	Log Dose organisms	No. of Mice infected	Number of deaths on day															Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			4	5	6	7	8	9	10	11	12	13	14	15	16	19	24					LD_{50}	ID_{50}
ORAL	8.8	12			1	8	1	2									SURVIVORS KILLED	12/12	0	0	12/12	5.0	4.8
	7.8	12				2	4	3	1		2							12/12	0	0	12/12		
	6.8	12			2	2	2	3	1	1								11/12	1	1	12/12		
	5.8	12				1	2	1	2	1	2							9/12	3	0	9/12		
	4.8	12					1		1		1	1		1	1			6/12	6	1	7/12		
	3.8	12														1		1/12	11	0	1/12		
INTRA PERITONEAL	2.8	8	2	3	3													8/8	0	0	8/8	<0.8	<0.8
	1.8	8	2	2	3	1												8/8	0	0	8/8		
	0.8	8		1	1	1	1		1	1		1	1					8/8	0	0	8/8		

Times to death at oral LD50

At log dose 5.8 = 7, 8, 8, 9, 10, 10, 11, 12, 12)
At log dose 4.8 = 8, 10, 12, 13, 15, 16) Mean 10.7 days

N = non-fimbriate

fl = flagellate

$<$ = less than

EXPERIMENT VII. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE/— FLAGELLATE AND THE PARENT NON-FIMBRIATE/— NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND INTRA-PERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. typhimurium strain 6351 N/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on 5th day	23rd day	Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
ORAL	8.4	12		SURVIVORS KILLED	0/12	12	2	2/12	>8.4	>8.4
	7.4	12	1		1/12	11	2	3/12		
	6.4	12			0/12	12	4	4/12		
	5.4	12			0/12	12	0	0/12		
	4.4	12			0/12	12	0	0/12		
	3.4	12			0/12	12	0	0/12		
INTRA PERITONEAL	2.4	12			0/12	12	5	5/12	>2.4	
	1.4	12			0/12	12	8	8/12		
	0.4	12			0/12	12	8	8/12		<0.4

Mean time to death at oral ID50 more than 23 days.

> = greater than < = less than

N = non-fimbriate

fl = flagellate

EXPERIMENT VII. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE/⟶ FLAGELLATE AND PARENT NON-FIMBRIATE/⟶ NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND INTRA-PERITONEAL ROUTES OF INFECTION. (b) Results obtained with S. typhimurium strain 6354 FT/flt.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																		Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose ID_{50}	Log dose ID_{50}
			3	4	5	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23						
ORAL	8.4	12								2			2	1	1				1	1	SURVIVORS KILLED	8/12	4	3	11/12	6.8	5.9
	7.4	12											1	2	2		1	2				8/12	4	2	10/12		
	6.4	12			1					1			1					1		1		5/12	7	4	9/12		
	5.4	12	1																			1/12	11	0	1/12		
	4.4	12															1	1		1		3/12	9	3	6/12		
INTRA PERITONEAL	3.4	12				2		1	1	1	1		1									7/12	5	5	12/12		
	2.4	12		1		2	2		1	2		3										11/12	1	1	12/12		
	1.4	12						2	1	1		1	1	1		1		2				10/12	2	1	11/12	< 1.4	< 1.4

Times to death at oral ID50

At log does 7.4 = 14, 15, 15, 16, 16, 18, 18, 19, (19) Mean 15.2 days
At log dose 6.4 = 5, 11, 14, 19, 22

FT = phage-transduced fimbriate
flt = phage-transduced flagellate
< = less than

EXPERIMENT VIII. COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE/— FLAGELLATE AND THE PARENT NON-FIMBRIATE/NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND THE INTRA-PERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. typhimurium strain 6351 N/nfl.

Route of infection	Log Dose organisms	No. of mice infected	Number of deaths on day									Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			8	10	11	12	13	15	16	21	23					LD_{50}	ID_{50}
ORAL	9.8	12				1	1		1		SURVIVORS KILLED	3/12	9	2	5/12	> 9.8	9.1
	8.8	12							1	1		2/12	10	1	3/12		
	7.8	12					1	1				2/12	10	2	4/12		
	6.8	12							1			1/12	11	1	2/12		
	5.8	12	1									1/12	11	1	2/12		
	4.8	12										0/12	12	0	0/12		
INTRA PERITONEAL	3.8	12			1	1			1			3/12	9	9	13/12	>3.8	
	2.8	12		1				1				2/12	10	10	12/12		
	1.8	12							1			1/12	11	9	10/12		<1.8

Times to death at oral LD50 = 12, 13, 16. Mean 13.3 days

> = more than < = less than

N = non-fimbriate nfl = non-flagellate

EXPERIMENT VIII. COMPARISON OF VIRULENCE OF A NON-FIMBRIATE / — — NON-FLAGELLATE S. TYPHIMURIUM AND ITS FIMBRIATE / FLAGELLATE PHAGE-TRANSDUCTANTS FOR WHITE MICE BY THE ORAL AND THE INTRAPERITONEAL ROUTES OF INFECTION. (b) Results obtained with S. typhimurium strain 6352 FT/nfl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day												Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			8	9	10	11	12	13	14	15	16	17	19	23					LD_{50}	ID_{50}
ORAL	8.9	12		1				1			1		1		4/12	8	8	12/12	8.4	
	7.9	12				3	1		1						5/12	7	5	10/12		
	6.9	12						2	1					KILLED	3/12	9	6	9/12		
	5.9	12									1				1/12	11	4	5/12		6.5
	4.9	12					1							SURVIVORS	1/12	11	0	1/12		
INTRA PERITONEAL	3.9	12		1	3				2	2			1		9/12	3	3	12/12		
	2.9	12		1	3	3	1		1						9/12	3	2	11/12		
	1.9	12	1		1	1	1	1	1	1		1			8/12	4	2	10/12	<1.9	<1.9

Times to death at oral LD50

At log dose 8.9 = 9, 13, 16, 19
At log dose 7.9 = 11, 11, 11, 12, 14 } Mean 12.9 days

< = less than

FT = phage-transduced fimbriate
nfl = non-flagellate

Experiment Salmonellosis I. Appendix 15.

EXPERIMENT VIII. COMPARISON OF VIRULENCE OF A NON-FIMBRIATE/ —— NON-FLAGELLATE S. TYPHIMURIUM AND ITS FIMBRIATE / FLAGELLATE PHAGE-TRANSDUCTANTS FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (c) Results obtained with S. typhimurium strain 6353 N/flt.

Routes of infection	Log dose organisms	No. of mice infected	Number of deaths on day													Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			7	8	9	10	11	12	13	14	15	16	19	20	23						
ORAL	8.7	12	1		2	1	1			1					SURVIVORS KILLED	6/12	6	6	12/12	8.2	
	7.7	12					1					1	1			3/12	9	7	10/12		
	6.7	12						1				1				2/12	10	4	6/12		
	5.7	12					1	1								2/12	10	3	5/12		6.4
	4.7	12														0/12	12	1	1/12		
INTRA PERITONEAL	3.7	12		2	3	2	3						1	1		12/12	0	0	12/12		
	2.7	12		1	2	2	2		2	2	1					12/12	0	0	12/12		
	1.7	12			1	3	2	1	2	1	1					11/12	1	1	12/12	<1.7	<1.7

Times to death at oral LD50

At log 8.7 = 7, 9, 9, 10, 11, 14
At log 7.7 = 11, 16, 19
} Mean 11.8 days

N = non-fimbriate
flt = phage-transduced flagellate
< = less than

EXPERIMENT IX. COMPARISON OF VIRULENCE OF A NON-FIMBRIATE S. TYPHIMURIUM 629N and ITS FIMBRIATE REVERTANT MUTANT 629MF (OBTAINED BY THE USE OF $MnCL_2$ AS A MUTAGENIC AGENT) FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. Typhimurium strain 629MF/fl.

Route of infection	Log Dose organisms	No. of Mice infected	Number of deaths on day									Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			5	6	8	9	11	13	14	16	24					LD_{50}	ID_{50}
ORAL	8.2	12									SURVIVORS KILLED	0/12	12	11	11/12	>8.2	5.9
	7.2	12										0/12	12	9	9/12		
	6.2	12		1								1/12	11	7	8/12		
	5.2	12										0/12	12	3	3/12		
	4.2	12										0/12	12	1	1/12		
INTRA PERITONEAL	3.2	12					1	1	1			3/12	9	9	12/12	2.9	
	2.2	12			2	1	1	1		1		6/12	6	6	12/12		
	1.2	12	1		1							2/12	10	10	12/12		<1.2

Mean time to death at oral LD50 >24 days >= more than <= less than

MF = revertant fimbriate fl = flagellate

EXPERIMENT IX. COMPARISON OF VIRULENCE OF A NON-FIMBRIATE S. TYPHIMURIUM 629N AND ITS FIMBRIATE REVERTANT MUTANT 629RF (OBTAINED BY THE USE OF $MnCL_2$ AS A MUTAGENIC AGENT) FOR WHITE MICE BY THE ORAL INTRAPERITONEAL ROUTES OF INFECTION. (b) Results obtained with S. typhimurium strain 629 N/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																	Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			3	6	7	8	9	11	12	13	14	15	16	17	19	20	24	24						LD_{50}	ID_{50}
ORAL	9	12							1	1								SURVIVORS KILLED		2/12	10	10	12/12	8.6	5.2
	8	12			1											1	1			3/12	9	9	12/12		
	7	12	1					1				1		1						4/12	8	7	11/12		
	6	12							1	1						1	1			4/12	8	7	11/12		
	5	12														1				1/12	11	5	6/12		
	4	12																		0/12	12	0	0/12		
INTRA PERITONEAL	3	12								1	1									2/12	10	10	12/12	2.5	
	2	12				1	2	2					2		1					8/12	4	4	12/12		
	1	12		1								1	1							3/12	9	9	12/12		<1.0

Times to death at oral LD_{50}

At log dose 9 = 12, 13
At log dose 8 = 7, 20, 24 } Mean 15.2 days

<= less than

N = non-fimbriate fl = flagellate

EXPERIMENT X. COMPARISON OF VIRULENCE OF A PHAGE-TRANSDUCED FIMBRIATE/FLAGELLATE STRAIN, A NON-FIMBRIATE/FLAGELLATE STRAIN AND THE PARENT NON-FIMBRIATE/NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. typhimurium strain SW573 N/nf1.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day												Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			3	6	7	11	12	13	15	16	19	20	21	22					LD_{50}	ID_{50}
ORAL	9.0	12			1			2						KILLED	3/12	9	6	9/12	>9.0	6.6
	8.0	12								1	1		1		3/12	9	7	10/12		
	7.0	12	1												1/12	11	7	8/12		
	6.0	12								1					1/12	11	5	6/12		
	5.0	12													0/12	12	1	1/12		
	4.0	12												SURVIVORS	0/12	12	0	0/12		
INTRA PERITONEAL	3.0	12		1		1		1	1		1	1			6/12	6	5	11/12	2.5	
	2.0	12			1	1	1			1	1				5/12	7	7	12/12		
	1.0	12						1		1					2/12	10	7	9/12		<1.0

Mean time to death at oral LD50 = >22 days >= more than <= less than

N = non-fimbriate nf1 = non-flagellate

EXPERIMENT X. COMPARISON OF VIRULENCE OF A PHAGE-TRANSDUCED FIMBRIATE/FLAGELLATE STRAIN, A NON-FIMBRIATE/FLAGELLATE STRAIN AND THE PARENT NON-FIMBRIATE/NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (b) Results obtained with S.typhimurium strain SW 576 N/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day														Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			6	7	8	9	10	12	13	14	15	16	17	20	21	22					LD_{50}	ID_{50}
ORAL	8.0	12						3									3/12	9	7	10/12	>8.0	5.7
	7.0	12									1	1					2/12	10	10	12/12		
	6.0	12												1			1/12	11	7	8/12		
	5.0	12															0/12	12	3	3/12		
	4.0	12															0/12	12	1	1/12		
INTRA PERITONEAL	3.0	12	1	1	2		1	1		1			1	1			9/12	3	3	12/12	<1.0	<1.0
	2.0	12			1	1		1	2	2			1				8/12	4	4	12/12		
	1.0	12			1	1					1		2		1		6/12	6	3	9/12		

Mean time to death at oral LD50 = >22 days

>= more than

<= less than

N = non-fimbriate

fl = flagellate

EXPERIMENT X. COMPARISON OF VIRULENCE OF A PHAGE-TRANSDUCED FIMBRIATE/FLAGELLATE STRAIN, A NON-FIMBRIATE/FLAGELLATE STRAIN AND THE PARENT NON-FIMBRIATE/NON-FLAGELLATE S. TYPHIMURIUM FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.

(c) Results obtained with S. typhimurium strain SW576 FT/flt.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day													Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			3	4	6	7	8	9	10	12	13	14	15	17	22						
ORAL	7.7	12				1									SURVIVORS KILLED	1/12	11	7	8/12	>7.7	5.9
	6.7	12														0/12	12	9	9/12		
	5.7	12											1			1/12	11	6	7/12		
	4.7	12														0/12	12	3	3/12		
	3.7	12														0/12	12	0	0/12		
INTRA PERITONEAL	2.7	12			1			1	1	2		1		1		7/12	5	4	11/12	1.7	
	1.7	12	1	1		1	1	2		2						8/12	4	3	11/12		
	0.7	12									1					1/12	11	7	8/12		<0.7

Mean time to death at oral LD50 is >22 days

> = more than

< = less than

FT= phage-transduced fimbriate flt = phage-transduced flagellate

Experimental Salmonellosis. Appendix 21.

EXPERIMENT XI. COMPARISON OF VIRULENCE OF FIMBRIATE S. TYPHIMURIUM (OBTAINED BY THE USE OF $MnCl_2$ AS A MUTAGENIC AGENT) AND THE NON-FIMBRIATE PARENT STRAIN, FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (a) Results obtained with S. typhimurium strain 1436 MF/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																		Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose LD_{50}	Log dose ID_{50}
			3	4	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22						
ORAL	8.3	12								1		1	1			1				SURVIVORS KILLED	4/12	8	8	12/12	7.3	4.9
	7.3	12						1	1		1				1			1			5/12	7	7	12/12		
	6.3	12											1		1				2		4/12	8	5	9/12		
	5.3	12												1	1			1			3/12	9	6	9/12		
	4.3	12						1		1						1					3/12	9	1	4/12		
INTRA PERITONEAL	3.3	12		1		1	1	1	3	1	1	1		1							11/12	1	1	12/12		
	2.3	12	1		1	3			2			1		1			1				10/12	2	2	12/12		
	1.3	12			1	2	1	1	1	1	1			1							9/12	3	2	11/12	< 1.3	< 1.3

Times to death at oral LD50

At log dose 7.3 = 9, 10, 12, 17, 20 Mean 13.6 days MF = mutagenic fimbriate fl = flagellate = less than

EXPERIMENT XII. COMPARISON OF VIRULENCE OF FIMBRIATE S. TYPHIMURIUM (OBTAINED BY THE USE OF $MnCl_2$ AS A MUTAGENIC AGENT) AND THE NON-FIMBRIATE PARENT STRAIN FOR WHITE MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION. (b) Results obtained with S. typhimurium strain 1436 NF/fl.

Route of infection	Log dose organisms	No. of mice infected	Number of deaths on day																Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			3	5	7	8	9	10	11	12	13	14	15	16	17	18	20	22					LD_{50}	ID_{50}
ORAL	9.6	12		1	1		2		1	1	1	2						SURVIVORS KILLED	9/12	3	3	12/12	7.8	5.7
	8.6	12									1	1	1	1	1				5/12	7	6	11/12		
	7.6	12													1				1/12	11	9	10/12		
	6.6	12	1		1		1										1		4/12	8	7	11/12		
	5.6	12		2					1								1		4/12	8	2	6/12		
	4.6	12		1															1/12	11	2	3/12		
INTRA PERITONEAL	3.6	12	1		2	2	1	3	1		1			1					12/12	0	0	12/12		
	2.6	12				3	3	2	1					1					10/12	2	1	11/12		
	1.6	12						2	1	1	1		1		1	1	1		9/12	3	2	11/12	<1.6	<1.6

Times to death at oral LD50

At log dose 7.6 = 17
At log dose 8.6 = 13, 14, 15, 16, 17 } Mean = 15.3 days

NF = non-fimbriate
fl = flagellate
< = less than

Experimental Salmonellosis III. Appendix 23.

THE RESULTS OF INTRA-PERITONEAL INFECTION OF ALBINO RATS WITH THE PARENT NON-FIMBRIATE STRAIN AND A PHAGE-TRANSDUCED FIMBRIATE STRAIN OF S. TYPHIMURIUM 1289.

Test strain	Log dose organisms	¥ No. of rats infected	Number of deaths on day					Mortality	Survivors	No. of Survivors Infected	Infectivity	Log dose	
			10	12	13	16	30					ID_{50}	ID_{50}
Non-fimbriate S. typhimurium 1289N/fl	7.2	6				1	KILLED	1/6	5	1	2/6	>7.2	>7.2
	6.2	6						0/6	6	0	0/6		
	5.2	6						0/6	6	0	0/6		
	4.2	6						0/6	6	0	0/6		
	3.2	6						0/6	6	0	0/6		
Phage-transduced fimbriate S.typhimurium 1289FT/fl	8.2	6	1	1	1		SURVIVORS	3/6	3	1	4/6	8.2	7.5
	7.2	6						0/6	6	2	2/6		
	6.2	6						0/6	6	1	1/6		
	5.2	6						0/6	6	1	1/6		
	4.2	6						0/6	6	0	0/6		

N = non-fimbriate strain FT = phage-transduced fimbriate strain fl = flagellate >= more than

¥ 3 of each sex were infected with one dose

Experimental Salmonellosis V. Appendix 24.

Experiment No. 1. RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF S. TYPHIMURIUM ON LEUCOCYTES IN VITRO.

Test preparation	Period of test (Time of reading) Min.	No. of viable leucocytes* of the 25 observed in each case	Percent. leucocytes killed and mean percentage mortality during first 2 hour observation		Remarks
Leucocyte control suspension	10	15	40)		
	45	15	40)	40	
	80	15	40)		
	110	15	40)		A few moribund
	180	10	60		Almost all moribund
S. typhimurium LT2 fimbriate strain mixed with leucocyte suspension	20	15	40)		Bacterial adhesion +++
	60	7	72)	64	
	85	8	68)		)Many moribund
	120	6	76)		) leucocytes
	185	5	75		) seen
S. typhimurium 7471 non-fimbriate strain mixed with leucocyte suspension	35	14	44)		No bacterial adhesion
	67	15	40)	55	A few small clumps seen
	95	7	72)		
	125	9	64)		
	190	2	92		All moribund

* As judged by observation of oscillation of leucocytic granules (see Methods)

Experiment No. II. RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF S. TYPHIMURIUM 1287 on LEUCOCYTES IN VITRO.

Test preparation	Period of test (Time of reading) Min.	No. of viable leucocytes* of the 25 observed in each case	Percentage of dead leucocytes and mean percentage mortality during first 2 hr. of observation	Remarks
Leucocyte control suspension	15 120	18 13	28) 48) 38	A few clumps seen
S. typhimurium 1287 FT/f1 phage-transduced fimbriate strain mixed with leucocytic suspension	25 60 105	5 2 4	75) 92) 84 84)	Bacteria adhering to leucocytes ++ Clumps ++ Moribund leucocytes seen
S. typhimurium 1287 N/f1 non-fimbriate parent strain mixed with leucocytic suspension	30 70 115	4 10 11	44) 60) 53 56)	A few clumps of leucocytes seen

* See Appendix 24.

Experimental Salmonellosis V. Appendix 26.

Experiment No. III. RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF SHIGELLA FLEXNERI on LEUCOCYTES IN VITRA.

Test preparation	Period of test (Time of reading) Min.	No. of viable leucocytes¥ of the 25 observed in each case	Percentage of dead leucocytes and mean percentage mortality during first 2 hr. of observation	Remarks
Leucocyte control suspension	20 50 85 135	20 20 22 19	20) 20) 12) 24) 18.5	No clumps seen
S. flexneri Fla 1. fimbriate (living) mixed with leucocytic suspension	28 58 88 120 202	16 14 13 9 6	36) 44) 48) 48 64) 76	Bacteria adhering leucocytes seen. Clumps ++ Many moribund leucocytes seen
Heated+ S.flexneri Fla 1. mixed with leucocytic suspension	33 64 110 145 210	20 16 10 7 1	20) 36) 60) 47 72) 96	Bacteria adhering to leucocytes and clumps seen Moribund leucocytes+
S. flexneri Fla 2. non-fimbriate mixed with leucocytic suspension	40 62 112 152 213	22 21 23 15 14	12) 16) 8) 19 40) 44	Bacteria adhering to leucocytes and clumps seen
Heated+ S.flexneri Fla 2. mixed with leucocytic suspension	45 75 123 158 218	22 22 19 10 11	12) 12) 24) 27 60) 56	- do -

+ 56°C. for 30 min.

¥ See Appendix 24

Experiment No. IV. RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF S. TYPHIMURIUM 1289 ON LEUCOCYTES IN VITRO.

Test preparation	Period of test (Time of reading) Min.	No. of viable leucocytes¥ of the 25 observed in each case	Percentage of dead leucocytes and mean percentage mortality during first 2 hr. of observation		Remarks
Leucocyte control suspension	20 60 185 240	14 15 13 8	44) 40) 48) 68	44	
S. typhimurium 1289 FT/f1 phage-transduced fimbriate strain mixed with leucocytic suspension	35 65 165	13 13 7	48) 48) 68)	55	Bacteria adhering to leucocytes and clumps seen. Moribund leucocytes seen.
Heated+ S. typhimurium 1289 FT/f1 phage-transduced fimbriate strain mixed with leucocytic suspension	45 75 170 280	13 13 8 6	48) 48) 68) 76	55	- do -
S. typhimurium 1289 N/f1 non-fimbriate strain	50 80 175 285	13 15 18 8	48) 40) 60) 68	49	Clumps or bacteria adhesion not seen
Heated+ S. typhimurium 1289 N/f1 non-fimbriate mixed with leucocytic suspension	55 88 180 288	13 12 11 9	48) 52) 56 66	55	- do -

+ 56°C. for 30 min.

¥ See Appendix 24.

Experimental salmonellosis VII. Appendix 28.

Experimental Immunity I. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY AVIRULENT NON-FIMBRIATE/NON-FLAGELLATE STRAIN OF S. TYPHIMURIUM (6351NF/nfl, phage type 2a/13). THE MICE WERE IMMUNISED BY A SUBCUTANEOUS INJECTION, AND WERE CHALLENGED, ON THE 15th DAY, BY INTRAPERITONEAL INJECTION OF A HETEROLOGOUS STRAIN (S. TYPHIMURIUM 1566F, phage type 1a/U57).

Vaccine preparation	Active immunisation: Log dose of vaccine (No. of organisms)	Active immunisation: * No. of mice	Number of deaths prior to challenge	Intraperitoneal challenge after 15 days: Log dose (No. of organisms)	Intraperitoneal challenge after 15 days: No. of mice	Following challenge, number of deaths on day: 3	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Mortality	Survivors	Survivors infected	Mortality percentage	Percentage infectivity	Mean time to death in days
Heat-killed phenol-preserved	3	36	0	3.1	18		1				2	1	2	1		1		1	1	1			11/18	7	7	66.1	100	14.4
				5.1	18				3	2	1	1	1	1	2	2				1			14/18	4	4	77.8	100	13.3
	5	36	0	3.1	18				1	3	1	1		1	1	1			1				10/18	8	8	55.5	100	13.3
				5.1	18		1	1	3	1	1	1	1	1	1			1	1				14/18	4	4	77.8	100	12.4
	7	36	2	3.1	17						1	2	1			1							5/17	12	12	29.4	100	13
				5.1	17			1		1	1	1	1	2	2			1		1	2		13/17	4	4	76.5	100	14.2
Acetone-killed freeze-dried	3	36	1	3.1	17						1	3		1	3		1						9/17	8	8	52.9	100	13.8
				5.1	18			2	2	1	2	3		2		1			1				14/18	4	4	77.8	100	12.4
	5	36	0	3.1	18					1	1	5	1					1	3				12/18	6	5	66.6	93.6	13.8
				5.1	18		1		1		5		3	1			1	1		1			14/18	4	4	77.8	100	13.8
	7	36	0	3.1	18					1		3	1	3	1		1						10/18	8	8	55.5	100	13.5
				5.1	18		1	1		2	1	2	2	3	1				1				14/18	4	4	77.8	100	12.1
Control: a group of 36 unvaccinated mice			0	3.1	18	1		1				1		2	1		1	1			2		10/18	8	8	55.5	100	13.3
				5.1	18		1			1	2	2		2	1		1	1		1			12/18	6	6	66.6	100	13.7

* Each group contained an equal number of male and female mice; the mice weighed 19 to 22 grams each and the groups were balanced by weight.

F = fimbriate NF = non-fimbriate nfl = non-flagellate

Experimental salmonellosis VII. Appendix 29.

Experimental Immunity III. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF VACCINES PREPARED FROM A RELATIVELY VIRULENT S. TYPHIMURIUM 6354 (A PHAGE-TRANSDUCED FIMBRIATE/FLAGELLATE STRAIN DERIVED FROM THE PARENT STRAIN OF S. TYPHIMURIUM 6351NF/nf1). THE MICE WERE IMMUNISED BY A SINGLE SUBCUTANEOUS INJECTION AND WERE CHALLENGED ON THE 15th DAY BY INTRAPERITONEAL INJECTION OF A HETEROLOGOUS STRAIN (S. TYPHIMURIUM 1566F, PHAGE TYPE 1a/U57).

Vaccine preparation	Immunisation: Log dose vaccine (No. of organisms)	Immunisation: No. of mice	Deaths prior to challenge	Intraperitoneal challenge after 15 days: Log dose (No. of orgs.)	Intraperitoneal challenge after 15 days: No. of mice	Following challenge, number of deaths on day 3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Cort-like	Survivors	Surv. infected	Effectivity	Effectivity cort-like	Effectivity infected	Mean time to death in days
Heat-killed phenol-preserved	3	24	0	3.2	12						1	3	1	3						1	1				10/12	2	2	12/12	83.3	100	13.2
				5.2	12	1				1	1	1	1	2	1						1				9/12	3	3	12/12	75	100	10.6
	5	24	2	3.2	11			1				2	2			1	1								7/12	5	5	12/12	58.3	100	11.2
				5.2	11	2	1				1	2	1	2		1	1								11/11	0	0	11/11	100	100	9.9
	7	24	0	3.2	12									1				1		1			1		4/12	8	7	11/12	33.3	91.6	16.8
				5.2	12				1		2			1	1						1				6/12	6	6	12/12	50	100	10.0
Acetone-killed freeze-dried	3	24	0	3.2	12								2	1	1	2	1						1		9/12	3	3	12/12	75	100	15.0
				5.2	12					1	1	2		1		1									6/12	6	6	12/12	50	100	10.6
	5	24	2	3.2	11			1			3	1	1	1			1		1	1	1				11/11	0	0	11/11	100	100	13.3
				5.2	11					1	5	2	1	1											10/11	1	1	11/11	90.9	100	10
	7	24	4	3.2	10			1			1	1		1				1							5/10	5	5	10/10	50	100	10.6
				5.2	10				2		1	1		1								2			7/10	3	3	10/10	70	100	11.6
Control: a group of 24 unvaccinated mice		2		3.2	11				1		1	1	2	1						1		1			8/11	3	3	11/11	72.7	100	12.6
				3.2	11				1	2			1	3	1		1			1					10/11	1	1	11/11	90.9	100	12.0

NF = non-fimbriate nf1 = non-flagellate FT = phage-transduced fimbriate F = fimbriate
ftl = phage-transduced flagellate

Experimental salmonellosis VII. Appendix 30.

Experimental Immunity V. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES (WITH AND WITHOUT FREUND'S INCOMPLETE ADJUVANT) PREPARED FROM A RELATIVELY AVIRULENT S. TYPHIMURIUM 6351 (A NON-FIMBRIATE/NON-FLAGELLATE STRAIN OF PHAGE TYPE 2a/13). THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS OF LOG 7 and LOG 8 ORGANISMS RESPECTIVELY. THE INTERVAL BETWEEN INJECTIONS WAS 14 DAYS AND THE MICE WERE CHALLENGED INTRAPERITONEALLY 7 DAYS AFTER THE 2nd INJECTION WITH S. TYPHIMURIUM 1564F, PHAGE TYPE 1a/U57.

Vaccine preparation	No. of mice immunised	Deaths prior to challenge 1st dose	Deaths prior to challenge 2nd dose	Challenge I.P. 7 days after 2nd injection Log dose	Challenge I.P. 7 days after 2nd injection No. mice	Following challenge number of deaths on day 3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	23	24	26	29	Mortality	Survivors	Survivors infected	Infectivity	Percentage mortality	% Infectivity	Mean time to death in days
Heat-killed phenol preserved (HP)	40	2	0	3.5	19					1	2	1	2	1									1			SURVIVORS FILED	8/19	11	11	19/19	42.1	100	122
				5.5	19		1				1	1	4	2		1	2				1			1				14/19	5	5	19/19	73.6	100	132
HP vaccine +Freund's inc'plete adjuvant	40	2	1	3.5	18						1	1	1	3	2				1				1				10/18	8	7	17/18	55.5	94.4	136
				5.5	19				2	1	2	4	2			1											12/19	7	7	19/19	63.1	100	9.8
Acetone-killed freeze-dried (AK)	40	2	0	3.5	19						1		3		4	2			1		1	1			1		14/9	5	5	19/19	73.6	100	164
				5.5	19			1	1	2	2	3		2	2	1	1	1									16/9	3	3	19/19	86.1	100	110
AK vaccine + Freund's inc'plete adjuvant	40	4	1	3.5	18				1	1		2	2	1	2	2	1	2		1							15/18	3	3	18/18	83.3	100	124
				5.5	17					1	5	2	1	3			1						1				15/17	2	2	17/17	88.2	100	126
Control: a group of 20 unvaccinated mice		1	0	3.5	10							1	4		1												6/10	4	4	10/10	60	100	107
				5.5	9						1	1	2	3				1										8/9	1	1	9/9	89.1	100	116

Each group contained an equal number of male and female mice which weighed 18-22 grams each and the groups were balanced by weight. F= fimbriate NF = non-fimbriate nfl = non-flagellate

Experimental salmonellosis VII. Appendix 31.

Experimental Immunity VII. COMPARISON OF PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY VIRULENT S. TYPHIMURIUM 6354 FT/flt PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS OF LOG 7 AND LOG 8 ORGANISMS RESPECTIVELY. THE INTERVAL BETWEEN THE TWO INJECTIONS WAS 14 DAYS, AND THE MICE WERE CHALLENGED INTRAPERITONEALLY 10 DAYS AFTER THE 2nd IMMUNISING DOSE WITH S. TYPHIMURIUM 1566F, PHAGE TYPE 1a/U57.

Vaccine preparation	No. of mice immunised	Deaths prior to challenge		Challenge I.P. 10 days after 2nd dose		Following challenge number of deaths on day																		Mortality	Survivors	Survivors infected	Infectivity	Percentage mortality	Percentage infectivity	Mean time to death in days
		1st dose	2nd dose	Log dose	No. mice	3	6	8	9	10	11	12	13	14	16	17	18	20	21	22	23	24	25	29							
Heat-killed phenol preserved	40	NIL		3.0	20				1					2		1			1			1		SURVIVORS CHALLENGED	6/20	14	14	20/20	30	100	19
				5.0	20						2	1	1		1				1	1					7/20	13	13	20/20	35	100	15.8
Acetone-killed freeze-dried	40	NIL		3.0	20			2			1	1		1		1							1		8/20	12	11	19/20	40	95	15.3
				5.0	20	1	1			1	2				1					2	3	1			12/20	8	8	20/20	60	100	14.4
Control: a group of 20 unvaccinated mice		NIL		3.0	10			2	2	2				1	1			1							9/10	1	1	10/10	90	100	12.8
				5.0	10		2	1		1	2	2	1				1								10/10	0	0	0	100	100	11.1

Each group contained an equal number of male and female mice and groups were balanced by weight. The mice weighed from 18 to 22 grams each.

FT = phage-transduced fimbriate flt = phage-transduced flagellate

Experimental salmonellosis VII. Appendix 32.

Experimental Immunity X. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM S. TYPHIMURIUM STRAINS 6351N/nf1 and 6354FT/f1t. THE MICE WERE IMMUNISED BY TWO SUBCUTANEOUS INJECTIONS OF LOG 7 and LOG 8 ORGANISMS RESPECTIVELY. THE INTERVAL BETWEEN THE INJECTIONS WAS 14 DAYS AND THE MICE WERE CHALLENGED ORALLY 10 DAYS AFTER THE SECOND IMMUNISING DOSE WITH S. TYPHIMURIUM 1566F/91.

Vaccine preparation	No. of mice immunised	Deaths prior to challenge 1st dose	Deaths prior to challenge 2nd dose	Oral challenge 10 days after 2nd injection Log dose	Oral challenge 10 days after 2nd injection No. mice	Following challenge number of deaths on day 3	5	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Mortality	Survivors	Survivors Infected	Infectivity	Percentage Mortality	Percentage Infectivity	Mean time to death in days
Heat-killed phenol preserved (HP) strain 6351N/nf1	24	0	0	6.1	12							1				1							SURVIVORS KILLED	2/12	10	9	11/12	16.6	91.6	14
				8.1	12											1		1						2/12	10	10	12/12	16.6	100	17
Acetone-killed freeze-dried(AK) strain 6351N/nf1	24	1	0	6.1	11																			0/12	12	11	11/12	0	91.6	23
				8.1	12			1			1	1												3/12	9	9	12/12	25	100	10.3
Heat-killed phenol preserved (HP) strain 6354FT/f1t	24	2	0	6.1	11	1																		1/11	10	8	9/11	9.1	81.9	3
				8.1	11					1		1									1	1		4/11	7	6	10/11	36.4	90.9	16.2
Acetone-killed freeze-dried strain6354FT/f1t	24	1	3	6.1	11					1														1/11	10	8	9/11	9.1	81.9	10
				8.1	9					1					1									2/9	7	5	7/9	22.2	77.7	12.5
Control: a group of 24 unvaccinated mice		0	0	6.1	12				1	1		1				1			1					5/12	7	7	12/12	41.7	100	13.2
				8.1	12		1		2	1				1				2	1	1				9/12	3	3	12/12	75	100	13.6

Each group contained an equal number of male and female mice which weighed from 16-22 grams each. The groups were balanced by weight. N = non-fimbriate FT = phage-transduced fimbriate F = fimbriate nf1 = non-flagellate f1t = phage-transduced flagellate f1 = flagellate

Experimental Immunity II. COMPARISON OF PROTECTIVE POTENCY FOR SWISS WHITE MICE OF A LIVE ATTENUATED (SINGLE STEP STREPTOMYCIN-RESISTANT MUTANT) VACCINE PREPARED FROM A RELATIVELY AVIRULENT *S. TYPHIMURIUM* 6351 (NON-FIMBRIATE AND NON-FLAGELLATE: PHAGE TYPE 2a/13) BY THE SUBCUTANEOUS AND ORAL ROUTES OF IMMUNISATION. ONE GROUP OF ORALLY IMMUNISED MICE WERE STREPTOMYCIN-TREATED. THE MICE WERE CHALLENGED ON THE 15th DAY BY AN INTRAPERITONEAL INJECTION OF A HETEROLOGOUS STRAIN (*S. TYPHIMURIUM* 1566F, PHAGE TYPE 1a/U57).

Vaccine preparation	Active immunisation: Route	Log dose vaccine	No. of mice	No. deaths prior to challenge	Intraperitoneal challenge after 15 days: Log dose	No. Mice	Following challenge, number of deaths on day: 3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	Mortality	Survivors	Survivors infected	Survivors not infected	Percentage mortality	Percentage infectivity	Mean time to death in days
Live attenuated vaccine single step streptomycin-resistant mutant 6351/SR	Subcutaneous	0.9	24	0	3.2	12										1	1							SURVIVORS KILLED	2/12	10	9	1	16.5	91.6	13.5
					5.2	12			1		1									1					3/12	9	9	0	25	100	10.3
		1.9	24	2	3.2	11						1													1/11	10	10	0	9.1	100	9
					5.2	11				1									1						2/11	9	9	0	18.2	100	11.5
		2.9	24	3	3.2	10																			0/10	10	9	1	0	90	>22
					5.2	11																			0/11	11	11	0	0	100	>22
-do-	Oral	0.9	24	2	3.2	11						1			3							1			5/12	7	6	1	41.6	91.6	13.3
					5.2	11					2			3	1					1	1				8/12	4	4	0	66.6	100	13.2
		1.9	24	2	3.2	11	1					1		1	3	1					1		1		9/11	2	2	0	81.9	100	12.4
					5.2	11						1	3	1		1							1		7/11	4	4	0	63.7	100	12.8
		2.9	24	3	3.2	10									2	1									3/10	7	7	0	30	100	12.5
					5.2	11					2	2	1	1		1	1			1		1			10/11	1	1	0	91	100	12.7
-do-	Oral streptomycin treated mice	0.9	24*	1	3.2	11																			0/11	11	11	0	0	100	>22
					5.2	12								1						1					2/12	10	10	0	16.5	100	14
		1.9	24*	0	3.2	12																			0/12	12	11	1	0	91.6	>22
					5.2	12																			0/12	12	12	0	0	100	>22
		2.9	24*	0	3.2	12																			0/12	12	11	1	0	91.6	>22
					5.2	12						1													1/12	11	10	1	8.2	91.6	9
Control: a group of 24 unvaccinated mice				1	3.2	11		1				1	2			2		1	1			1			9/11	2	2	0	81.9	100	12.6
					5.2	12		1		1	1	3	1	1	1			1		1					11/12	1	1	0	91.6	100	10.4

* The mice in these groups were treated with streptomycin orally on the day prior to vaccination. = more than

ó Each group contained an equal number of male and female mice; the mice weighed 19-22 g. each and the groups were balanced by weight.

NF = non-fimbriate nfl = non-flagellate SR = streptomycin-resistant

Experiment Immunity IV. COMPARISON OF THE PROTECTIVE POTENCY FOR SWISS WHITE MICE OF ALIVE ATTENUATED VACCINES (SINGLE STEP STREPTOMYCIN-RESISTANT MUTANTS), PREPARED FROM S. TYPHIMURIUM STRAINS 6351 NF/nfl and 6354 FT/flt OF PHAGE TYPE 2a/13. THE MICE WERE IMMUNISED BY A SINGLE SUBCUTANEOUS INJECTION. THE VACCINES WERE EMPLOYED IN THREE STRENGTHS. THE MICE WERE CHALLENGED ON THE 15th DAY OF IMMUNISATION BY AN INTRAPERITONEAL INJECTION OF S. TYPHIMURIUM 1566F, PHAGE TYPE 1a/U57. THE SURVIVORS WERE KILLED ON THE 30th DAY FOLLOWING CHALLENGE.

Attenuated alive S.typhimurium vaccines	Immunisation (single subcutaneous injection) Log dose	No. of mice	Deaths prior to challenge	I.P.challenge1566F on the 15th day Log dose	No.of mice	Following challenge, number of deaths on day 3	4	5	6	7	8	9	10	11	12	13	14	16	17	20	25	30	Mortality	Survivors No.survivors infected	Infectivity	Percentage mortality	Percentage infectivity	Mean time to death in days
6351 NF/nfl/SR	1.1	24	0	5.3	9					1					1		1				1	SURVIVORS KILLED	4/9	5	5	9/9	44.4	100	14.5
				7.3	9	1	1		5				2										9/9	0	0	9/9	100	100	5.8
	2.1	24	1	5.3	9																		0/9	9	9	9/9	0	100	>30
				7.3	9		1	2	2	2													7/9	2	2	9/9	77.7	100	5.8
	3.1	24	0	5.3	9																		0/9	9	9	9/9	0	100	>30
				7.3	9	2		1		1	2					1							7/9	2	2	9/9	77.7	100	7.2
6354 FT/flt/SR	1.0	24	0	5.3	9						4		1	3				1					9/9	0	0	9/9	100	100	11.3
				7.3	9	1		3	1	1	1		2										9/9	0	0	9/9	100	100	6.5
	2.0	24	0	5.3	9						2			1				1	1				5/9	4	4	9/9	55.5	100	13.0
				7.3	9		2	1	5		1												9/9	0	0	9/9	100	100	5.8
	3.0	24	0	5.3	9						2	3	2							1			8/9	1	1	9/9	88.8	100	11.8
				7.3	9		1	2	5	1													9/9	0	0	9/9	100	100	5.8
Control: a group of 24 unvaccinated mice			0	5.3	9					2	3	2	1					1		1	1		11/12	1	1	12/12	91.6	100	13.6
				7.3	9		2	3	5	1					1								12/12	0	0	12/12	100	100	6.8
6351NF/nfl/SR immunised control: not challenged				-	6																		0/6	6	1	1/6	0	16.6	>30
				-	5																		0/5	5	0	0/5	0	0	
				-	6																		0/6	6	0	0/6	0	0	
6354FT/flt/SR immunised control: not challenged				-	6																		0/6	6	0	0/6	0	0	
				-	6																		0/6	6	0	0/6	0	0	
				-	6																		0/6	6	0	0/6	0	0	

Log doses of the vaccine and the challenge strain were estimated by the Miles and Misra method.

NF = non-fimbriate nfl = non-flagellate SR = streptomycin-resistant
FT = phage-transduced fimbriate flt = phage-transduced flagellate

>= more than

Experimental salmonellosis VII. Appendix 35.

Experimental Immunity XI. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF ALIVE ATTENUATED VACCINES (SINGLE STEP STREPTOMYCIN-RESISTANT MUTANTS) PREPARED FROM S. TYPHIMURIUM STRAINS 6351N/nf1 and 6354FT/f1t. THE MICE WERE IMMUNISED BY A SINGLE SUBCUTANEOUS INJECTION OF LOG 3 ORGANISMS. THEY WERE CHALLENGED ORALLY ON THE 15th DAY OF IMMUNISATION WITH S. TYPHIMURIUM STRAIN 1566F/f1. THE SURVIVORS WERE KILLED ON THE 23rd DAY FOLLOWING THE CHALLENGE.

Attenuated vaccine preparation	Immunisation No. of mice	Deaths prior to challenge	Oral challenge on 15th day		Following challenge, number of deaths on day																	Mortality	Survivors	No. survivors infected	Infectivity	Percentage		Mean time to death in days
			Log dose	No of mice	5	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23					Mortality	Infectivity	
6351N/nf1/SR	24	1	6.1	11					1							1					SURVIVORS KILLED	2/11	9	6	8/11	18.2	72.7	14.5
			8.1	12		1									1							2/12	10	5	7/12	16.6	58.3	12.5
6354FT/f1t/SR	24		6.1	12										1								1/12	11	10	11/12	8.3	91.6	16
			8.1	12			1					1			2	1						5/12	7	7	12/12	41.7	100	15
Immunised control: (6351 N/nf1/SR)	12	0	-																			0/12	12	3	3/12	0	24.9	>23
not challenged (6354 FT/f1t/SR)	12	0	-																			0/12	12	1	1/12	0	8.3	>23
Control: a group of 24 unvaccinated mice		1	6.1	12		1		1				1		1				1				5/12	7	7	12/12	41.7	100	13.8
			8.1	11	1	1				1		1		1		1		1	1			8/11	3	3	11/11	72.7	100	14.4

Each group contained an equal number of male and female mice which weighed 16-22 grams each. The groups were balanced by weight.

Log dose vaccines and challenge were estimated from viable counts by Miles and Misra method.

N = non-fimbriate FT = phage-transduced fimbriate F = fimbriate nf1 = non-flagellate
f1t = phage-transduced flagellate f1 = flagellate SR = streptomycin-resistant >= more than