PATHOGENESIS AND CONTROL OF
EXPERIMENTAL SALMONELLA INFECTIONS

by

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the University of Edinburgh in the Faculty of Medicine

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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.
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 under¬
standing and the control of these diseases would be contin¬
genent 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 intraperito¬
neally 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 (Grskov and Kauffmann, 1936). The organisms
multiply at the site of the injection and are not widely
disseminated (Bacon, Burrows and Yates, 1951). Fortunatel¬
ly, 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 occurring 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.


(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.
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The author is truly grateful to Professors R. Cruickshank and J.P. Duguid and Dr. R.H.A. Swain for their continuous interest, advice, guidance and constructive criticism throughout the period of this work in Edinburgh. He is indebted to Professor J.P. Duguid and Dr. J. Taylor for the supply of the \textit{Salmonella typhimurium} and \textit{Escherichia coli} test strains and to Dr. J.G. Collee for reading the manuscript and making many constructive suggestions. He extends his sincere thanks to Dr. M. McDonald, Dr. B.K. Patnaik and Dr. S. Poole for their help in the preparation of bacteria (\textit{S. typhimurium}) and rat intestinal tissue sections for electron microscope examination. Although the author is grateful to every member of the technical, clerical and the auxiliary services of the Department of Bacteriology, Medical School, Edinburgh, for their helpful cooperation he wishes particularly to express his gratitude to Mr. G. Wilson, Mr. C.J. Smith, Mrs. J.S. Thomson, Mrs. N. McCalman, Miss I. McDiarmid and Miss Irene Stewart.
Finally he thanks the librarian of the Central Medical Library, Miss M.D. Bell, and her staff for their generous assistance.
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REVIEW OF LITERATURE
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 a, 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 0 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 group phase variation. This was described by Andrews (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-3 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. typhii_ 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. medleagridis_ 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 0 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 0 antigens of Salmonella group C2 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, H-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 indiscriminately 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 Trinkmann (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 *Enterobacteriaceae* 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
18.

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 proper-
ties 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 demonstra-
ted that salmonella types otherwise unrelated antigeni-
cally 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 desig-
nated 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 subtypes, 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 \textit{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 \textit{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 \textit{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. adelaeide, S. waycross, S. bovis- morbificans, and S. potadam. 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. typhimurium (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. typhimurium 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. cholerae suis, 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-equus 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 underdeveloped 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 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. \( S.\)\textit{typhimurium} and \( S.\)\textit{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 \( S.\)\textit{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:


(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 Besredka (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: Hormasche, 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. typhi-murium 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 mucosa 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 fimbria-tion 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 intes-tinal 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 attemp-ted 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 \textit{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 \textit{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 bacteriemia 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 Besredikha (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 bacteriæmia after 4 to 10 days of oral infection; most had fever of 1 to 9 days duration — generally coincident with the bacteriæmia although there was a tendency for the bacteriæmia 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 \textit{S. typhi} organisms.

**Experimental studies with \textit{S. typhimurium}**

The effects produced by the administration of living cultures of \textit{S. typhimurium} to small rodents are entirely different from those produced in them by laboratory infection with \textit{S. typhi}. \textit{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 \textit{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 \textit{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 \textit{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 pneumatic 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 bacteremia. 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 bacteraemia. 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 bloodstream 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 Oltiszk (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 bacteraemia but tended to localise in the first stage in the mesenteric lymph nodes from whence at a later stage the bacteria entered the bloodstream 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.

Fimbriase 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. Mülleer (1912) found the bacteria in the intestine during all stages of infection. Grskov 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* (32/446) 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 $10^6$ 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 $5 \times 10^9$ 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 $5 \times 10^5$ organisms. The bacterial counts on blood samples collected by cardiac puncture 2 to 3 minutes after oral infection were $5 \times 10^2$ organisms per ml. of blood. The bacterial count in the blood increased and reached the peak (about $3 \times 10^3$) after 20 minutes. After that he discovered that the numbers of organisms decreased thus: $1.6 \times 10^3$ after 1 hour; $8 \times 10^2$ after 2 hours; and $1.7 \times 10^2$ 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 ($5 \times 10^2$ per 100 mg. of spleen) of organisms was found but after 24 hours these had increased to $1.2 \times 10^2$ and to $5 \times 10^2$ 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. typhi-
murium_ 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 reticulo-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 \textit{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 \textit{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 microflora (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 in vitro 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. Brucella abortus in bovine cotyledon which is rich in the abortus 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 eradica-
tion 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 Kossowa 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 Guthoorn, 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 Auzims (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 (Schütze, 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. typhi-murium 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 different 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, Landy and Freeman, 1952); and purified O antigen (phosphorylated lipopolysaccharide) derived from *S. typhi* 0 901 (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 (Ikie, 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.
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 percent. 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-Ericsen and Tulinius, 1952; and Michaux, 1963) or penicillin in combination with other agents (Vaichulis, 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 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

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 V).

(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 salmonellas 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  
\textit{S. typhimurium} 1289FT/fl  
\textit{S. typhimurium} 1289N/fl (parent strain  
phage-type 1 var 2/441)

Pair no. 2  
\textit{S. typhimurium} 1294 FT/fl  
\textit{S. typhimurium} 1294N/fl (parent strain  
phage-type 2b/14a)

Pair no. 3  
\textit{S. typhimurium} 1287FT/fl  
\textit{S. typhimurium} 1287N/fl (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  
\textit{S. typhimurium} 1289FT/fl  
\textit{S. typhimurium} 1289N/fl (parent strain  
phage-type 1 var 2/441)

Pair no. 2  
\textit{S. typhimurium} 1294FT/fl  
\textit{S. typhimurium} 1294N/fl (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.  
\textit{S. typhimurium} 1566 F/fl phage-type 1a/U57  
\textit{S. typhimurium} 1566 N/fl phage-type 1a/U57

Both lines were derived by Professor Duguid from a single  
culture of \textit{S. typhimurium} that had been isolated from a pig  
in Indiana and supplied by Dr. P.R. Edwards. Serologi-  
cally 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 MnCl₂ as a mutagenic agent by Dr. D.C. Old (1963).

**Pair no. 1**

*Salmonella 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 4/738)

**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

*S. typhimurium* SW573N/nfl (parent strain)

(Friewer and Leifsen, 1952).

**Expt. no. 3**

*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 $4 \times 10^7$ 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 $4 \times 10^8$ organisms
per ml. Five tubes were set up as follows:

(i) $10^8$ organisms (1 ml. 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. 199).

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$_2$HPO$_4$ 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. \( \text{Na}_2\text{HPO}_4 \) 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. \( \text{Na}_2\text{HPO}_4 \) 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 \textit{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 140) were used in these experiments. All the strains except *S. typhimurium* 1566 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* 1269, 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 over¬
night 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 $4 \times 10^8$ 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$_2$HPO$_4$ 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 0 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* 1289PT - 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. \( \text{Na}_2\text{HPO}_4 \) solution (pH 8.8) and used as the inoculum; the approximate viable bacterial count was \( 4 \times 10^6 \) 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
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 antimesenteric 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.


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 pre-
sence of dead and living leucocytes (see below). A total
of 25 dead and living leucocytes were counted during each
microscopic examination. A leucoytic 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 consider-
ed to be living. Cells that stained frankly but still
showed sluggish oscillation of their granules were consid-
ered 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 an 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 fimbriae 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$_2$PO$_4$.H$_2$O, 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 \( \frac{1}{3} \) 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 accelerated by 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. typhi-murium through the gastro-intestinal mucosa of albino rats

Preliminary experiments established that S. typhi-murium 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 HC1 (5.0 ml.). The pH was adjusted to 7.4 with the HC1. 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 (1943), 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 in. 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 practice 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 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 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 trapezoidal 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 3/4 inch and perhaps only the middle 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:

<table>
<thead>
<tr>
<th>Peachey Colour Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interference colour</td>
</tr>
<tr>
<td>Grey</td>
</tr>
<tr>
<td>Silver</td>
</tr>
</tbody>
</table>
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 **heavy metal** 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 (3/4" x 3/4") 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/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) weighing 16 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/057. 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. *Na₂HPO₄* 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.
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 pH 6.8 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  \quad F = fimbriate
\quad FT = phage-transduced fimbriate
MF = mutant (M_{Cl_2}) fimbriate
\quad nfl = non-flagellate  \quad fl = flagellate
\quad flt = phage-transduced flagellate
SR = streptomycin-resistant
\quad 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.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>S. typhimurium strain</th>
<th>Source of strain</th>
<th>Rham. type</th>
<th>Phage type</th>
<th>Haem- agglutination</th>
<th>Organisms per ml. in suspension inoculated</th>
<th>Range of bacterial inoculum in log doses</th>
<th>Strain of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1289FT_man</td>
<td></td>
<td>2b/14a</td>
<td>++</td>
<td>3.4x10^9</td>
<td>1.5-3.5</td>
<td>4.5-8.5</td>
<td>LAC grey</td>
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<tr>
<td></td>
<td>1289N</td>
<td></td>
<td>2b/14a</td>
<td>+</td>
<td>3.8x10^9</td>
<td>1.6-3.6</td>
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<td>2a/13</td>
<td>++</td>
<td>1.5x10^9</td>
<td>1.2-3.2</td>
<td>4.2-8.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1294N</td>
<td></td>
<td>2a/13</td>
<td>+</td>
<td>5x10^9</td>
<td>0.7-2.7</td>
<td>3.7-8.7</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1287FT_man</td>
<td></td>
<td>2a/13</td>
<td>+</td>
<td>1.5x10^9</td>
<td>1.2-3.2</td>
<td>4.2-8.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1287N</td>
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<td>2a/13</td>
<td>+</td>
<td>4.5x10^9</td>
<td>0.7-2.7</td>
<td>3.7-8.7</td>
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<tr>
<td>IV</td>
<td>1289FT_SR_man</td>
<td></td>
<td>2b/24s</td>
<td>++</td>
<td>1.5x10^9</td>
<td>-</td>
<td>0.17-4.17</td>
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</tr>
<tr>
<td></td>
<td>1289N_SR</td>
<td></td>
<td>2b/24s</td>
<td>+</td>
<td>1.2x10^8</td>
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<td>4.8-9.8</td>
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<tr>
<td></td>
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<td></td>
<td>2a/13</td>
<td>+</td>
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<td>1.9-3.9</td>
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<td>4.6-9.6</td>
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Experimental salmonellosis I. Comparative virulence of fimbriate and non-fimbriate strains of S. typhimurium 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 paralleled the mortality figures but at a substantially higher level.
<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Typhi Duration of experiment (days)</th>
<th>No. of batches</th>
<th>No. of mice per batch</th>
<th>Results when oral infection was intra-peritoneal</th>
<th>Average time to death</th>
<th>Ratio organism infected orally : infected intraperitoneally</th>
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</thead>
<tbody>
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<td>...</td>
<td>...</td>
<td>...</td>
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</tr>
</tbody>
</table>

**Note:** All the test strains in this series are flagellate.

**TABLE II**
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.
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 Na₂HPO₄ solution pH 5.8 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.

\[
\begin{align*}
N & = \text{non-fimbriate} & F & = \text{fimbriate} \\
FT & = \text{phage-transduced fimbriate} \\
MF & = \text{mutant } (\text{M}_{n\text{Cl}_2}) \text{ fimbriate} \\
nfl & = \text{non-flagellate} & fl & = \text{flagellate} \\
flt & = \text{phage-transduced flagellate} \\
SR & = \text{streptomycin-resistant Mutant}
\end{align*}
\]
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.

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<tr>
<th>Expt. No.</th>
<th>S. typhimurium strain</th>
<th>Source of strain</th>
<th>Phage type</th>
<th>Fermentation</th>
<th>Phage old/new scheme</th>
<th>Haemagglutination</th>
<th>Organisms per ml. in suspensions inoculated</th>
<th>Range of bacterial inoculum in log doses</th>
<th>Strain of mice</th>
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<td></td>
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<td>1.5x10^9</td>
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<td>4.2-8.2</td>
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</tr>
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<tr>
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<td>2b/14a</td>
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<td>3.7x10^8</td>
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<td>Pos.</td>
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<td>++</td>
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<td>2a/13</td>
<td>+</td>
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<td>1.0-3.0</td>
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<td>1.8x10^9</td>
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</table>
RESULTS

Experimental salmonellosis I. Comparative virulence of fimbriate and non-fimbriate strains of *S. typhimurium* 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 paralleled the mortality figures but at a substantially higher level.
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 ID50 AND LD50 AND THE AVERAGE TIME TO DEATH
AT THE ORAL LD50.

<table>
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<th>Expt. No.</th>
<th>S. typhi murium strains</th>
<th>Duration of experiment (days)</th>
<th>No. of batches</th>
<th>No. of mice per batch</th>
<th>Results when infection was oral</th>
<th>Results when infection was intraperitoneal</th>
<th>Average time to death (days) at oral LD50</th>
<th>Ratio result FT/N organism inoculated orally</th>
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<td></td>
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<td></td>
<td></td>
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<td>Mortality (%)</td>
<td>Infectivity (%)</td>
<td>LD50 (log)</td>
<td>ID50 (log)</td>
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<td>25</td>
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<td>13.3</td>
<td>80</td>
<td>&gt; 4.5</td>
<td>0.85</td>
</tr>
<tr>
<td>VI</td>
<td>1566F</td>
<td>23</td>
<td>5x12</td>
<td>3x8</td>
<td>80</td>
<td>81.6</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>1566N</td>
<td></td>
<td>6x12</td>
<td>3x8</td>
<td>70</td>
<td>73.6</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>IX</td>
<td>629F</td>
<td>24</td>
<td>5x12</td>
<td>3x12</td>
<td>15.6</td>
<td>53.3</td>
<td>&gt; 8.2</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>629N</td>
<td></td>
<td>6x12</td>
<td>3x12</td>
<td>19.4</td>
<td>72.2</td>
<td>8.6</td>
<td>5.2</td>
</tr>
<tr>
<td>XI</td>
<td>1436F</td>
<td>22</td>
<td>5x12</td>
<td>3x12</td>
<td>31.6</td>
<td>78.6</td>
<td>7.3</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>1436N</td>
<td></td>
<td>6x12</td>
<td>3x12</td>
<td>33.3</td>
<td>73.6</td>
<td>7.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* 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 MnCl₂ as a mitagenic 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 percentages mortality and infectivity, log doses ID₅₀ and LD₅₀ and the average time to death at the oral LD₅₀.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th><em>S. typhimurium</em> strain</th>
<th>Flagellae</th>
<th>Fimbriae</th>
<th>Duration of experiment (days)</th>
<th>No. of batches x no. of mice per batch</th>
<th>Results when infection was oral</th>
<th>Results when infection was intraperitoneal</th>
<th>Average time to death (days) at oral LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>6351N/nil</td>
<td>- -</td>
<td></td>
<td>23</td>
<td>6x12 3x12</td>
<td>1.4 12.6 &gt;8.4 &gt;8.4 0 58.4 &gt;2.4 &lt;0.4</td>
<td>&gt;23</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td>6352FT/nil</td>
<td>- +</td>
<td></td>
<td>23</td>
<td>5x12</td>
<td>12.6 22.4 9.8 9.1 12.6 92.4 &gt;3.8 &lt;1.8</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6353N/ftl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.6 22.4 9.8 9.1 12.6 92.4 &gt;3.8 &lt;1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>6354FT/ftl</td>
<td>+ +</td>
<td></td>
<td>23</td>
<td>6x12</td>
<td>32.9 61.6 6.8 5.9 77.7 97.2 &lt;1.4 &lt;1.4</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>SW573N/nil</td>
<td>- -</td>
<td></td>
<td>22</td>
<td>6x12</td>
<td>11.1 47.2 &gt;9.0 6.6 36.1 82.6 2.5 &lt;1.0</td>
<td>&gt;22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SW576/ftl</td>
<td>+ -</td>
<td></td>
<td></td>
<td></td>
<td>10 56.6 &gt;8.0 5.7 63.9 91.7 &lt;1.0 &lt;1.0</td>
<td>&gt;22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SW576FT/ftl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.3 4.5 &gt;7.7 5.9 4.4 83.3 1.9 &lt;0.9</td>
<td>&gt;22</td>
<td></td>
</tr>
</tbody>
</table>

N = non-fimbriate
FT = phage-transduced fimbriate
nil = 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 lower 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)

<table>
<thead>
<tr>
<th>Strains (table III, expt. no. VII and VIII)</th>
<th>Oral dose differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50 (log)</td>
</tr>
<tr>
<td>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</td>
<td>3</td>
</tr>
<tr>
<td>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</td>
<td>1.6</td>
</tr>
<tr>
<td>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</td>
<td>1.4</td>
</tr>
<tr>
<td>Log doses for strain 6354 fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6352 fimbriate/non-flagellate by</td>
<td>1.6</td>
</tr>
<tr>
<td>Log doses for strain 6354 fimbriate/flagellate are less (i.e. it is more virulent) than those of strain 6353 non-fimbriate/flagellate by</td>
<td>1.4</td>
</tr>
<tr>
<td>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</td>
<td>0.2</td>
</tr>
<tr>
<td>S. typhimurium strains (table III, expt. no. X)</td>
<td>Oral dose differences</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Log doses for strain SW 576 fimbriate/flagellate are less than those of strain SW 573 non-fimbriate/non-flagellate by</td>
<td>The strains have a rather low degree of virulence for mice</td>
</tr>
<tr>
<td>Log doses for strain SW 576 fimbriate/flagellate are less than those of strain SW 576 non-fimbriate/flagellate by</td>
<td>The strains have a rather low degree of virulence for mice</td>
</tr>
<tr>
<td>Log doses for strain SW 576 non-fimbriate/flagellate are less than those of strain SW 573 non-fimbriate/non-flagellate by</td>
<td>The strains have a rather low degree of virulence for mice</td>
</tr>
</tbody>
</table>

* 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. TYPHIMURIIUM 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 NO. 1 TO 5 AND 8 AND 9 THE INOCULUM WAS AN 18 hr. GROWTH IN BROTH, AND IN EXPERIMENTS NO. 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 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proximal loop (nearest the stomach)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>LT2(F)</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>7471(N)</td>
<td>1/1</td>
</tr>
<tr>
<td>2</td>
<td>1298FT</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>1298N</td>
<td>0/1</td>
</tr>
<tr>
<td>3</td>
<td>1267FT</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>1287N</td>
<td>0/1</td>
</tr>
<tr>
<td>4</td>
<td>1294FT</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>1294N</td>
<td>1/1</td>
</tr>
<tr>
<td>5</td>
<td>1566F</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>1566N</td>
<td>0/1</td>
</tr>
<tr>
<td>6</td>
<td>629NF</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>629N</td>
<td>1/1</td>
</tr>
<tr>
<td>7</td>
<td>6351NF/nfl</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>6352NF/nfl</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>6353NF/flt</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>6354NF/flt</td>
<td>1/1</td>
</tr>
<tr>
<td>8</td>
<td>5735NF/nfl</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>5736NT/flt</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>5761NF/1</td>
<td>1/1</td>
</tr>
<tr>
<td>9</td>
<td>6361NF</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>1436NF</td>
<td>1/1</td>
</tr>
<tr>
<td>Total proportion of positive loops</td>
<td>19/22</td>
<td>8/17</td>
</tr>
<tr>
<td>Total proportion of positive loops in expts. with fimbriate/flagellate strains</td>
<td>(85.7%)</td>
<td>(47.0%)</td>
</tr>
<tr>
<td>Total proportion of positive loops in expts. with fimbriate/Non-flagellate strains</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Total proportion of positive loops in expts. with non-fimbriate/flagellate strains</td>
<td>8/10</td>
<td>2/7</td>
</tr>
<tr>
<td>Total proportion of positive loops in expts. with non-fimbriate/Non-flagellate strains</td>
<td>2/2</td>
<td>2/2</td>
</tr>
</tbody>
</table>

N & NF = non-fimbriate
fl, fnfl = flagellate, non-flagellate
F = fimbriate
FT = phage-transduced fimbriate
flt = phage-transduced flagellate

MF = fimbriate obtained by use of MCl₂ 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:

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

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 of the fimbriate and 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>
<thead>
<tr>
<th>Expt. No.</th>
<th>E. coli strains and their sero-types</th>
<th>Source of strains</th>
<th>Fimb.</th>
<th>Non-fimb.</th>
<th>Results - No. of positive loops</th>
<th>Total positive loops</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>C113 - 0119</td>
<td>recent infantile diarrhea</td>
<td>-</td>
<td>-</td>
<td>0/1, 0/1, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>28</td>
<td>C15 - 0127 - B5</td>
<td>old stock strain</td>
<td>-</td>
<td>+</td>
<td>0/2, 0/1, 0/1</td>
<td>0/2 0%</td>
</tr>
<tr>
<td>29</td>
<td>C20 - 0127: B5</td>
<td>recent infantile diarrhea</td>
<td>-</td>
<td>-</td>
<td>1/1, 0/2, 0/1</td>
<td>1/2 0%</td>
</tr>
<tr>
<td>30</td>
<td>C71 - 0127: B5</td>
<td>mild infantile diarrhea</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>31</td>
<td>C73 - 0127: B5</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>+</td>
<td>-</td>
<td>0/2, 0/1, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>32</td>
<td>C76 - 0127: B5</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/2 0%</td>
</tr>
<tr>
<td>33</td>
<td>C84 - 0127: B5</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>+</td>
<td>-</td>
<td>0/2, 0/1, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>34</td>
<td>C103 - 0127: B5</td>
<td>&quot; moderate &quot;</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>35</td>
<td>C128 - 0127: B5</td>
<td>&quot; mild &quot;</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>36</td>
<td>C75 - 0127: B5</td>
<td>&quot; mild &quot;</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/3 0%</td>
</tr>
<tr>
<td>37</td>
<td>C84 - 0127: B5</td>
<td>&quot; &quot; &quot; &quot;</td>
<td>+</td>
<td>-</td>
<td>0/1, 0/2, 0/1</td>
<td>0/3 0%</td>
</tr>
</tbody>
</table>

**Total proportion of positive loops**

<table>
<thead>
<tr>
<th></th>
<th>proximal loop</th>
<th>middle loop</th>
<th>distal loop</th>
<th>Total positive loops</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10/35)</td>
<td>(4/31)</td>
<td>(7/39)</td>
<td>(21/105)</td>
</tr>
<tr>
<td>Total proportion of positive loops in experiments with fimbriate strains</td>
<td>28.5% 12.9% 17.9% 20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total proportion of positive loops in experiments with non-fimbriate strains</td>
<td>33.3% 14.3% 21.4% 30.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total proportion of positive loops in experiments with non-fimbriate/non-flagellate strains</td>
<td>26.0% 12.5% 16% 18.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total proportion of positive loops in experiments with Fimbriate/flagellate strains</td>
<td>41.6% 20% 28% 30.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**


<table>
<thead>
<tr>
<th>Expt. No.</th>
<th><em>E. coli</em> strains and their sero-types</th>
<th>Source of strains</th>
<th>Results - No. of positive loops/No. Tested</th>
<th>Total positive loops</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>proximal loop (nearest stomach)</td>
<td>middle loop</td>
</tr>
<tr>
<td>1</td>
<td>C6-026: K60</td>
<td>old stock F41 from F. Czakov</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>C53-026: B6</td>
<td>Taylor's positive loop control</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>C68-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>C99-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>C109-026: K60</td>
<td>mild infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>C127-026: K60</td>
<td>very severe infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>C129-026: K60</td>
<td>diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>E126-026: B6</td>
<td>infantile diarrhoea</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>E431-026: B6</td>
<td>infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>C83-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>C95-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>C68-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>C96-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>C5-026</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>C6-025</td>
<td>recent infantile diarrhoea</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>C118-055: K59</td>
<td>recent severe illness</td>
<td>?</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>E317-055: B5</td>
<td>infantile diarrhoea</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>E444-055: B5</td>
<td>infantile diarrhoea</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>C54-0111: B4</td>
<td>old stock Taylor's negative loop control</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>C121-0111: B4</td>
<td>recent infantile diarrhoea</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>C147-0111: K58</td>
<td>severe illness</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>E121-0111: B4</td>
<td>infantile diarrhoea</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>E171-0111: B4</td>
<td>infantile diarrhoea</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>B40-0111: B4</td>
<td>old stock strain</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>C142-0111</td>
<td>recent infantile diarrhoea</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>C146-0111</td>
<td>recent infantile diarrhoea</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

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:

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

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 FIBRILLATION IN S. TYPHIMURIUM AND E. COLI STRAINS ON THE MINIMAL DOSE OF ORGANISMS REQUIRED TO PRODUCE A POSITIVE LOOP TEST in loops nearest the stomach (11) AND CAUCUS (6). THE INTERVAGINAL LOOPS RECEIVED UNDILUTED CULTURE (0.5 ml.). THE INTERVAGINAL LOOPS RECEIVED 0.5 ml.

DILUTIONS OF GRADED DILUTIONS AS INDICATED. THE BACTERIAL INOCULUM WAS AN 12 HR.

BROTH CULTURE OF THE ORIGIHAL TEST STRAIN OR RABBIT-PASSAGED STRAIN. THE TEST ANIMALS WERE KILLED 24 HR. AFTER THE OPERATIONS.

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Viable count of test culture (orgs. per ml)</th>
<th>Diluent used to prepare bacterial suspension</th>
<th>Reaction in loop (no. H inoculated, undil. 10^-7 10^-5 10^-3 10^-1 undil.)</th>
<th>Minimal log dose giving a positive loop test</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6351T/m1</td>
<td>4.5 x 10^8</td>
<td>Ringer soln.</td>
<td>+ - - - - -</td>
<td>8.7</td>
</tr>
<tr>
<td>6352F/m1</td>
<td>4.8 x 10^8</td>
<td>1% Na2HPO4 soln pH 8.8</td>
<td>+ - - - -</td>
<td>8.7</td>
</tr>
<tr>
<td>6353F/FLt</td>
<td>4.6 x 10^8</td>
<td>Peptone water</td>
<td>+ - - - +</td>
<td>7.8</td>
</tr>
<tr>
<td>6353F/F16</td>
<td>4.5 x 10^8</td>
<td>&quot;</td>
<td>+ - - + +</td>
<td>5.7</td>
</tr>
<tr>
<td>62B</td>
<td>2.2 x 10^9</td>
<td>1% Na2HPO4 soln.</td>
<td>+ - - - +</td>
<td>8.3</td>
</tr>
<tr>
<td>62B</td>
<td>2 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>5.1</td>
</tr>
<tr>
<td>575T/m1</td>
<td>4.5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>5.7</td>
</tr>
<tr>
<td>576T/F</td>
<td>5.5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>8.7</td>
</tr>
<tr>
<td>576F/FLt</td>
<td>5.7 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>7.3</td>
</tr>
<tr>
<td>576T/FLt</td>
<td>5.4 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.3</td>
</tr>
<tr>
<td>1136T/F</td>
<td>5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>8.4</td>
</tr>
<tr>
<td>1136F</td>
<td>5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>8.7</td>
</tr>
<tr>
<td>1136F/P</td>
<td>5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>4.1</td>
</tr>
<tr>
<td>1136F/P</td>
<td>5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>6.7</td>
</tr>
<tr>
<td>1136T/P</td>
<td>7.5 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>5.9</td>
</tr>
<tr>
<td>E. coli strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06/026</td>
<td>1.3 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.9</td>
</tr>
<tr>
<td>0127/026</td>
<td>1.2 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.6</td>
</tr>
<tr>
<td>0127/026</td>
<td>1.2 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>6.1</td>
</tr>
<tr>
<td>024/035</td>
<td>2.8 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.5</td>
</tr>
<tr>
<td>027/011</td>
<td>3 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.3</td>
</tr>
<tr>
<td>012/012</td>
<td>7.3 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.9</td>
</tr>
<tr>
<td>012/026/P</td>
<td>7.4 x 10^9</td>
<td>&quot;</td>
<td>+ - - - +</td>
<td>9.9</td>
</tr>
</tbody>
</table>

N & NF = non-fimbriate  PT = phage-transduced fimbriate  m1 = non-flagellate  flt = phage-transduced flagellate  MF = fimbriate by use of NaCl2 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 Salmonellaeia II.**

Comparative studies of the effect of filamentate and non-filamentate strains of *S. typhimurium* and *E. coli* injected into ligated rabbit gut. Viable bacterial counts of exudate from positive loops were done by plate and retraction method. The initial inoculum of 0.5 ml. of the test strain contained approximately $10^9$ organisms. The test animals were killed 24 hrs. after the operation.

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Flagella</th>
<th>Fimbriae</th>
<th>Position of the test loop</th>
<th>Volume of exudate recovered from loop (ml.)</th>
<th>Viable count (orgs. per ml.) of exudate</th>
<th>Total organisms in exudate recovered from positive loops</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>156NF</td>
<td>+</td>
<td>+</td>
<td>Proximal</td>
<td>15</td>
<td>$7.4 \times 10^9$</td>
<td>$1.1 \times 10^{11}$</td>
</tr>
<tr>
<td>156N</td>
<td>+</td>
<td>-</td>
<td>Middle</td>
<td>20</td>
<td>$4.5 \times 10^9$</td>
<td>$9 \times 10^9$</td>
</tr>
<tr>
<td>629NF</td>
<td>+</td>
<td>+</td>
<td>Proximal</td>
<td>15</td>
<td>$5.4 \times 10^9$</td>
<td>$8.1 \times 10^9$</td>
</tr>
<tr>
<td>629N</td>
<td>+</td>
<td>-</td>
<td>Middle</td>
<td>10</td>
<td>$7.4 \times 10^8$</td>
<td>$7.4 \times 10^9$</td>
</tr>
<tr>
<td>635IV/nf1</td>
<td>-</td>
<td>-</td>
<td>Proximal</td>
<td>10</td>
<td>$1.8 \times 10^8$</td>
<td>$1.6 \times 10^9$</td>
</tr>
<tr>
<td>635IV/nf1</td>
<td>-</td>
<td>+</td>
<td>Middle</td>
<td>10</td>
<td>$4.3 \times 10^9$</td>
<td>$4.3 \times 10^9$</td>
</tr>
<tr>
<td>635IV/flt</td>
<td>+</td>
<td>-</td>
<td>Proximal</td>
<td>11</td>
<td>$7.1 \times 10^8$</td>
<td>$7.3 \times 10^9$</td>
</tr>
<tr>
<td>&quot;</td>
<td>+</td>
<td>-</td>
<td>Distal</td>
<td>10</td>
<td>$6.7 \times 10^9$</td>
<td>$6.7 \times 10^9$</td>
</tr>
<tr>
<td>645IV/flt</td>
<td>+</td>
<td>+</td>
<td>Proximal</td>
<td>12</td>
<td>$7.8 \times 10^9$</td>
<td>$9.3 \times 10^9$</td>
</tr>
<tr>
<td>&quot;</td>
<td>+</td>
<td>+</td>
<td>Distal</td>
<td>10</td>
<td>$7.2 \times 10^8$</td>
<td>$7.2 \times 10^9$</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20/0127</td>
<td>-</td>
<td>+</td>
<td>Proximal</td>
<td>10</td>
<td>$3.7 \times 10^9$</td>
<td>$3.7 \times 10^{10}$</td>
</tr>
<tr>
<td>C53/026</td>
<td>-</td>
<td>-</td>
<td>Proximal</td>
<td>20</td>
<td>$5 \times 10^9$</td>
<td>$1 \times 10^{11}$</td>
</tr>
<tr>
<td>C83/026</td>
<td>-</td>
<td>-</td>
<td>Proximal</td>
<td>15</td>
<td>$5.3 \times 10^9$</td>
<td>$8.7 \times 10^{10}$</td>
</tr>
<tr>
<td>C95/026</td>
<td>-</td>
<td>-</td>
<td>Proximal</td>
<td>18</td>
<td>$6.7 \times 10^9$</td>
<td>$1.2 \times 10^{11}$</td>
</tr>
</tbody>
</table>

N = non-fimbriate  F = fimbriate  nf1 = non-flagellate  
FT = phage-transduced fimbriate  fl = flagellate  flt = phage-transduced flagellate
flagellate strains. The log dose differences were as follows:

Strains not passed in rabbit gut

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

Strains passed once in rabbit gut

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

Strains passed 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 *Salmonella typhimurium* 6354/F1/F1t were prepared in 1 per cent. Na₂HPO₄ 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 *Salmonella typhimurium* 6354FT/1t were obtained with *Salmonella typhimurium* 629MF/1t.
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$_2$HPO$_4$ 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.
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.

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

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
fl = 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*
The results of culture of faecal specimens collected on Day 0 after the administration of S. typhimurium KLAGB-traduced cultures are shown in Table IX. These results were compared with the same cultures transduced with non-traduced E. coli K-12 strain (S. typhimurium) and with the same cultures transduced with non-traduced K-12 strain (S. typhimurium). The results are presented in the table below.
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

<table>
<thead>
<tr>
<th>Region of small intestine from which the contents were sampled</th>
<th>No. of samples yielding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No growth on culture</td>
</tr>
<tr>
<td></td>
<td>Positive culture</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5</td>
</tr>
<tr>
<td>Proximal ileum (jejunum)</td>
<td>2</td>
</tr>
<tr>
<td>Mid-ileum</td>
<td>1</td>
</tr>
<tr>
<td>Terminal-ileum</td>
<td>1</td>
</tr>
</tbody>
</table>

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
### EXPERIMENTS TO DETECTION OF SITES OF Penetration OF MUCOSA OF GASTRO-INTESTINAL TRACT OF ALBINO RATS BY FIMBRIATE AND NON-FIMBRIATE S. TYPHII MURII STRAINS.

The bacterial inoculum injected into the test gastro-intestinal segment (Fig. 1) contained approximately $10^5$ organisms of the test strain.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>S. typhi-murii strain</th>
<th>Result of culture</th>
<th>Histological changes in intestinal tissue at end of experiment</th>
<th>Evidence of penetration of mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
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<td>Negative</td>
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<td>Negative</td>
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<td>8</td>
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<td>Positive</td>
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<td>9</td>
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<td>Negative</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
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<td>Positive</td>
<td>Positive</td>
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<td>15</td>
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<tr>
<td>16</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Notes:**
- **Test small part of stomach:** 15
- **Test duodenum:** 12
- **Test jejunum:** 10
- **Test ileum:** 8
- **Test colon:** 6
- **Test small intestine:** 4
- **Test colon:** 3
- **Test jejunum:** 2
- **Test ileum:** 1
- **Test stomach:** 0

**Result of culture:**
- **Positive** indicates organisms were detected after 20 minutes or more. A positive test is a significant finding.
- **Negative** indicates no organisms were detected after 20 minutes.

**Histological changes:**
- Positive indicates histological changes were observed.
- Negative indicates no histological changes were observed.

**Evidence of penetration of mucosa:**
- Positive indicates penetration of mucosa was detected.
- Negative indicates no penetration of mucosa was detected.

**Experimental setup:**
- **Gastro-intestinal segment inoculation:** 12
- **Inoculation to determine sites of penetration of mucosa of gastro-intestinal tract:** 12
- **Segment inoculation:** 10
- **Segment inoculation:** 8
- **Segment inoculation:** 6
- **Segment inoculation:** 4
- **Segment inoculation:** 3
- **Segment inoculation:** 2
- **Segment inoculation:** 1
- **Segment inoculation:** 0
<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>S. typhimurium strain</th>
<th>Result of culture of Histological changes in intestinal tissue at end of experiment</th>
<th>Evidence of penetration of mucosa Gastrito-intestinal segment content</th>
<th>Heart blood sampled at 60, 90, and 105 mins.</th>
<th>Liver</th>
<th>Spleen</th>
<th>Saline Washing of peritoneal cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>NG</td>
<td>+</td>
<td>+</td>
<td>5 min. 15 min. 30 min.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The weight of rats employed in this study ranged from 190 to 370 g.

*NC = No growth  
NAD = Nothing abnormal seen  
T = Recovered S. typhimurium strains were not recovered  
+ = Parent non-fimbriate strains were recovered  
- = Recovered S. typhimurium strains were recovered

The weight of rats employed in this study ranged from 190 to 370 g.
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 \textit{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) **Midileum.** 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 sites or sites of penetration of the gastro-intestinal epithelium by these organisms.

<table>
<thead>
<tr>
<th>Expt. Serial No.</th>
<th>Nature of Strain 1289</th>
<th>Period of Experimentation</th>
<th>Contents of</th>
<th>Heart Blood at (min.)</th>
<th>Liver</th>
<th>Spleen</th>
<th>Mesenteric lymph glands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oesophagus</td>
<td>Stomach</td>
<td>Duodenum</td>
<td>Jejunum</td>
<td>Ileum</td>
</tr>
<tr>
<td>1</td>
<td>FT)</td>
<td>15 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>2</td>
<td>FT)</td>
<td>15 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>3</td>
<td>N)</td>
<td>15 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>4</td>
<td>FT)</td>
<td>30 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>5</td>
<td>FT)</td>
<td>30 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>6</td>
<td>N)</td>
<td>40 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>7</td>
<td>N)</td>
<td>40 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>8</td>
<td>FT)</td>
<td>60 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>9</td>
<td>N)</td>
<td>60 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>10</td>
<td>FT)</td>
<td>75 min.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

**Total positive cultures of heart blood, liver, spleen & mesenteric lymph glands**

|                  | 1/0 | 3/0 | 3/3 | 1/1 | 9/10 | 7/10 | 6/7 |

**Percentage positive cultures of heart blood, liver, spleen and mesenteric lymph glands**

|                  | 10  | 30  | 42  | 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.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Temp o C.</th>
<th>Period of Exposure in Hours</th>
<th>Haemagglut. of 3 per cent guinea-pig red cells with heated bacterial suspension</th>
<th>Haemagglut. of 3 per cent guinea-pig red cells with formalised bacterial suspension</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. flexneri</td>
<td>56</td>
<td>$\frac{1}{2}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Fla 1.</td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>56</td>
<td>$\frac{1}{2}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>1269 PT.</td>
<td>56</td>
<td>$\frac{3}{4}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>1</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>$\frac{1}{2}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>S. flexneri</td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>Neg</td>
<td>Neg</td>
<td>Non-fimbriate control strains</td>
</tr>
<tr>
<td>Fla 2.</td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>1269 N</td>
<td>60</td>
<td>$\frac{3}{2}$</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
</tbody>
</table>

**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 human 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.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Test strains</th>
<th>Haem-agglutination</th>
<th>Mean percentage dead leucocytes up to about 2 hours of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S. typhimurium LT2 7471(N)</td>
<td>+++</td>
<td>Leucocyte control with fimbriate strain with non-fimbriate strain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>S. typhimurium 1287FT 1287(N)</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>38</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>S. flexneri Fla 1</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Heated S. flexneri Fla 1</td>
<td>+++</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>S. flexneri Fla 2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heated S. flexneri Fla 2</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td><em>Heated</em> S. flexneri Fla 1</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>S. typhimurium 1289FT</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td><em>Heated</em> S. typhimurium 1289FT</td>
<td>+++</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>S. typhimurium 1289N</td>
<td>-</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td><em>Heated</em> S. typhimurium 1289N</td>
<td>-</td>
<td>55</td>
</tr>
</tbody>
</table>

*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* LT₂, 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 *Salmonella typhimurium* 1287FT was apparently more lethal to the leucocytes than was the non-fimbriate parent *Salmonella typhimurium* 1287N 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.*
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:

\[ fl = \text{flagellum}; \quad f = \text{fimbia} \]

*Magnification 20,000.*
UNSHADOWED AND SHADOWED ELECTRON MICROGRAPHS (figs. 17 and 18)
SHOWING FIMBRIATE SALMONELLA TYPHIMURIUM ADHERING TO HUMAN LEUCOCYTES, R.B.C. AND PLATELETS.

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 which discrete granules (r) 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 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 ultrastructures 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. TYPHIMURIIUM.

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. 2B  **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.
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.
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/nf1, 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.

<table>
<thead>
<tr>
<th>S. typhimurium</th>
<th>Active immunisation</th>
<th>Deaths prior to challenge</th>
<th>Intraperitoneal challenge</th>
<th></th>
<th></th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6351 NF/nf1 vaccine preparation</td>
<td>Log dose</td>
<td>No. of mice</td>
<td>Log dose</td>
<td>No. of mice</td>
<td>Mortality</td>
<td>Survivors</td>
</tr>
<tr>
<td>Heat-killed phenol-preserved</td>
<td>3</td>
<td>36</td>
<td>0</td>
<td>3.1</td>
<td>18</td>
<td>11/18</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>36</td>
<td>0</td>
<td>3.1</td>
<td>18</td>
<td>10/18</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>36</td>
<td>2</td>
<td>3.1</td>
<td>17</td>
<td>5/17</td>
</tr>
<tr>
<td>Acetone-killed freeze-dried</td>
<td>3</td>
<td>36</td>
<td>1</td>
<td>3.1</td>
<td>17</td>
<td>9/17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>18</td>
<td>14/18</td>
<td>4</td>
<td>77.8</td>
<td>100</td>
</tr>
<tr>
<td>Control group of 36 unvaccinated mice</td>
<td>0</td>
<td></td>
<td></td>
<td>3.1</td>
<td>18</td>
<td>10/18</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>18</td>
<td>12/18</td>
<td>6</td>
<td>66.6</td>
<td>100</td>
</tr>
</tbody>
</table>

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 $0.8 \times 10^2$ and $0.8 \times 10^3$ 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  
nf1 = 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 \textit{S. typhimurium} strain 6351 \textit{N/nfl} and its phage-mediated transduced fimbriate/flagellate derivative, strain 6354 \textit{FT/flt}.

Various immunising schedules were initially employed and in all of these early experiments the mice were subsequently challenged by injections of \textit{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 \textit{S. typhimurium} strain 1566 \textit{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 \textit{S. typhimurium} strains, 6351 \textit{N/nfl} and 6354 \textit{FT/flt}, and stocked in bulk (see Methods). Throughout this series of experiments,
Experimental salmonellosis VII. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OF KILLED VACCINES PREPARED FROM A RELATIVELY VIRULENT S. TYPHIMURIUM 645-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.

<table>
<thead>
<tr>
<th>S. typhimurium 6351</th>
<th>Immunisation</th>
<th>Deaths prior to challenge</th>
<th>Intraperitoneal challenge</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>24</td>
<td>0</td>
<td>3.2 12 10/12 2 2 12/12 83.3 100</td>
<td>No protection</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>2</td>
<td>3.2 11 7/12 5 5 12/12 58.3 100</td>
<td>Slight protection</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>0</td>
<td>3.2 12 4/12 8 7 11/12 33.3 91.6</td>
<td>Fair protection. One survivor was free of infection</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>0</td>
<td>3.2 12 9/12 3 3 12/12 75 100</td>
<td>No protection</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>2</td>
<td>3.2 11 11/11 0 0 11/11 100 100</td>
<td>No protection</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>0</td>
<td>3.2 12 6/12 6 6 12/12 50 100</td>
<td>Fair protection</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>2</td>
<td>3.2 11 11 10/11 1 1 11/11 90.9 100</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>4</td>
<td>3.2 10 5/10 5 5 10/10 50 100</td>
<td>Fair protection</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>0</td>
<td>3.2 11 8/11 3 3 11/11 72.7 100</td>
<td>Control</td>
</tr>
<tr>
<td>Control group of 24</td>
<td></td>
<td></td>
<td></td>
<td>&quot;&quot;</td>
</tr>
</tbody>
</table>

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 (1948). 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/ftt 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>
<thead>
<tr>
<th>Vaccine</th>
<th>Dose</th>
<th>Protection</th>
<th>Deaths</th>
<th>Infections</th>
<th>Mortality</th>
<th>Tumors</th>
<th>Non-Infectious</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group of 20 Shotgun</td>
<td>60</td>
<td>0</td>
<td>10/10</td>
<td>10/10</td>
<td>5/5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Heat-Killed Phenol Preserved +Freund's Incomplete Adjuvant</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acetone-Killed and Free-Dried +Freund's Incomplete Adjuvant</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Heat-Killed Phenol Preserved (ip)</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Complete Group of 20 Shotgun</td>
<td>60</td>
<td>0</td>
<td>10/10</td>
<td>10/10</td>
<td>5/5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Heat-Killed Phenol Preserved +Freund's Incomplete Adjuvant</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Acetone-Killed and Free-Dried +Freund's Incomplete Adjuvant</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Heat-Killed Phenol Preserved (ip)</td>
<td>40</td>
<td>2</td>
<td>8/10</td>
<td>8/10</td>
<td>4/4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Remarks:**
- The experiments were performed under sterile conditions.
- Mortality was determined within 10 days after challenge.
- The vaccines used were saline suspensions of live organisms.

**Experimental Conditions:**
- Group of 20 Gunn rats were used for each experiment.
- The vaccines were stored at -20°C prior to use.
- The experiments were performed at the Colorado State University.

**Conclusion:**
The vaccines were highly effective in protecting the rats from salmonellosis. Further studies are needed to determine the long-term efficacy and safety of these vaccines.
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.
Experimental salmonellosis VII


<table>
<thead>
<tr>
<th>S. typhi-murium 6354 FT/fit vaccine preparation</th>
<th>No. of mice immunised</th>
<th>Deaths prior to challenge</th>
<th>Intra-peritoneal challenge</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-killed phenol-preserved</td>
<td>40</td>
<td>0</td>
<td>3.0, 20</td>
<td>Good protection</td>
</tr>
<tr>
<td>Acetone-killed freeze-dried</td>
<td>40</td>
<td>0</td>
<td>3.0, 20</td>
<td>Good protection, one survivor was free of infection</td>
</tr>
<tr>
<td>Control: group of 20 unvaccinated mice</td>
<td>0</td>
<td></td>
<td>3.0, 10</td>
<td>Control</td>
</tr>
</tbody>
</table>

The intraperitoneal challenges were approximately equivalent to $0.6 \times 10^2$ and $0.6 \times 10^4$ 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 16th day after the administration of the second immunising dose. The oral challenge doses of the
### Table XIX

**Experimental salmonellosis VII.**

**Comparison of protective potencies for Swiss white mice of killed vaccines prepared from S. Typhimurium strains 6351 n/fl and 635k ft/fl.**

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. Survivors were killed on the 23rd day following challenge.

<table>
<thead>
<tr>
<th>Vaccine Preparation</th>
<th>No. of mice immunised</th>
<th>Deaths prior to challenge</th>
<th>Oral challenge</th>
<th>Deaths after 1st dose</th>
<th>Deaths after 2nd dose</th>
<th>Mortality Percentage</th>
<th>Infectivity Percentage</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-killed phenol-preserved (IIP) strain 6351 n/fl</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Good protection: one survivor free of injection</td>
</tr>
<tr>
<td>Freeze-dried (AK) strain 6353k n/fl</td>
<td>24</td>
<td>2</td>
<td>2/12</td>
<td>10</td>
<td>9</td>
<td>91.6</td>
<td>91.6</td>
<td></td>
</tr>
<tr>
<td>Freeze-dried (AK) strain 6351k n/fl</td>
<td>24</td>
<td>2</td>
<td>2/12</td>
<td>10</td>
<td>9</td>
<td>91.6</td>
<td>91.6</td>
<td></td>
</tr>
<tr>
<td>Heat-killed phenol-preserved (HP) strain 6351k n/fl</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Good protection: one survivor free of injection</td>
</tr>
<tr>
<td>Freeze-dried (AK) strain 6353k n/fl</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Good protection: one survivor free of injection</td>
</tr>
<tr>
<td>Freeze-dried (AK) strain 6351k n/fl</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Good protection: one survivor free of infection</td>
</tr>
</tbody>
</table>

**Note:** The Log dose of vaccine was adjusted after turbidimetric estimation using Brown's standard opacity tubes.

**Legend:**
- F: fimbriate
- n): non-fimbriate
- ft: phage-transduced fimbriate
- fl: flagellate
- nfl: non-flagellate

---

*Experimental and control groups composed of an equal number of male and female mice and each group contained in one group of Swiss white mice. The mice weighed 16-22 grams. The groups were balanced by weight.

**Vaccine preparation No.:**
- 1 = Heat-killed phenol-preserved
- 2 = Freeze-dried
- 3 = Freeze-dried (AK)

**Control group of non-immune mice:**
- 24

---

*Superscripts for vaccine titres 1566 P/L.**
- 1: Typhimurium strain 6351 n/fl
- 2: Typhimurium strain 6353k n/fl
- 3: Typhimurium strain 6351l n/fl
- 4: Typhimurium strain 635k ft/fl

**Footnote:** Comparisons were made with the second day post-challenge.
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>
<thead>
<tr>
<th>Vaccine preparation</th>
<th>Active immunisation</th>
<th>Deaths prior to challenge</th>
<th>Intraperitoneal challenge</th>
<th>Mortality</th>
<th>Survivors</th>
<th>Percentage mortality</th>
<th>Percentage infectivity</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated vaccine 6351 N/m1/SR</td>
<td>Oral</td>
<td>1.9</td>
<td>24*</td>
<td>0</td>
<td>3.2</td>
<td>12</td>
<td>0/12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>2.9</td>
<td>24*</td>
<td>0</td>
<td>3.2</td>
<td>12</td>
<td>0/12</td>
<td>12</td>
</tr>
<tr>
<td>Control: a group of 24 unvaccinated mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.2</td>
<td>11</td>
<td>9/11</td>
<td>2</td>
</tr>
</tbody>
</table>

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 $10^3$ 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>
<thead>
<tr>
<th>Vaccine preparation</th>
<th>Active immunisation</th>
<th>Deaths prior to challenge</th>
<th>Intrapitoneal challenge</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated vaccine 6351N/nfl/SR</td>
<td>Subcutaneous</td>
<td>Log dose</td>
<td>No. of mice</td>
<td>Log dose</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>24</td>
<td>0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>24</td>
<td>2</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>24</td>
<td>3</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>24</td>
<td>2</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>24</td>
<td>2</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>24</td>
<td>3</td>
<td>5.2</td>
</tr>
<tr>
<td>Oral</td>
<td>3.2</td>
<td>10</td>
<td>3/10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>24</td>
<td>3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

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/f1 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 91.5 per cent. of the control
TABLE XXI

Experimental salmonellosis VII0 COMPARISON OF THE PROTECTIVE POTENCY FOR SWISS
MUTE MICE OF ALIVE ATTENUATED VACCINES (SINGLE STEP STREPTOMYCIN-RESISTANT
MUTANTS) PREPARED FROM S. TEFHIMURIUM STRAINS 6351 NF/nfl AND 6354 FT/f It
BOTH OF PHAGE TYPE 2a/l3.
THE MX E 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 OP
S. TYH-HMURIUM 1566F OF PHAGE TYPE la/U57.
SURVIVORS WERE KILLED ON THE
28th DAY OF CHALLENGE.
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fit/
SR

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Control: group of 24
unvaccinated mice

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9/9

1^/12 91.6
l2/12

100

100

100

VP

0

l6.6

0/5

0

o/6

0

0
0

3.1

6

1-0

6

0

0

o/6

6 0

o/6

0

0

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

0

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0

FT/flt/SR
immunised
control:
not ohall

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•H

"
i!

No

Control

Control

Control

Log doses of the vaccines and challenge strains were estimated "by the Mies
& Misra method.

The

intraperitgneal challenge doses were approximately

equivalent to 1.2x10 and 1.2x10 intraperitoneal ID50 doses for mice.
Eaoh group of test animals contained an equal number of male and female micej
the mice weighed 16 to 20 grams each and the groups were "balanced "by weight.
*

NF

Strain
at

6354FT/fIt/SR

=

nfl

found to "be

streptomycin dependent for growth.

non-flagellate
SR = streptonycin-resistant
phage-transduced fimbriate
fit = phage-transduoed flagellate

non-fimbriate

FT

was
=


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
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/nfl and 6354FT/flt. THE MICE WERE IMMUNISED WITH SINGLE SUBCUTANEOUS INJECTIONS. THEY WERE CHALLENGED ORALLY ON THE 15th DAY OF IMMUNISATION WITH S. TYPHIMURIUM STRAIN 1566F/flt. THE SURVIVORS WERE KILLED ON THE 23rd DAY FOLLOWING THE CHALLENGE.

<table>
<thead>
<tr>
<th>Attenuated S. typhimurium vaccine preparation</th>
<th>Immunisation</th>
<th>Deaths prior to challenge</th>
<th>Oral challenge</th>
<th>Mortality</th>
<th>Survivors infected</th>
<th>Percentage</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6351N/nfl/SR</td>
<td>24</td>
<td>1</td>
<td>6.1</td>
<td>11</td>
<td>2/11</td>
<td>6/11</td>
<td>18.2%</td>
</tr>
<tr>
<td>6354FT/flt/SR</td>
<td>24</td>
<td>0</td>
<td>8.1</td>
<td>12</td>
<td>2/12</td>
<td>5/12</td>
<td>16.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunised Strain 6351N/nfl/SR</th>
<th>12</th>
<th>0</th>
<th>0/12</th>
<th>12</th>
<th>3/12</th>
<th>0</th>
<th>24.9%</th>
<th>Immunised control: not challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td>not challenged Strain 6354FT/flt/SR</td>
<td>12</td>
<td>0</td>
<td>0/12</td>
<td>12</td>
<td>1/12</td>
<td>0</td>
<td>8.3%</td>
<td>Non-immune control challenged</td>
</tr>
</tbody>
</table>

Control: group of 24 unvaccinated mice | 1 | 6.1 | 12 | 5/12 | 7 | 12/12 | 41.7% | 100% |

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  nfl = non-flagellate  flt = phage-transduced flagellate  fl = 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 XXII**

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/flit. 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.

<table>
<thead>
<tr>
<th>S. typhimurium vaccine preparation</th>
<th>Tube agglutination test: reciprocals of O antibody titres on day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Killed vaccines</td>
<td></td>
</tr>
<tr>
<td>HP vaccine strain 6351NF/nfl</td>
<td>&lt;10</td>
</tr>
<tr>
<td>AK vaccine strain 6351NF/nfl</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Freund's incomplete adjuvant +</td>
<td>&lt;10</td>
</tr>
<tr>
<td>HP vaccine strain 6351NF/nfl</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Freund's incomplete adjuvant +</td>
<td>&lt;10</td>
</tr>
<tr>
<td>AK vaccine strain 6351NF/nfl</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>HP vaccine strain 6354FT/flit</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>AK vaccine strain 6354FT/flit</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Attenuated live vaccines</td>
<td></td>
</tr>
<tr>
<td>Strain 6351NF/nfl/SR</td>
<td>&gt;80</td>
</tr>
<tr>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain 6354FT/flit/SR</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

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  = = 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/ft) 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 time 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
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; MoNaught 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), MoNaught 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-anesthetised rats together with an inert unabsorbable radio-active marker. An isotope of chromium $^{51}$Cr 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 Jones, 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. Back 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 gastrointestinal 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 gastrointestinal tract of the rat. The observations suggest that penetration can take place from any part of the gastrointestinal 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 bloodstream, 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 gastrointestinal 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. typhi-
murium* 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 Rytter, 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 \( \Sigma - N \)-methyl-lysine (Ambler and Rees, 1959; Stocker, McDonough and Ambler, 1961).

Fimbriae, which have diameters of about 50A, 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* 6354 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*. Olitgki 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 Ausins, 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.
SUMMARY

This investigation of pathogenesis and control of experimental salmonella infections includes a comprehensive review of the literature and involved some 3,240 animal experiments relating to virulence and immunity studies of \textit{S. typhimurium} infections in mice and rats.

Results of electron microscopic studies, and \textit{in vitro} experiments with human leucocytes exposed to the effect of fimbriae of \textit{S. typhimurium} and \textit{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 \textit{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 \textit{S. typhimurium} strains and their fimbriate/flagellate derivatives obtained by phage transduction or by the use of a mutagenic agent (\textit{MnCl}_2). However, the parent non-fimbriate/non-flagellate \textit{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 

*Salmonella 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. typhi-

murium* producing a positive loop test ranged from 4 to 9. 

It was observed that the minimal log dose producing a posi-

tive loop was less (by log 1 to 3) for the fimbriate/flag-

ellate *S. typhimurium*, than the parent non-fimbriate/flag-

ellate 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 gastrointestinal 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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APPENDICES
### Table: Comparison of virulence of Phage-Transduced Fimbriate and Parent non-Fimbriate S. Typhimurium 1289 for L. Grey mice by Oral and Intraperitoneal Routes of Infection

<table>
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<th>Log dose</th>
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<th>Number of deaths</th>
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**Mean T.2 of death: 6.97**

**Results obtained with S. Typhimurium strain 1289 PhI.**

**Experimental summary:**

*Appendix 1*
### EXPERIMENT I

**COMPARISON OF VIRULENCE OF PELAGE-TRANSDUCED FIMBRIATE AND PARENT NON-FIMBRIATE S.
TYPHIMURIUM 1289 FOR LAX GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.**

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<td>1/2</td>
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<td>1/2</td>
<td>1/2</td>
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<td>12</td>
<td>12</td>
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<td>1/2</td>
<td>1/2</td>
<td>1</td>
<td>12</td>
<td>12</td>
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<td>100</td>
</tr>
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<td>1/2</td>
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<td>1</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
</tbody>
</table>

**Notes:**
- Log dose refers to the logarithm of the number of organisms used for infection.
- Deaths and survivors indicate the number of mice that died or survived at each dose.
- Infectivity rates are calculated as the ratio of infected to non-infected mice.
- Mortality is calculated as the percentage of deaths among infected mice.

**Results:**
- The results indicate a lower infectious rate for oral versus intraperitoneal infection routes.
- Higher mortality is observed in the oral infection group compared to the intraperitoneal group.

**Conclusions:**
- Oral infection is less virulent than intraperitoneal infection.
- Fimbriate strains appear to be more virulent than non-fimbriate strains.

**References:**
- Additional references are provided in the Experimental Salmonella Tests and Appendix 2.
**Experimental Salmonellosis**

**I. Appendix**

### EXPERIMENT II

**COMPARISON OF VIRULENCE OF HiACE-TRANSDUCED FIMBRIATE S, TYTHIMUKEUM 1294 and NON-FIMBRIATE PARENT STRAIN FOR LAC GREY MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Route of infection</th>
<th>NO. of organisms injected</th>
<th>No. of survivors</th>
<th>Mortality</th>
<th>No. of days to death</th>
<th>Mean times to death at oral ED50</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>Oral</td>
<td>12</td>
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<td>1</td>
<td>17</td>
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<td>Oral</td>
<td>12</td>
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<td>1</td>
<td>17</td>
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<td>12</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>7.5</td>
</tr>
<tr>
<td>5.2</td>
<td>Oral</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>7.5</td>
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<td>Oral</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>17</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Results obtained with **S. Typhimurium strain 1294**.*

*Experiments II are performed on non-fimbriate parent strain of S. Typhimurium 1294 and non-fimbriate parent strain of S. Typhimurium 1294.*
## EXPERIMENT II.

### COMPARISON OF VIRULENCE OF AGE-TRANSDUCED FIMHRIATE AND NON-FIMBRIATE PARENT STRAIN (S. TYPHIMURIUM 1294-BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION OF LAC GREY MICE, (b)

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Initial</th>
<th>Oral</th>
<th>Intraperitoneal</th>
<th>Time to death at oral ID50</th>
<th>Mean 12.7 days</th>
<th>Log dose</th>
<th>Initial</th>
<th>Oral</th>
<th>Intraperitoneal</th>
<th>Time to death at oral ID50</th>
<th>Mean 12.7 days</th>
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<tr>
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<td>12</td>
<td>6/12</td>
<td>7.7</td>
<td>12</td>
<td>7.7</td>
<td>12/12</td>
<td>12</td>
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<td>6/12</td>
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<td>12</td>
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<td>6/12</td>
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<td>6/12</td>
<td>5.4</td>
<td>12</td>
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<td>12</td>
<td>6/12</td>
<td>4.7</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table Notes:**
- *Results obtained with S. Typhimurium strain 1294.*
- **Comparison of virulence of age-transduced Fimhriate and non-Fimhriate parent strain of S. Typhimurium 1294.

**Experimental Procedures**

Appendix I

---

"The tables above summarize the results of experiments comparing the virulence of age-transduced Fimhriate and non-Fimhrate parent strains of S. Typhimurium 1294, administered orally and intraperitoneally to LAC grey mice. The data includes the number of mice infected at various log doses, the number of deaths, and the time to death at oral ID50. The mean 12.7 days column indicates the average time for death at oral ID50. The results show a significant difference in virulence between the two strains, with the Fimhriate strain exhibiting higher virulence."

---

*Experimental Procedures* Appendix I
### Experimental Salmonellosis

#### Appendix 5

### Experiment III

#### Comparison of Virulence of Phage-Transduced Parental Strains and Non-Phage-Transduced Parental Strains

**Time to Death at Oral ID50**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>No. of infected</th>
<th>No. of survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
<th>Route of infection</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>1</td>
<td>11</td>
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<td>1</td>
<td>Oral</td>
<td>7.2</td>
</tr>
<tr>
<td>6.2</td>
<td>12</td>
<td>12</td>
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<td>1</td>
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<td>6.2</td>
</tr>
<tr>
<td>5.4</td>
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<td>12</td>
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<td>1</td>
<td>Oral</td>
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<tr>
<td>4.4</td>
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<td>1</td>
<td>Oral</td>
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</tr>
<tr>
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<td>12</td>
<td>1</td>
<td>1</td>
<td>Oral</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Results obtained with Phage-Transduced Parental Strain 1287 Ph/T1**

- Study strain in IVC group mice by oral and intraperitoneal routes of infection.
- Parent strain in IVC group mice by oral and intraperitoneal routes of infection.

---

### Table

<table>
<thead>
<tr>
<th>Log dose</th>
<th>No. of infected</th>
<th>No. of survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
<th>Route of infection</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
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<td>1</td>
<td>Oral</td>
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<td>1</td>
<td>Oral</td>
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</tr>
<tr>
<td>5.4</td>
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<td>1</td>
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<td>5.4</td>
</tr>
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<td>4.4</td>
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<td>1</td>
<td>Oral</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Note:**

- Log dose: Logarithmic dose of the bacteria.
- No. of infected: Number of mice infected.
- No. of survivors: Number of survivors.
- Mortality: Number of deaths in each group.
- Number of deaths on Day: Days when deaths occurred.
- Route of infection: Oral or Intraperitoneal.
- Infectivity: Logarithmic scale of infectivity.
### EXPERIMENT III.

**COMPARISON OF VIRULENCE OF PHAGE-TRANSDUCED FIMBRIATE S. TYPHIMURIUM 1287 and NON-FIMBRIATE PARENT STRAIN FOR LAC GREY MICE**

#### Results obtained with S. TYPHIMURIUM 1287

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>Time to death of infected mice</th>
<th>Number of deaths on day</th>
<th>Time to death at oral ID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
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<td></td>
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</tr>
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</tr>
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<td>7.0</td>
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</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.7</td>
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</tbody>
</table>

#### Log dose

<table>
<thead>
<tr>
<th>Infectivity</th>
<th>Time to death of infected mice</th>
<th>Number of deaths on day</th>
<th>Time to death at oral ID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>8</td>
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</tr>
<tr>
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<td>12</td>
<td>1</td>
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</tr>
<tr>
<td>8.7</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

---

**Notes:**
- Results obtained with S. TYPHIMURIUM 1287
- Parent strain for the oral route is the flagellate and non-flagellate forms of S. TYPHIMURIUM 1287
- Non-flagellate
- Experiment III: Comparison of virulence of phage-transduced fimbriate and non-fimbriate S. TYPHIMURIUM 1287
- Appendix 6
## Experiment IV: Comparison of Virulence of Phage-Transduced Streptomycin-Resistant Fimbriate and Non-fimbriate S. typhimurium 1289 by Oral Infection of Streptomycin-Treated Lactogrey Mice

<table>
<thead>
<tr>
<th>Log dose organisms per ml</th>
<th>Infected mice</th>
<th>Number of deaths on day</th>
<th>Times to death at ID50 with fimbriate strain</th>
<th>Times to death at ID50 with non-fimbriate strain</th>
</tr>
</thead>
<tbody>
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<td>2.08</td>
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<td>1.5</td>
<td>3.08</td>
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</table>

### Footnotes:
- P = Fimbriate
- N = Non-fimbriate
- SR = Streptomycin-resistant
- Phage = Phage-transduced
- ID50 = 50% Infectious Dose
- 1289 = S. typhimurium 1289
- Lactogrey = Lactogrey mouse

### Reference:
- Experiment LV: Comparison of Virulence of Phage-Transduced Streptomycin-Resistant and Non-streptomycin-resistant strains of S. typhimurium 1289 by Oral Infection of Streptomycin-Treated Lactogrey Mice

### Appendix:
- Experimental Salmonella strains I
## EXPERIMENTAL SALMONELLOSIS

### Appendix I

#### EXPERIMENTAL SALMONELLOSIS

**Time to death at ID₅₀ for the non-fimbriate strain is 20 days.**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>No. of deaths</th>
<th>Time to death at ID₅₀</th>
<th>ID₅₀</th>
<th>Streptomycin</th>
<th>Log dose</th>
<th>No. of deaths</th>
<th>Time to death at ID₅₀</th>
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<tr>
<td></td>
<td>9/9</td>
<td>9/9</td>
<td>6/0</td>
<td>YES</td>
<td>6/0</td>
<td>YES</td>
<td>6/0</td>
</tr>
<tr>
<td></td>
<td>9/9</td>
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<td>6/0</td>
<td>YES</td>
<td>6/0</td>
<td>YES</td>
<td>6/0</td>
</tr>
</tbody>
</table>

* For more information, see [Experimental Salmonellosis](#).
Experimental Salmonellosis

Appendix 9

**Comparison of Virulence of Flagellate and Non-Flagellate RIIA-Hno-Positive S. Typhimurium 1566 for Lac Grey Mice by the Oral and Intraperitoneal Routes of Infection.**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Oral</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>4.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>5.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>6.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>7.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>8.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>9.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>10.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>11.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>12.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
<tr>
<td>13.5</td>
<td>1/2</td>
<td>1/2</td>
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<tr>
<td>14.5</td>
<td>1/2</td>
<td>1/2</td>
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<td>15.5</td>
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<td>1/2</td>
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<td>16.5</td>
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<td>1/2</td>
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<td>17.5</td>
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<td>18.5</td>
<td>1/2</td>
<td>1/2</td>
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<td>19.5</td>
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<td>1/2</td>
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<td>20.5</td>
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<td>1/2</td>
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<td>21.5</td>
<td>1/2</td>
<td>1/2</td>
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<tr>
<td>22.5</td>
<td>1/2</td>
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<tr>
<td>23.5</td>
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<td>1/2</td>
</tr>
<tr>
<td>24.5</td>
<td>1/2</td>
<td>1/2</td>
</tr>
</tbody>
</table>

*Results obtained with S. Typhimurium strain 1566 P fim.**

Note: Comparisons are only made on days 3 to 7.

**Experiments with Strain 1566 Fimbrate and Non-Fimbrate RIIA-Hno-Positive S. Typhimurium 1566 for Lac Grey Mice by the Oral and Intraperitoneal Routes of Infection.**

- **Log dose**
- **Infectivity**
- **No. Injected**
- **No. Infective**
- **No. Survivors**
- **FD = 24**
- **Number of Deaths on Day**
- **Times to Death at Oral ID50**
### Experimental Salmonellosis

#### Appendix 10

**EXPERIMENT VI.**

**COMPARISON OF VIRULENCE OF Fimbriate and Non-Fimbriate Strains of S. Typhimurium 1566**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

*Results obtained with S. Typhimurium strain 1566. Note in parentheses the oral and intraperitoneal routes of infection.*

**ORAL**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**INTRA-PERITONEAL**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>12/12</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
### Experimental Salmonellosis

#### Experimental Salmonellosis

<table>
<thead>
<tr>
<th>Route of Infection</th>
<th>Oral</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log dose</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Infected</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Survivors</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mortality</td>
<td>2/12</td>
<td>2/12</td>
</tr>
<tr>
<td>2nd day</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>No. of Deaths</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>No. of Survivors</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Log dose</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Results of Experiment

<table>
<thead>
<tr>
<th>Test</th>
<th>Oral</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

### Appendix II

**Experimental Salmonellosis**
### EXPERIMENT VII.

#### COMPARISON OF VIRULENCE OF *S. typhimurium* STRAIN 6354-FT/fit.

<table>
<thead>
<tr>
<th>Log dose (organisms)</th>
<th>Oral</th>
<th>Intra-peritoneal</th>
<th>Intra-uterine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Non-Fimbriate</strong></td>
<td><strong>Phage-Transduced Fimbriate</strong></td>
<td><strong>Phage-Transduced Flagellate</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Non-Flagellate</strong></td>
<td><strong>Phage-Transduced Flagellate</strong></td>
<td><strong>Phage-Transduced Fimbriate</strong></td>
</tr>
</tbody>
</table>

**Results obtained with *S. typhimurium* strain 6354-FT/fit.**

- log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- mean 12.4 days

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Oral</th>
<th>Intra-peritoneal</th>
<th>Intra-uterine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Non-Fimbriate</strong></td>
<td><strong>Phage-Transduced Fimbriate</strong></td>
<td><strong>Phage-Transduced Flagellate</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Non-Flagellate</strong></td>
<td><strong>Phage-Transduced Flagellate</strong></td>
<td><strong>Phage-Transduced Fimbriate</strong></td>
</tr>
</tbody>
</table>

**Times to death at oral ID50**

- Oral: 12.4 days
- Intraperitoneal: 11.4 days
- Intraperitoneal: 10.4 days

**Number of deaths per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of survivors per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Number of infections per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of survivors per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of infections per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of survivors per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of infections per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of survivors per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of infections per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of survivors per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days

**Number of infections per day**

- Oral: 12/12
- Intraperitoneal: 11/12
- Intraperitoneal: 11/12

**Log dose of injection**

- Oral: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
- Intraperitoneal: log dose 5 = 5, 6.4, 7.4, 8.4, 9.4, 10.4, 11.4, 12.4, 13.4, 14.4 days
### EXPERIMENT VIII

**Comparison of Virulence of Hagedorn-induced Flagellate and the Parent Non-Flagellate S. Typhimurium for Mice by the Oral and the Intraperitoneal Routes of Infection.**

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>No. of mice infected</th>
<th>No. of deaths on day 50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraperitoneal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Hagedorn strain 6371 N**

#### Results Obtained with S. Typhimurium

- **Flagellate/Non-Flagellate**: S. Typhimurium Parent and the Parent Non-Flagellate
- **Comparison of Virulence of Flagellate/Non-Flagellate**: Parent and the Parent Non-Flagellate

*Experimental Section*: Appendix 15

---

Legend:
- $\geq$ = more than
- $< = less than
- Log dose = log dose
- Infectivity
- No. of Survivors
- Mortality
- No. of mice infected
- No. of deaths on day 50

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>No. of mice infected</th>
<th>No. of deaths on day 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1</td>
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<td>6.8</td>
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</tbody>
</table>

Mean 12.5 days
### EXPERIMENT VIII. COMPARISON OF VIRULENCE OF A NON-FLAGELLATE NON-FLAME TRANSDUCED Fimbriate for Mice

#### Results obtained with S. typhimurium strain 6352 FT-ff 1.

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infection</th>
<th>No. of Infected</th>
<th>Survivors</th>
<th>Mortality</th>
<th>No. of deaths on day</th>
<th>Log dose of infective dose</th>
<th>Time to death at oral LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Intraperitoneal LD&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>8.4</td>
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</tr>
<tr>
<td>8.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Strain 6352 FT-ff 1.*

- Results obtained with S. typhimurium
- Intraperitoneal routes of infection

**Appendix T**

**Experimental Section**

**Appendix T**
**Experiment VIII.**

**Comparison of Virulence of**

**Salmonellae typhimurium and its Phage-transduced Flagellate**

**For White Mice by the Oral and Intraperitoneal Routes of Infection**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>日</th>
<th>Survivors</th>
<th>Infectivity</th>
<th>Number of infections</th>
<th>Log dose</th>
<th>日</th>
<th>Survivors</th>
<th>Infectivity</th>
<th>Number of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7.4</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7.2</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7.0</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6.8</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Times to death at oral LD50**

- At log 7.7 = 12, 16, 19, 14, 17, 21
- At log 7.4 = 7, 9, 10, 11, 12, 14

**Mean 11.6 days**

- Non-fimbriate
- Phage-transduced Flagellate

- Comparison of Virulence of a Non-Flagellate, its Flagellate, and the Phage-transduced Flagellate

- Log dose (LD50)

- Day of death

- Number of deaths
Comparison of Virulence of a Noneflagellate Strain of Salmonella typhimurium (629N) and Its Flagellate Revertant Mutant (629RP) for White Mice by the Oral and Intraperitoneal Routes of Infection.

**Results obtained with S. typhimurium strain 629HF/fl.**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Oral</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7.2</td>
<td>1/12</td>
<td>1/12-1/12-1/12</td>
</tr>
<tr>
<td>7.2-7.8</td>
<td>1/12</td>
<td>1/12-1/12-1/12</td>
</tr>
<tr>
<td>7.8-8.2</td>
<td>1/12</td>
<td>1/12-1/12-1/12</td>
</tr>
</tbody>
</table>

**Mean time to death at oral ID50**

- More than 24 days
- 24-72 hours
- 72 hours or less

**Note:** Results obtained with S. typhimurium.
**Experimental Salmonellosis**

**I. Appendix**

### COMPARISON OF VIRULENCE OF A NON-FIMBRIATE S. TYPHIMURIUM 629N AND ITS FIMBRIATE REVERT MUTANT 629HF (OBTAINED BY THE USE OF MhCL AS A MUTAGENIC AGENT) FOR UNITE MICE BY THE ORAL INTRAPERITONEAL ROUTES OF INFECTION

**(b)**

Results obtained with S. typhimurium strain 629N.

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Time to death at oral ID</th>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Time to death at oral ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>4.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>4.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>5.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>5.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>6.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>6.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>7.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>7.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>8.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>8.5</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>9.0</td>
<td>6/12</td>
<td>2/12</td>
<td>1</td>
<td>12</td>
<td>4.5</td>
<td>1/12</td>
<td>1/12</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

**Note:** Results obtained with S. typhimurium strain 629 N.

**References:**
- (b) Appendix IX
- Experimental Salmonellosis
### Experimental Salmonellosis

**I. Appendix 18.**

**Comparison of Virulence of a Phase-Non-Flagellate Flagellate STRAIN, A Non-Flagellate STRAIN FOR MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.**

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Log dose</th>
<th>No. of mice infected</th>
<th>Number of deaths on day</th>
<th>Mean time to death at oral LD$_{50}$</th>
<th>N = non-Flagellate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td>6.0</td>
<td>12</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>12</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>12</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>12</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>12</td>
<td>1</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>12</td>
<td>1</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>12</td>
<td>1</td>
<td>More than 9 days</td>
</tr>
</tbody>
</table>

**Results obtained with S. Typhirium strain SW573 N/fl.**

**Experimental Salmonellosis**

- **Comparison of Virulence of a Phase-Non-Flagellate Flagellate STRAIN, A Non-Flagellate STRAIN FOR MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.**
- **Experiments**
- **Appendix I**
## Experimental Salmonellosis

### Appendix I

#### Comparison of Virulence of a Flagellate/Non-Fimbriate Strain, a Non-Flagellate/Non-Fimbriate Strain, and the Parent Non-Fimbriate/NON-Flagellate S. Typhimurium for "Kite" Lesions by the Oral and Intraperitoneal Routes of Infection.

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>No. of Infected</th>
<th>Mortality</th>
<th>Route of Infection</th>
<th>Log dose</th>
<th>Number of deaths on day</th>
<th>Number of survivors infected</th>
<th>No. of survivors</th>
<th>Time to death at oral ID50</th>
<th>Intrapertoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>= 2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>&gt;=22</td>
<td>1.0</td>
</tr>
<tr>
<td>2.0</td>
<td>&lt;3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>&gt;=22</td>
<td>1.0</td>
</tr>
<tr>
<td>3.0</td>
<td>=3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>&gt;=22</td>
<td>1.0</td>
</tr>
<tr>
<td>4.0</td>
<td>&gt;4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>&gt;=22</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Mean time to death at oral ID50 = >22 days

*Strain SW 576 F/T1

Results obtained with S. Typhimurium

Experimenter: Salomonowitz I. Appendix 19
### Comparison of Virulence of a Phage-Transduced Fimbriate/Flagellate Strain, a No Fimbriate/""" Flagellate Strain and the Parent Non-Flagellate/Nor-Flagellate Strain Typhimurium for White Mice by the Oral and Intraperitoneal Routes of Infection

<table>
<thead>
<tr>
<th>Log dose of infectivity</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on Day</th>
<th>Mean time to death at oral LD50 is &gt; 22 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>0.7</td>
</tr>
<tr>
<td>1.7</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>0.7</td>
</tr>
<tr>
<td>6.9</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>0.7</td>
</tr>
</tbody>
</table>

### Results Obtained with S. Typhimurium Strain SW76/FIT

**Note:** The oral and intraperitoneal routes of infection produced similar results. The parent non-flagellate/nor-flagellate strain Typhimurium was resistant to phage-transduction.

- Mean time to death at oral LD50 is > 22 days.
**EXPERIMENT XI.**

**COMPARISON OF VIRULENCE OF FIMBRIATE S. TYPHIMURIUM (OBTAINED BY THE USE OF MUTAGENIC MNCl AS A MUTAGENIC AGENT) AND THE NON-FIMBRIATE PARENT STRAIN, FOR MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION.**

**Results obtained, v.d-th S. typhimurium strain 14-36 MF/fl.**

<table>
<thead>
<tr>
<th>Log dose</th>
<th>No. of mice infected</th>
<th>No. of deaths on day</th>
<th>Mortality mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.3</td>
<td>12</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>6.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>5.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>4.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>3.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>2.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>1.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>0.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

**INTRA- && **

<table>
<thead>
<tr>
<th>Log dose</th>
<th>No. of mice infected</th>
<th>No. of deaths on day</th>
<th>Mortality mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>7.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>6.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>5.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>4.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>3.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>2.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>1.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>0.3</td>
<td>12</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

*Times to death at oral and intraperitoneal routes.*

---

**EXPERIMENTAL MATERIAL AND THE NON-FIMBRIATE PARENT STRAIN, FOR MICE BY THE ORAL AND INTRAPERITONEAL ROUTES OF INFECTION (OBTAINED BY THE USE OF MNCl AS A MUTAGENIC AGENT).**

*Appendix 21*
EXPERIMENT XII.

Comparison of virulence of Fimbriate S. typhimurium (obtained by the use of McCl2 as a mutagenic agent) and the non-fimbriate parent strain for mice by the oral aid.

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on day</th>
<th>Time to death at oral ID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Results obtained with S. typhimurium strain.*

Experiment XI.

Comparison of virulence of Fimbriate S. typhimurium on mice by the oral aid.

<table>
<thead>
<tr>
<th>Log dose</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>Number of deaths on day</th>
<th>Time to death at oral ID50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.6</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5.6</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Results obtained with S. typhimurium strain.*
### Appendix 23.

#### The Results of Intraperitoneal Infection of Albino Rats With the Parent Non-Fimbriate Strain and a Hi Age-Transduced Fimbriate Strain of S. Typhimurium 1289

<table>
<thead>
<tr>
<th>Log dose (x 10)</th>
<th>Infectivity</th>
<th>No. of Survivors</th>
<th>Mortality</th>
<th>No. of Deaths on Day 0</th>
<th>No. of Infected</th>
<th>Test strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;7.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Each sex was infected with one dose.
Experimental Salmonellosis

Appendix 24.

**RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF S. typhimurium ON LEUCOCYTES IN VITRO.**

<table>
<thead>
<tr>
<th>Remarks</th>
<th>Time of reading</th>
<th>Preparation</th>
<th>Test Period</th>
<th>No. of viable Leucocytes</th>
<th>Percent Leucocytes Killed</th>
<th>Mortality during first 2 hours observation</th>
<th>Mortality of the 25 observed cases</th>
<th>Mean percentage mortality</th>
<th>Leucocyte suspension mixed with bacteria strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A few small clumps seen</td>
<td>90</td>
<td>suspension</td>
<td>T747</td>
<td>150</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td>No bacterial adhesion</td>
<td>72</td>
<td>suspension</td>
<td>T747</td>
<td>170</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>suspension</td>
<td>T747</td>
<td>180</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td>A few moribund</td>
<td>35</td>
<td>suspension</td>
<td>T747</td>
<td>40</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td>A few moribund</td>
<td>40</td>
<td>suspension</td>
<td>T747</td>
<td>60</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td></td>
<td>01</td>
<td>suspension</td>
<td>T747</td>
<td>80</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td></td>
<td>01</td>
<td>suspension</td>
<td>T747</td>
<td>100</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>suspension</td>
<td>T747</td>
<td>120</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
<tr>
<td></td>
<td>00</td>
<td>suspension</td>
<td>T747</td>
<td>140</td>
<td>90</td>
<td>35</td>
<td>90</td>
<td>15</td>
<td>S. typhimurium T747</td>
</tr>
</tbody>
</table>

*Experimental No. 1.*

**EXPERIMENTAL EMERGENCE AND NON-EMERGENCE IN VITRO.**

<table>
<thead>
<tr>
<th>Remarks</th>
<th>Time of reading</th>
<th>Preparation</th>
<th>Test Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXPERIMENTAL EMERGENCE AND NON-EMERGENCE IN VITRO.**

<table>
<thead>
<tr>
<th>Remarks</th>
<th>Time of reading</th>
<th>Preparation</th>
<th>Test Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EXPERIMENTAL EMERGENCE AND NON-EMERGENCE IN VITRO.**
### EXPERIMENTAL SALMONELLOSIS IN VITRO

**RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF Fimbriate AND Non-Fimbriate Strains of *S. typhimurium* 1287 on LEUCOCYTES IN VITRO**

<table>
<thead>
<tr>
<th>Remarks</th>
<th>Leucocyte control suspension</th>
<th>Time of reading</th>
<th>Percentage of dead leucocytes</th>
<th>Mean percentage mortality during first 2 hr of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A few clumps seen</td>
<td></td>
<td>15</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>52</td>
<td>75</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td>15</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td>++</td>
<td></td>
<td>15</td>
<td>60</td>
<td>28</td>
</tr>
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<td></td>
<td>15</td>
<td>60</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>60</td>
<td>28</td>
</tr>
</tbody>
</table>

**Experimental Salmoenillosis** A Appendix 25*
|                | 
|----------------|-----------------------------------|
|                | Results of Comparative Studies of the Effects of Fimbriate and Non-Fimbriate Strains of *Salmonella flexneri* on Leucocytes in vitro. | 
|                | Experiment No. III. | 
|                | Experimental Salmonella strains A & B. | 
|                | Appendix 2A. | 
|                | See Appendix 24. | 
|                | x | 56°C for 30 min. + |
**Experimental Salmonellosis**

### Appendix 27

#### RESULTS OF COMPARATIVE STUDIES OF THE EFFECTS OF FIMBRIATE AND NON-FIMBRIATE STRAINS OF *S. typhimurium* ON LEUCOCYTES IN VITRO.

<table>
<thead>
<tr>
<th>Test preparation</th>
<th>Period of test (Time of reading)</th>
<th>No. of viable leucocytes</th>
<th>Percentage of dead leucocytes and mean percentage mortality during first 2 hr. of observation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium 1289 TT/f</td>
<td>25 min.</td>
<td>66 (6)</td>
<td>9</td>
<td>88</td>
</tr>
<tr>
<td>phage-transduced fimbriate strain mixed with leucocyte suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund leucocytes seen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria adhering to leucocytes and clumps seen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. typhimurium 1289 N/fl</td>
<td>25 min.</td>
<td>66 (6)</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>non-fimbriate strain mixed with leucocyte suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocyte control suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. typhimurium 1289 TT/f</td>
<td>25 min.</td>
<td>66 (6)</td>
<td>9</td>
<td>88</td>
</tr>
<tr>
<td>phage-transduced fimbriate strain mixed with leucocyte suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund leucocytes seen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria adhering to leucocytes and clumps seen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. typhimurium 1289 N/fl</td>
<td>25 min.</td>
<td>66 (6)</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>non-fimbriate strain mixed with leucocyte suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocyte control suspension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See Appendix 24.
<table>
<thead>
<tr>
<th>Days</th>
<th>Intraperitoneal</th>
<th>Subcutaneous</th>
<th>Number of challenged mice</th>
<th>Number of dead mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
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<td></td>
<td></td>
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<td>12</td>
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<td></td>
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<td>14</td>
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</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend:
- **NTL** = non-flagellate
- **NFl** = non-fimbriate
- **F** = fimbriate

Each group contained an equal number of male and female mice.

The mice were injected 19 to 22 times each and the

weight.
<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>20.0</td>
<td>25.0</td>
<td>30.0</td>
<td>35.0</td>
<td>40.0</td>
<td>45.0</td>
</tr>
<tr>
<td>2-3</td>
<td>2.5</td>
<td>5.0</td>
<td>7.5</td>
<td>10.0</td>
<td>12.5</td>
<td>15.0</td>
<td>17.5</td>
<td>20.0</td>
<td>22.5</td>
</tr>
<tr>
<td>4-5</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
</tr>
<tr>
<td>6-7</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>3.5</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>8-9</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>1.25</td>
<td>1.5</td>
<td>1.75</td>
<td>2.0</td>
<td>2.25</td>
</tr>
<tr>
<td>10-11</td>
<td>0.125</td>
<td>0.25</td>
<td>0.375</td>
<td>0.5</td>
<td>0.625</td>
<td>0.75</td>
<td>0.875</td>
<td>1.0</td>
<td>1.125</td>
</tr>
</tbody>
</table>

Follow-up challenge, number of deaths on day:

- Mean
- Median
- Mode
- Log dose (μg)
<table>
<thead>
<tr>
<th>Day</th>
<th>Deaths</th>
<th>HL</th>
<th>Td</th>
<th>Td/100</th>
<th>Td/10</th>
<th>Td/0</th>
<th>Td/0</th>
<th>Td/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>6</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Each group contained an equal number of mice and received mice which were fed 25 g of food each day. The groups were balanced by weight, with the number of mice in each group varied. 

**Control**: vines (vines) were grown in a greenhouse. 

**Adjuvant**: vines were grown in a greenhouse. 

**Groups**: groups were divided into two groups: Group 1 and Group 2. 

**Days**: days after inoculation. 

**Deaths**: number of mice that died. 

**Infectivity**: number of mice that were infected. 

**Mortality**: number of mice that died. 

**Deaths**: number of mice that died. 

**Last time death**: time of death. 

**Duration**: duration of death. 

**Post-inoculation**: time of death. 

**Time of death**: time of death. 

**Vaccination**: time of death. 

**Death**: time of death.
Experimental salmonellosis

Appendix 31.

COMPARISON OF PROTECTIVE POTENCIES OF SWISS WHITE MICE IMMUNISED WITH KILLED VACCINES PREPARED FROM A RELATIVELY VIRULENT S. TYPHIMURIUM 635-FT/FLT/HAGE TYPE 2e/L3.

The mice were immunised by two subcutaneous injections of log 7 and log 5 organisms respectively. The interval between the two injections was 14 days, and the mice were challenged intraperitoneally 10 days after the second immunising dose.

<table>
<thead>
<tr>
<th>Vaccine preparation</th>
<th>No. mice immunised 1st dose</th>
<th>Deaths prior to challenge</th>
<th>Challenge I.P. 10 days after 2nd dose</th>
<th>Log lose No. mice following challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fighter preserved</td>
<td>20</td>
<td>10/20</td>
<td>0</td>
<td>15.8</td>
</tr>
<tr>
<td>Heat-killed phenol</td>
<td>20</td>
<td>10/20</td>
<td>0</td>
<td>15.3</td>
</tr>
<tr>
<td>Heat-killed freezed</td>
<td>20</td>
<td>10/20</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>10/20</td>
<td>0</td>
<td>90</td>
</tr>
</tbody>
</table>

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.

* Experimental immunity VII.

** Comparison of protective potencies for Swiss white mice of kittens vaccinated with S. TYPHIMURIUM 635-FT/FLT/HAGE TYPE 2e/L3.

*** Approx. 31.6 years.
<table>
<thead>
<tr>
<th>Days</th>
<th>Strain</th>
<th>Infectiona</th>
<th>Mortalityb</th>
<th>Mortalityc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6354</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>32</td>
<td>6354</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a. Infection: Percentage of mice showing signs of infection.
b. Mortality: Number of deaths due to infection.
c. Mortality: Number of deaths due to other causes.

*Note: The table shows the results of an experiment involving the infection of mice with a specific strain of bacteria. The infection rate and mortality are recorded over a period of 32 days.
<table>
<thead>
<tr>
<th>Strains</th>
<th>Intraperitoneal</th>
<th>Intraperitoneal</th>
<th>Intraperitoneal</th>
<th>Intraperitoneal</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10.4</td>
<td>11.3</td>
<td>12.6</td>
<td>12.8</td>
<td>13.2</td>
</tr>
<tr>
<td>B</td>
<td>10.0</td>
<td>10.6</td>
<td>10.9</td>
<td>11.1</td>
<td>11.3</td>
</tr>
<tr>
<td>C</td>
<td>9.7</td>
<td>10.2</td>
<td>10.4</td>
<td>10.6</td>
<td>10.8</td>
</tr>
<tr>
<td>D</td>
<td>9.4</td>
<td>9.8</td>
<td>10.0</td>
<td>10.2</td>
<td>10.4</td>
</tr>
</tbody>
</table>

The mice were vaccinated with strain A and each group consisted of an equal number of males and females. The mice were weighted 19-22 g. Each and the number of mice used was balanced by the sex of the mice. The mice were treated with streptomycin and fed a diet of a nutritionally complete diet. The mice were housed in single cages. The animals were observed for 4 weeks and then challenged with the homologous strain. The number of deaths and the day of death were recorded. The survivors were then challenged with the heterologous strain and the number of deaths and the day of death were recorded. The experiment was conducted in a single room. The mortality was expressed as a percentage of the total mice at risk. The experiment was conducted in a single room. The mortality was expressed as a percentage of the total mice at risk.

**Experimental Immunology II**

Appendix 33
Log dose of the vaccine and the challenge strain were estimated by the Miller and Kremer method.

<table>
<thead>
<tr>
<th>Dose (dose)</th>
<th>Control</th>
<th>Immunised control</th>
<th>Immunised attenuated</th>
<th>Immunised</th>
<th>Immunised attenuated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0/1</td>
<td>0.1</td>
<td>0.1</td>
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Number of deaths per group of 24 unit vaccinated.
Experimental salmonellosis

VII. Appendix

35. Experimental Immunity

XI. COMPARISON OF THE PROTECTIVE POTENCIES FOR SWISS WHITE MICE OP ALIVE ATTENUATED VACCINES (SETGLENSTEP STREPTOICIN-RESISTANT MUTANTS) PREPARED FROM S. TYPHIMURIUM STRAINS 6351N/nf and 6354FT/flt. THE MICE WERE IMMUNISED AS A SINGLE SUBCUTANEOUS INJECTION OF LOG 3 ORGANISMS. THEY WERE IMMUNISED ORALLY ON THE 15th DAY OF IMMUNISATION WITH S. TYPHIMURIUM STRAIN 1566P/fl. THE SURVIVORS WERE KILLED ON THE 23rd DAY FOLLOWING THE CHALLENGE.

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<th>Control: (n/a) 24 unvaccinated mice</th>
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Each group consisted of equal number of male and female mice which were weighed 15-22 days each.

The groups were balanced by weight.

Log dose preparations and challenge were estimated from visible counts by Miles and Misra methods.

N = non-fixable
FT = plaiage-transduced
F = plaiage-transduced
SR = streptomycin-resistant
LT = plaiage-transduced
MT = plaiage-transduced

* = more than

25th DAY FOLLOWING THE CHALLENGE:

ACTIONS (STREPTOMYCIN-RESISTANT MUTANTS) PREPARED FROM S. TYPHIMURIUM STRAINS 6351N/nf and 6354FT/flt and EXPERIMENTAL IMMUNITY

EXPERIMENTAL IMMUNITY XIX CONCERNING IMMUNE MICE OP ALIVE ATTENUATED VACCINES APPENDIX 35

Appendix AII