GENERAL INTRODUCTION
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The problems of salmonellosis are numerous. Infections due to *Salmonella* though controlled considerably in developed countries are still potential public health hazards in developing countries.

Infection due to *Salmonella weltevreden* in India was detected quite late (Freeman, 1953). But during recent years the rate of infection due to *S. weltevreden* both in man and animals is increasing at an alarming rate. In 1965, Singh (Singh, 1968) placed *S. weltevreden* as one of the first ten sero-types of *Salmonella* in order of frequency. In 1970 this sero-type was placed 8th amongst *Salmonella* isolations from human sources and fifth from other sources (Nath, Singh and Bhandari, 1970). In 1971 Basu, Lahadevan and John (1971) found *S. weltevreden* to rank second amongst all *Salmonella* isolations in India.

Garg and Singh (1971) developed a phage-typing scheme for *S. weltevreden*. This scheme has not been thoroughly assessed by the authors. Moreover, they noted that "different strains of *S. weltevreden* from various host species belonged to a single phage-type........". They could only associate two strains of *S. weltevreden* as both were isolated on the same day from a slaughter house and were of the same phage type, out of 149 strains tested by them. Therefore, this scheme does not seem to have much practical utility. In 1973, the
same authors developed another phage-typing scheme based on identification of temperate phages (Garg and Singh, 1973). This scheme also did not have any epidemiological significance as they observed that "lysis patterns of symbiotic phages derived from various \textit{S. weltevreden} strains showed no correlation with either the source or the geographic origin of \textit{S. weltevreden} strains".

It may, therefore, be concluded that no phage-typing scheme of practical epidemiological value has been developed for \textit{S. weltevreden} so far. The present thesis incorporates the results of studies aiming at the development of a phage-typing scheme for \textit{S. weltevreden}.

The introduction of this thesis is presented in four sections:

Section I \textit{Salmonella weltevreden}.

Section II Present state of knowledge about bacteriophage.

Section III Present state of knowledge about phage-typing.

Section IV Plan of work.
SECTION I

SALMONELLA WELTEVREDEN

Salmonella weltevreden, 3,10:1:e, belongs to sero-group E₁ of Kauffmann - White Schema. Biochemically it belongs to sub-genus I of the genus Salmonella (Kauffmann, 1960). It was first isolated by Erber (1941) in Batavia in Indonesia (Netherland East Indies) from cases of human gastro-enteritis. At present this sero-type has a very wide host range and has been isolated from many countries of the world. It has been held responsible for quite a few food-borne infections in man and for infections in various animal species and thus poses both zoonotic and epidemiological problems.

Sources of Salmonella weltevreden:

Salmonella weltevreden has been isolated from a wide range of sources. It has been isolated from man (Erber, 1941; Freeman, 1953; Ganguli, 1958; Nathur, 1959; Agarwal, 1963; Datta and Singh, 1964; Kakhalia and Singh, 1964; Nath et al., 1966; Morahan, 1967, 1968; Nath, Singh and Bhandari, 1970), sheep (Nath et al., 1966; Sethi, Nath and Shrivastava, 1968), goat (Sharma and Singh, 1961a, 1961b; Agarwal, 1962, 1963; Nath et al., 1966; Nath, Singh and Bhandari, 1970), cattle (Khera and Dhanda, 1958;

Apart from these sources a number of other vehicles and reservoirs have been incriminated e.g. Harvey and Phillips (1961) isolated *S. weltevreden* from pig slaughter drain of an abattoir; Randhawa and Kalra (1970) isolated it from goat meat and meat shopping block. Taylor et al. (1965)
enlist its isolation from piggery drain, bone meal, coconut, calf-sweetbread and sea water and Garg and Singh (1973) from frog's leg and from sea foods like frozen prawns and oysters. It has also been isolated from sewage (Datta, Sharma and Singh, 1964; Sharma and Singh, 1967; Nath, Singh and Bhandari, 1970; Garg and Singh, 1973).

**Geographical Distribution of Salmonella weltevreden:**

*Salmonella weltevreden* is one of those *Salmonella* sero-types which exhibit patterns of geographical and regional distribution. For example, during the *Salmonella* Surveillance Programme in the United States during the year 1970, Hawaii reported 93 of the 104 isolations of *S. weltevreden*. A similar pattern of high incidence of this sero-type in Hawaii as compared to other States was observed in earlier years also and has remained unchanged in recent years (Fox, Leewenstein and Martin, 1972).

In Papua and New Guinea, Morahan and his colleagues isolated *Salmonellae* from human carriers, hospital specimens, rodents, water and other sources. It was found in various studies (Egerton and Hambling, 1963; Morahan, 1967, 1968, 1969a, 1969b) that *S. weltevreden* was the most predominant sero-type isolated both from human and
rodent sources. In the Nowak Hospital series, *S. weltevreden* emerged as not only the most common organism isolated but also the only one associated with mortality (isolated from three patients who subsequently died) and it was concluded by Korahan (1969b) that "*S. weltevreden* must be considered to be the most important agent of human salmonellosis in this area at the present time".

*S. weltevreden* has been isolated from many countries of the world and particularly from the Asian countries. These include Australia, Borneo, Canada, Ceylon, England, Gan Island, Germany, Ghana, Hongkong, India, Japan, Kenya, Kuwait, Malaya, Malaysia, Mauritius, Holland, New Zealand, Pakistan and Iran, Papua and New Guinea, Philippines, Sweden, Switzerland, South Rhodesia, Taiwan, Tanzania, Thailand, the United States of America and Vietnam (Taylor et al., 1968; Singh and Garg, 1971; Garg and Singh, 1973).

In India though strains have been isolated from a number of States, viz., Andhra Pradesh, Bihar, Delhi, Haryana, Himachal Pradesh, Madhya Pradesh, Maharashtra, Punjab, Kerala, Tamil Nadu, Uttar Pradesh and West Bengal the preponderance of isolations has been from the Punjab–Haryana–Himachal Pradesh–Delhi regions in the north, from Tamil Nadu in the south and from Maharashtra in the west.
From the northern regions most of the isolations from human sources have been from and around Ludhiana district in the Punjab State. In the south the majority of the isolations from human sources have been from Vellore in Tamil Nadu State. No strains have been reported from Assam, Gujarat, Jammu and Kashmir, Karnataka, Manipur, Meghalaya, Nagaland, Rajasthan, Tripura, Arunachal Pradesh, Goa, Lakshadweep and Mizoram. Salmonella weltevreden in India:

The first isolation of *S. weltevreden* in India was reported in 1953 by Freeman (1953) from a patient of gastro-enteritis who also had hyper-pyrexia. Ganguli (1958) reported its isolation from human cases of diarrhoea at Poona and Vellore and from guinea pigs at Poona and Delhi. Its isolation has also been reported by Khera and Dhanda (1958) from fatal cases of gastro-enteritis amongst calves. Mathur (1989) described a localised outbreak of food poisoning which involved five individuals of a family and their servant who suffered from severe gastro-enteritis due to this sero-type after consumption of milk.

Rao and Gupta (1961) reported outbreak of salmonellosis due to this sero-type in the second generation of the imported chicks and Jayaraman, Krishnan and Sethumadhavan (1964) reported its isolation from
guinea pigs. Sharma, Datta and Singh (1964) reported its isolation from the septicemic infection of a rabbit. Datta and Singh (1965) could culture it from heart blood and internal organs of dead chickens without the help of enrichment medium. Its isolation from various other sources has already been described.

Singh (1968) found *S. weltevreden* to belong to the first 10 *Salmonella* sero-types in order of frequency and to constitute 11.1% of all *Salmonella* isolations in India, up to June 1966. Nath, Singh and Rhandari (1970) found that amongst 1700 strains of various *Salmonella* sero-types encountered during July, 1965 to December, 1969 from various sources, *S. weltevreden* ranked 8th amongst isolations from human sources and 5th amongst isolations from non-human sources. More recently, it has been found to rank second amongst all *Salmonella* isolations reported in India (Basu, Mahadevan and John, 1971).

Since the number of *S. weltevreden* strains isolated in India has increased markedly during recent years, it has become one of the few sero-types of major public health significance.

The incidence of *S. weltevreden* in India during the last 10 years as compared to other *Salmonella* in man and animals is presented in the Table.
# Table

Relative incidence of *Salmonella weltevreden* in man and animals during 1964–1973 as recorded at the National Salmonella and Escherichia Centre, Kasauli (India)

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>No. of <em>Salmonella weltevreden</em> isolated from</th>
<th>No. of <em>Salmonella</em> strains other than <em>S. weltevreden</em> isolated from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Man</td>
<td>Animals</td>
</tr>
<tr>
<td>1964</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>1965</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1966</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>1967</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>1968</td>
<td>17</td>
<td>30</td>
</tr>
<tr>
<td>1969</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>1971</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>1972</td>
<td>77</td>
<td>50</td>
</tr>
<tr>
<td>1973 (upto June)</td>
<td>21</td>
<td>25</td>
</tr>
</tbody>
</table>
SECTION II

PRESENT STATE OF KNOWLEDGE ABOUT BACTERIOPHAGE

Iwanowski (1892) first demonstrated the presence of ultramicroscopic filter-passing organisms in tobacco plants with mosaic disease. He transmitted the disease to healthy plants through the bacteria-free filtrate of sap from diseased plants. The phenomenon of viruses attacking bacteria was first observed by Twort in 1915. In 1917, d'Herelle independently discovered a virus active against Shigella shige and named it "bacteriophage". Bacteriophages (or phages for short) are viruses parasitic on bacterial cells. In 1920 d'Herelle discovered bacteriophages active against Vibrio cholerae in the stool of cholera convalescent patients (d'Herelle, 1923). He found that the bacterial lysis caused by phage is accompanied by production of more phage.

Because of the observations on the susceptibility of V. cholerae to the lytic action of phage, d'Herelle thought that they play some role in the resistance to and recovery from cholera. This concept stimulated much research since 1920 on the possible prophylactic and therapeutic value of the phages; much fundamental work was also done on their
biological characteristics. Except Pastukov (1959) and Sayasov (1963) most of the recent workers have found that bacteriophages are of little value in the prophylaxis and therapy of cholera. Trials in East Pakistan (now Bangla Desh) have demonstrated conclusively that cholera phages are of practically no therapeutic value (Monsur et al., 1970).

More recently, research on phages has been directed mainly towards their application in epidemiological surveys by phage-typing and their use as a research tool in studies in molecular genetics. They also furnish ideal materials for studying host-parasite relationships and virus multiplication. Information gained from studies on phage may in future help solve many problems in the control of animal and plant viruses.

**Morphology of bacteriophages:**

The morphology of bacteriophage was first studied under the electron microscope by Ruska in 1940 (Ruska, 1940). Luria and Anderson (1942) and Luria, Delbrück and Anderson (1943) studied the electron micrograph of coliphage \( \phi_2 \) and showed that it is not a simple sphere but a complex structure which consists of a 'head' and a 'tail'. Since then many bacteriophages have been studied by this method. Much variation in size and morphology
has been observed amongst them. Delbrück (1946) believed that the morphology of phages is likely to be a valuable taxonomic criterion.

Bacteriophages are usually found to consist of a head and a tail of varying shape and size. The head consists of a tightly packed core of nucleic acid, surrounded by a protein coat or 'capsid'. The protein capsid of the head is made up of identical subunits called 'capsomeres', packed to form a prismatic structure, usually hexagonal in cross-section.

Electron micrographs, made on material prepared in various ways, have revealed that the heads of different phages assume a number of geometrical forms. (Horne and Wildy, 1961). Kosloff and Henderson (1965), Kellenberger and Arber (1955), Williams and Fraser (1953, 1956) and Brenner et al. (1959) studied the head morphology of the T-even coliphages and found them to be bipyramidal hexagonal prisms. Bradley and Kay (1960) and Chiossotto et al. (1960) found VI 1 phage and a phage of B. megaterium to possess icosahedral heads. Octahedral heads have been described for typhoid phage 2 and in other phages various ovoid heads have been noted (Bradley and Kay, 1960). The well-defined symmetrical
shapes of their heads indicate that bacteriophages possess an orderly arrangement of substructures (Horne and Wildy, 1961).

The tail structures of the T-even coliphages have been well studied. In the process of infection most of the phage protein remains outside the bacterial host (Hershey and Chase, 1952) in the form of empty ghosts (Kellenberger and Arber, 1955). The DNA is squirted into the bacterial cell as though the phage were a micro-syringe. The mechanism responsible for thrusting the tail into the bacterium may equally well be likened to that of a spring gun (Horne and Wildy, 1961).

The phage tail varies greatly in its complexity from one phage to another. The most complex tail is found in phage T2 and in a number of other coli and typhoid phages. In these phages, the tail consists of at least 3 parts: a hollow core, ranging from 6-10 nm in width; a contractile sheath, ranging from 15-25 nm in width; and a terminal base plate, hexagonal in shape, to which may be attached prongs, tail fibres, or both (Jawetz, Melnick and Adelberg, 1970).

Electron micrographs of phage preparations have revealed the phages to exist in two states: in one, the sheath is expanded, and the base-plate appears to have
a series of prongs. In the second state the sheath is contracted and the base-plate has 6 fibres attached to it (Champs, 1963). Phages also vary with respect to the terminal structure of the tail; some have base-plates, some have 'knobs', and others lack the specific terminal structures altogether. Amongst the last mentioned group are the tail-less phages which fall under three categories. The first with large capsomeres, such as φX174 which is in the form of an icosahedron with 12 apical capsomeres and containing a circular single-stranded DNA of $1.7 \times 10^6$ daltons molecular weight (Sinsheimer, 1959a, 1959b), the second containing ribonucleic acid, such as f2 (Loeb and Zinder, 1961) which is an icosahedron with small capsomeres and with RNA genome of about $10^6$ daltons molecular weight, and the third being of filamentous type (Marvin and Hoffmann-Berling, 1963). The filamentous, male specific bacteriophages Fd, f1, M13 and their relatives contain one piece of single-stranded circular DNA of molecular weight $2 \times 10^6$ daltons (Marvin and Hohn, 1969).

Chemical and biochemical characteristics:

Phage particles contain only protein and one kind of nucleic acid. Most phages contain only DNA; however,
a group of phages which specifically attack male
strains of Escherichia coli contains only RNA. The nucleic
acid of the phage is in the form of a long filamentous
molecule and may be of a double- or single- stranded
type (Bradley, 1967). DNA for most phages is double-
stranded but some like \( \lambda \) contain single- stranded
DNA. The nucleic acid makes up about 50% of the dry
weight.

In the T-even phages (T2, T4 and T6), the
double- stranded DNA consists of a single molecule with
a molecular weight of \( 1.3 \times 10^8 \) daltons. These phages
are distinctive in that they contain 5-hydroxy methyl-
cytosine as one of the pyrimidine bases in their DNA
in place of cytosine (Stent, 1963). This pyrimidine
has not been found in the nucleic acid of the bacterial
host.

The proteins which make up the head membrane, the
core, the sheath, and the tail fibres are distinct from
each other; in each case, however, the structure appears
to be made up of repeating subunits. The head membrane
of a T-even phage has a particle weight of about \( 85 \times 10^6 \)
to \( 90 \times 10^6 \) daltons and is composed of about 1000 subunits
of polypeptides of 80,000 daltons molecular weight (Van
The tail sheath has a particle weight of about \( 7.8 \times 10^6 \)
to \( 12 \times 10^6 \) daltons and is composed of 140 to 200
polypeptide subunits. The tail fibre has a particle weight of 400,000 daltons (Champa, 1963). It is, therefore, clear that the proteins composing the head, sheath and tail fibres of a T-even phage have different primary structures (Brenner et al., 1959). The total bacteriophage protein is composed of the twenty 'standard' amino acids, which are found in the proteins of all viruses — plant, animal and bacterial (Crick, 1958).

The so-called 'internal' protein, which constitutes 5% of the total phage protein, is not visible in the electron microscope and is released from the phage along with the DNA into the host cell and can also be released by osmotic shock. This protein is of low molecular weight and is composed of small peptides containing principally glutamic acid, aspartic acid and lysine and two polyamines (Ames, Dublin and Rosenthal, 1968).

Small bacteriophages containing ribonucleic acid in the nucleus, which attack the male strains of E. coli K-12 have been isolated by Loeb (1960); Loeb and Zinder (1961); Dettori, Maccacaro and Piccinin (1961). The coliphages containing RNA, unlike the majority of bacteriophages, do not adsorb to specific receptor sites in the cell wall (Bradley, 1967).
**Enzyme activities:**

Although the phage particle is known to be metabolically inactive, certain enzymatic activities have been demonstrated. Koch and Dreyer (1958) isolated lysozyme of molecular weight about 15,000 daltons from a purified T2 phage. Probably this enzyme is located at the distal end of the tail i.e. the tail plate.

Phosphatase activity of T2 and T4 phages has been reported by Dukes and Kosloff (1959), which is capable of hydrolysing ATP, Desoxy-ATP, ADP and a number of other phosphate-esters. The phosphatase activity of T5 phage is different from T2 and T4 phages even though all the phages are propagated on the same host strain. The phosphatase of T-even phage may be identical with or contained in the contractile sheath of the phages (Champe, 1963).

The empty ghost cells of bacteriophages obtained by osmotic shock (Anderson, 1949 and Hotchin, 1954) and treatment with deoxyribonuclease contain most of the phage proteins and retain the adsorption property and killing power for the host bacteria.

**Phage Reproduction and Life Cycle of Bacteriophage:**

**Phage Reproduction:**

**Adsorption:**

The adsorption of phage to the host bacterium has been shown to be proportional to the concentration of
both the phage and the bacterium. Krueger (1931) investigated the adsorption of a phage to living and heat-killed Staphylococcus and found that with an excess of bacteria the adsorption follows the kinetics of first order reaction.

The adsorption rate is markedly affected by environmental conditions such as salt concentration, pH and temperature. Some phages also require the presence of specific cofactors particularly inorganic ions and amino acids before adsorption occurs. Each phage is quite specific with regard to the cofactors required for adsorption but the cofactor requirement for adsorption is subject to modification by mutations of the phage (Adams, 1959).

Different bacterial strains are highly specific with regard to the phages which they will adsorb. This specificity has been found to reside in the cell wall. The factors in the cell wall responsible for adsorption appear to be discrete, localised 'receptors'; the receptors for phages T3, T4 and T7 reside in the lipopolysaccharide layer, whereas the receptors for phages T2 and T6 reside in the lipoprotein layer. Ability to adsorb phage is obviously a factor in the determination of bacterial sensitivity to infection (Jawetz, Kelnick and Adelberg, 1970).
Intracellular development of phage:

After attachment to the bacterial cell there is a period called the 'eclipse phase' when no phage can be detected within the host cell. During this period the synthesis of bacterial nucleic acid and protein is halted and the activities of the host cell are directed towards synthesizing the specific proteins and nucleic acids of the phage. The data about the intracellular development of phage has been derived mostly from experiments with isotopically labelled phage and labelled metabolites, and from experiments involving premature lysis of infected cells by artificial means.

The time interval between the adsorption of phage to the host cell and the lysis of the cell liberating phage progeny is known as the 'latent period' and is measured by the 'one-step growth experiment'. The latent period depends on the phage and host strains and also on the environmental conditions.

Bacterial lysis and liberation of new phage:

When the number of mature phage particles within the bacterial cell reaches a critical level the bacterium lyses liberating phage particles which may
then attack further cells. The cell lysis occurs as a result of osmotic pressure after the cell wall has been weakened by the phage lysozyme.

The number of phage particles liberated per infected bacterium is known as the 'burst size' and is characteristic for each phage strain. The average burst size is also determined by the one-step growth experiment and depends on the phage strain, the host cell and the environmental conditions.

**Life cycle of bacteriophage:**

When a bacteriophage particle comes in contact with a susceptible bacterial cell it attaches itself to the bacterial cells by the tip of its tail. The tail pierces through the cell wall of the bacterium by mechanical action (Williams and Fraser, 1986), which might be aided by some enzymatic process, and the DNA contained in the head of the phage particle is ejected into the bacterial cells. The empty protein sheath or the 'ghost' falls off in the medium and ultimately gets lysed. The phage DNA in the infected bacterium may assume either of two phases - the vegetative phase or the lysogenic phase.
THE LIFE CYCLE OF A TEMPERATE BACTERIOPHAGE
THE PROPHAGE IS REPRESENTED BY A DASH. b=BACTERIUM.
Vegetative phase:

The DNA which has been ejected into the bacterial cell by the phage appears to lose its infectivity for several minutes. Then half-way through the latent period, material serologically recognisable as phage begins to form and almost immediately afterwards infective phage itself appears. The amount of infective phage then increases for some minutes and finally the cell lyses releasing a large number of mature phage particles.

Lysogenic phase:

In this phase the genetic material does not enter the vegetative phase. It comes in intimate relation with the bacterial gene and is converted into prophage and the bacterium remains alive and lysogenic. A lysogenic bacterium generally divides with the prophage without producing mature bacteriophage particles. But it can produce mature phage spontaneously under certain conditions. Phage production in a lysogenic bacterium can be induced by agents like ultraviolet light, gamma radiation, etc., and mature phage particles are produced which ultimately lyse the bacterium.

The two phases in the life cycle of phage are represented in the Figure.
Chang of antigenic structure of Salmonellae induced by bacteriophage:

Zinder and Lederberg (1952) showed that the temperate phage PLT 22 which was active on sero-groups A, B and D of Salmonellae could introduce genetic material into susceptible cells and hence effect transfer of H antigens from donor to recipient cells. In 1953, Lederberg and Edwards (1953) applied the technique of genetic transduction to the exchange of flagellar antigens among various sero-types of sero-groups B and D and Kauffmann (1953) effected similar changes in members of sero-groups A and B with different temperate phages. Edwards, Davis and Cherry (1955) characterised temperate phages that were capable of transducing H antigens in other sero-groups also.

Lederberg (1955) and Barksdale (1959) considered the phage genes as such to function as part of the bacterial cells. The state of lysogenicity per se, caused by infection with a phage that has converting properties, brings about changes in the O antigens. As long as the organism remains lysogenic these changes persist, and the particular O antigen can be demonstrated. Conversely, when the state of lysogenicity is lost the O antigen involved can no longer be detected (Edwards and Ewings, 1972).
Since each lysogenized organism undergoes antigenic change in lysogenic conversion, it has probably bearing the most on serotypic identity and epidemiology.

A classic example was the presence of both *S. anatum* (3,10 : e,h : 1,6) and *S. newington* (3,15 : e,h : 1,6) in the outbreak of disease in which both types first were isolated (Rettger and Seeville, 1920; Edwards, 1937). The two types often have been found in association and now it is apparent that the factor determining their identity is infection of sero-type *S. newington* by phage $\varepsilon^{15}$. The conversion of *Salmonella* strains producing the antigens 3,10 to produce antigens 3,15 by lysogenization with $\varepsilon^{15}$ phage was demonstrated by Iseki and Sakai (1953) and studied in detail by Uetake, Nakagawa and Akiba (1955) and Uetake, Luria and Burrous (1956). A second conversion in the E group *Salmonella* is brought about by $\varepsilon^{34}$ phage which adds antigen 34. The interrelationship between $\varepsilon^{15}$ and $\varepsilon^{34}$ was investigated by Uetake and Hagiwara (1960) who demonstrated that $\varepsilon^{15}$ is required for the $\varepsilon^{34}$ genome to be expressed.

Following infection or lysogenization, *Salmonella* phages $\varepsilon^{15}$, $\varepsilon^{34}$ and $\varepsilon^{341}$ (conversion phages) are capable of bringing about alterations in chemical structure of O-antigen polysaccharides (Matsuyama and
Phage conversions in *Salmonella* have been reported in group A (Zinder, 1957), group B (Iseki and Kashiwagi, 1957; Zinder, 1957; Stecker, 1958), group C (Iseki and Kashiwagi, 1957; Baron, Formal and Washington, 1957), group D (Iseki and Kashiwagi, 1957; Zinder, 1957) and group E (Iseki and Sakai, 1953; Robbins and Uchida, 1962). All of these O-antigen conversions lead to the addition of an antigenic component with the exception of the $E_4$ to $E_2$ conversion by $\varepsilon^{15}$. 

Uetake, 1972]. These changes can be visualized by serological reactions or by phage adsorptions (Uetake, Nakagawa and Akiba, 1955; Uetake, 1957; Hagiwara, Uetake and Takeda, 1966). Phage $\varepsilon^{15}$ exerts repression of cellular transacetylation of galactose residues on the antigenic polysaccharide, inhibition of $\alpha$-polymerase, and synthesis of $\beta$-polymerase; phage $E^{34}$ synthesizes two types of glucosyl transferase that are required to link the short side chain, glucose, to the carbon 4 of galactose present on the antigenic polysaccharide; and phage $E^{34}$ inhibits cellular transacetylation of galactose residues on the antigenic polysaccharide (Uchida, Robbins and Luria, 1963; Robbins and Uchida, 1965; Bray and Robbins, 1967; Losick and Robbins, 1967; Wright, 1971; Hagiwara, Uetake and Takeda, 1966; Matsuura and Uetake, 1972).
SECTION III

PRESENT STATE OF KNOWLEDGE ABOUT

PHAGE-TYPING

Bacteriophages are known to have relatively limited range of lytic activity and to possess different degrees of lytic specificity for different strains of their bacterial hosts belonging to the same species. By studying their comparative sensitivity to bacteriophages it is possible to distinguish the strains of a given species of bacterium, in a manner which is not possible by any other laboratory technique. It has also been shown that strains of bacteria isolated from groups of infections originating from a common source have an identical phage susceptibility pattern. These characteristics form the scientific basis of the technique of phage-typing for identification of bacterial strains in epidemiological studies. The practical value of phage-typing for identification of sources of origin and lines of spread of an infectious disease has been established in numerous studies, and in recent years phage-typing has proved to be one of the most important advances in the methodology of epidemiological research.
Historical:

The practicability of the phage-typing scheme depends on the host specificity. This was first recognised by Sommenschein (1925, 1928) who isolated specific phages for *Salmonella paratyphi* B and *S. typhi* and suggested their use for rapid identification of strains. Marcuse (1931) used specific phages to identify strains of *Shigella flexneri*. Following the discovery of Vi antigen of *S. typhi* by Felix and Pitt (1934), phages specific for the Vi forms of *S. typhi* were isolated by Craigie and Brandon (1936), Sertio and Boulgakov (1936a) and Scholtens (1936).

In 1938, Craigie and Yen (Craigie and Yen, 1938) first introduced a phage-typing scheme for *S. typhi* using four Vi phages designated I to IV. They observed that the Vi phage II possessed the unusual property of acquiring high specificity for the strains on which it had been propagated. By using different strains of host bacteria isolated from different outbreaks, for propagation of phages, they obtained a number of phage preparations which not only became specific for the particular type of strains on which they had been propagated but also behaved in an identical manner with strains related epidemiologically. On this basis they identified a number
of distinct Vi phage-types of \textit{S. typhi}. On further study of 708 strains from different sources they also found phage-typing of \textit{S. typhi} to be of definite epidemiological significance.

The practical value of the phage-typing technique for strain identification developed by Craigie and Yen, was confirmed by workers like Felix (1943). In 1947, Craigie and Felix put forward suggestions for the standardization of the method. In 1950, the International Committee for Enteric Phage-typing adopted the phage-typing scheme for \textit{S. typhi} and in 1953 approved the scheme of extended typing (Felix, 1955) which recognised 33 types of Vi typhoid bacilli. As a result of further investigations on typhoid phage-typing, additional types and sub-types of strains have been identified.

The practical value of the phage-typing scheme has been proved beyond doubt in tracing chronic carriers and groups of cases originating from them (Felix, 1943; Bradley, 1943; Cruickshank, 1947) and is now regarded as a valuable tool in the epidemiological study and control of typhoid fever.

The Vi phage-typing scheme for \textit{S. typhi} formed the model for subsequent schemes for the typing of other pathogenic bacteria. The scope of this new technique
has been studied in many bacterial species and the phage-typing of a number of bacterial pathogens has been standardized by internationally agreed methods.

**Schemes of phage-typing:**

Different techniques have been found suitable for different bacterial species. These techniques are based on two approaches. One depends on the susceptibility to lysis of the bacterial strains by bacteriophages, while the other depends on identification of the particular temperate phage carried by lysogenic bacterial strains.

In the former approach, bacteria possessing Vi-antigens are typed by monospecific Vi phages. These phages are derived from a single phage by adaptation on strains originally resistant to the lytic action of the parent phage. Bacterial species devoid of the Vi-antigen and some with Vi-antigen also are classified on the basis of their patterns of sensitivity to groups of non-specific anti-O phages.

In the second approach, lysogenic bacteria are classified by a mark, signifying the type of the temperate phage as identified after isolation from the bacteria. They may also be grouped by the patterns of lysis of selected sets of indicator strains.
Different phage-typing schemes have been developed according to their suitability to particular bacterial species. For certain species, more than one typing scheme has been worked out, each one of which has given results of epidemiological significance. But such multiplicity of schemes for individual species may lead to confusion and, therefore, for the same species particular schemes have been adopted by international agreement.

Phage-typing schemes, using nonspecific phage preparations, have been developed for *S. typhi* (Craigie and Yen, 1938; Craigie and Felix, 1947) and to a limited extent for *S. paratyphi B*, *S. typhimurium* and *S. thompson* (Felix and Callow, 1943, 1951; Felix, 1956; Gershman, 1972). But for most bacterial species, phage-typing schemes have been based on their patterns of lysis by a set of nonspecific anti-O phages. These include *S. paratyphi A* (Banker, 1955), *S. typhimurium* (Lilleengen, 1948), *S. pullorum* and *S. gallinarum* (Lilleengen, 1952), *S. thompson* (Williams Smith, 1951b), *S. dublin* (Lilleengen, 1950; Williams Smith, 1951a), *Shigella sonnie* (Hamnarström, 1947, 1949), *Escherichia coli*
of infantile gastro-enteritis (Nicolle et al., 1952),
*E. coli* of cattle (Williams Smith and Crabb, 1956),
*Staphylococcus aureus* (Fisk, 1942; Wilson and Atkinson,
1945 and Williams and Hippom, 1952), *Corynebacterium
diphtheriae* (Fahey, 1952; Thibaut and Fredericq, 1956),
*Vibrio cholerae* (Nicolle, Gallut and Le Kinor; Nicolle et al., 1962; Mukerjee, 1963; Newman and Eisenstark, 1964)
and *V. cholerae* bio-type El Tor (Basu and Mukerjee, 1969).

Methods of phage-typing based on identification
of the temperate phages have been classified under two
heads: (1) Boyd's Scheme (Boyd, 1950; Boyd, Parker and
Kair, 1951) of isolation and identification of the
temperate phage as applied in phage-typing of *S. typhi-
murium* and (ii) Atkinson's scheme based on lytic patterns
of temperate phages from lysogenic strains on indicator
strains as used for phage-typing for *S. adelaide* (Atkinson,
1955; Atkinson and Klaus, 1955) and *S. bovis-morbificans*
(Atkinson, 1956a, 1956b). Atkinson (1957) tried to
simplify Boyd's scheme by growing the test strain and a
set of indicator strains together in mixed culture and
extracting the mixed growth in a little broth. The broth
extract was then centrifuged and lytic action of the
supernatant was noted on both the indicator and the test
strains. The grouping of a test strain was determined
from the pattern of results of lysogenicity tests on a set of indicator strains. In contrast to Boyd's Schema of marking strains by identification of their temperate phage, the carried phage was not actually identified in the phage grouping scheme of Atkinson but was recognized by its range of activity in lysogenicity tests on a series of indicator strains representing the established phage groups.

Sechter and Gerichter developed phage-typing schemes for \textit{S. braenderup} (Sechter and Gerichter, 1968) and \textit{S. blockley} (Sechter and Gerichter, 1969) using both the methods of phage-typing, that is, by using a set of anti-\(O\) phages as well as by identifying the temperate phages. They have classified bacterial strains into phage-types by means of lytic affinity of bacterial strains to a set of anti-\(O\) phages and further subclassified the phage-types by means of lytic patterns of temperate phages carried by them on a set of indicator strains.

Although both methods of phage-typing, i.e. sensitivity to lysis by typing phages and identification of the temperate phages, give results of epidemiological value, the first approach is generally simpler and less time-consuming for routine application. Anderson and
Felix (1953) have pointed out that different Vi phage-types of *S. typhi* may carry the same phage (determining phage) and if they are classified on the basis of the temperate phages, they will be classified into one type. Moreover, it is not always possible to isolate temperate phages from cultures as in *S. typhi* and in such cases the method of typing by identification of temperate phages fails altogether (Anderson, 1959). So Anderson (1959) suggested that a combination of the two methods offers the most precise means of strain characterization, but for routine use the determination of the spectrum of phage sensitivity of bacterial strains is the more practical and reliable approach.
SECTION IV

PLAN OF WORK

The studies comprised in this thesis are presented in three parts:

Part I: Studies on Salmonella weltevreden:

Isolation, identification and sero-typing of S. weltevreden have been carried out. All the 607 strains of S. weltevreden available have been studied for their epidemiological history.

Part II: Phage-typing of Salmonella weltevreden:

Three hundred and ninety strains of S. weltevreden have been tested for lysogeny and all of them found to be lysogenic. A phage-typing scheme has been developed by lysogenicity patterns of temperate phages on four indicator strains. However, phage-typing by this method has not been found to be very satisfactory.

Seventy-eight strains of bacteriophages, from lysogenic bacteria, stool samples of man and animals and from sewage and natural water sources have been isolated and purified. From amongst these phages six phages were selected for their suitability as typing phages of S. weltevreden.
Using these phages, a phage-typing scheme has been developed for strain identification of *S. weltevreden*. A total of 607 strains from different sources isolated during 1953-1973 have been classified into types and sub-types. The epidemiological value of the phage-typing scheme has been studied in outbreaks originating from single sources and in cases and their contacts.

**Part III: Studies on the biological characteristics of the Salmonella weltevreden typing phages:**

Some of the biological properties of the six typing phages described in Part II have been investigated in some detail.