CHAPTER III.

IMMUNITY IN TYPHOID FEVER
The concept of immunity stems from the observation of the survival of the fittest in the struggle for existence. It denotes a resistance or refractoriness of an organism to the aggression offered to it by a parasite. During this struggle both the parasite and the host undergo adaptation, the former to more effectively invade the host and the latter to more effectively resist it.

From the earliest times it had been observed that individuals who had once suffered from small-pox became relatively immune to it. The practice of variolation, which could induce this immunity, was prevalent in far removed countries and its origin is lost in antiquity. In the absence of proper controls, this could be a hazardous procedure and fulminating disease, leading to fatality often developed. An improved and safer method was clearly desirable.

This need was first supplied as the result of the work of Jenner (1796). He crystallised his observations and practice of 20 years into the process of vaccination. This took the form of using virus from cowpox infection to provide induced immunity. Such was his faith in this, that despite discouragement by the Royal Society, he persisted in it, but no general acceptance of his findings was forthcoming till the establishment of bacteriology as a science.

Eighty-four years intervened between Jenner and Pasteur. During this period the basis of enquiry changed from the empirical to the scientific. In 1860, Pasteur accidentally discovered that cultures of chicken cholera bacillus which had been left in the laboratory for several months had lost their virulence and could no longer produce a
fatal disease in chicken. When these chicken which had received the attenuated organism were inoculated with the fresh virulent cultures, they did not develop the infection. That is, they had developed immunity. He immediately saw the analogy between this phenomenon and Jenner's observations and called the process of inoculation with virulent cultures "Vaccination". Turning his attention next to anthrax he proceeded to develop a vaccine for it. The changed basis of thought is amply demonstrated by the fact that although he was greeted with the same scepticism as Jenner, he not only did not give up, but converted his critics by conducting his historical experiment at Pouilly-le-Fort in the presence of a distinguished gathering. Pasteur's work revolutionised thinking on the subject, and opened up new avenues of enquiry in the phenomenon of immunity.

A host of discoveries followed. Nuttal (1888) clearly showed by experiments in-vitro that whole blood had the property of destroying organisms seeded in it. Buchner (1889), extended Nuttal's work with whole blood to serum and observed that although fresh serum would destroy certain bacteria, this property was lost when the serum was heated to 55°C. In 1890, Von Behring and Kitasato showed that by giving repeated injections of non-lethal doses of tetanus or diphtheria toxin to animals, the serum acquired the property of specifically neutralising these toxins. Pfeiffer (1894) demonstrated the gradual lysis and disintegration of Vibrio cholera when introduced into the peritoneal cavity of immunised animals. In 1895, Bordet published his classical paper on the properties of immunised sera and attributed the bactericidal action of immune sera to two factors.
one a thermolabile factor present in normal serum and, the other a thermostable factor present in immune serum.

The demonstration of a direct lethal action on bacteria, led to channelising of views on the mechanism of immunity into two main theories. The adherants of the 'humoral theory' felt that both specific and natural protection of animals was due to the presence of bactericidal substances in the serum. They quoted Pfeiffer's findings in support of this view.

The rival 'cellular' theory was propounded by Katchnikoff, a zoologist. From observations of the process of ingestion and disintegration of food particles in amoeba, he held that in the course of cellular differentiation the process had become adapted to the combating of invading micro-organisms (Florey, 1958). He noted these phagocytic activities in the Daphnia. Further support for the theory came from studies on mammalian leucocytes and later the phagocytic cells of the tissues. Katchnikoff believed these cells to be the chief means of protection of the animal body against infection. He attributed the incontrovertible fact of bactericidal action of serum to an origin in cell ferments.

The controversy was finally resolved when Wright and Douglas (1903) demonstrated that even in the apparently purely cellular phenomenon of phagocytosis the presence of opsonins in serum was imperative. Heufold & Sinpou (1904) showed that the humoral factors in serum can be increased with immunisation. Thus a synthesis of the two schools of thought came about and has resulted in
The fundamental mechanism of the phenomenon of immunity however, continues to elude the efforts of the immunologist even today. The union between the specific part of the antibody molecule and the radical of the antigen which determines the specificity, probably plays the primary role. The reconstruction of events after this primary reaction is, however, not simple. Only very few of the protective antibodies are known to bring about the death of susceptible bacteria and in all the known phenomena, the elimination or inhibition of bacteria calls for the participation of host factors like phagocytic cells or complement.

The nature of the bactericidal power which is exhibited by freshly drawn blood or by serum is not known. It may be due to physiological constituents of the serum or to actual bacteriolysins produced as the result of constant stimulation by a variety of antigens during life. The role of immune bacteriolysis in determining the resistance to infection has been adequately demonstrated by the Pfeiffer's phenomenon.

Phagocytosis is a general biological phenomenon and is undoubtedly a most important mechanism of defence against infection. It is not surprising therefore, that many types of bacteria are engulfed by the phagocytic cells. This brings about rapid death of certain organisms but the outcome is not universal as some organisms like the gonococci and meningococci become adapted to an intracellular life; Nor do all organisms exhibit an equal degree of susceptibility to...
phagocytosis. The virulent forms particularly are resistant to this action. The specific antiserum enhances the phagocytosis of otherwise resistant forms by supplying the specific bacteriotoxin.

Like other infections, in typhoid fever, a relative insusceptibility or refractoriness of some individuals was noticeable in all epidemics, these being characterised by extreme variability of the course of the disease in different individuals. In some, it took the form of a mild abortive attack which could even pass unnoticed, while in others, it was severe and prolonged. Gay (1916), has cited an interesting observation made by Denemark on 229 people who ate potato salad prepared by a typhoid carrier. Twenty-two of these had a typical attack, while fifty-nine had no fever but antibodies against the organisms could be demonstrated, and in four of these typhoid bacilli were found in the stools.

Racial susceptibility was also at one time thought to play an important role in the epidemiology of typhoid fever. It was generally held that white people were more liable to typhoid fever than the darker races. This view is no longer tenable, as mortality figures during World War I indicated that in Mesopotamia among the troops two to three times more Indians succumbed to the disease than Europeans. (H.R.C., 1929.) Enteric fever was also encountered among Chinese and Negroes in all the theatres of the war.

Attempts to reproduce the disease in experimental animals occupied an important part in the study of typhoid fever. Records of these ventures can be traced even into the pre-bacteriological era.
(Magenie, 1823 & Murchison, 1858). After the discovery of the causative organism, several workers have made unsuccessful efforts to produce the analogous syndrome in laboratory animals (Caffky, 1884; Gilbert & Girodé, 1891 & Sanarelli, 1892). The closest approximation to it, as described by Metchnikoff and Besredka (1911), was attained in the anthropoid apes by administering massive doses orally. In other laboratory animals, however, oral administration usually does not give rise to any harmful effects at all. A fatal infection can, however, be produced by an intravenous or intraperitoneal injection of living typhoid bacilli in adequate doses. The organisms can be recovered from the blood and tissues postmortem but no typical lesions are produced and the death appears to be toxemic (Wilson & Miles, 1955). These experimental failures demonstrate that typhoid fever is essentially a human disease and other animals possess natural immunity to it.

In the small rodents of the laboratory, a natural disease resembling typhoid fever in man can be produced by administering S. typhi-murium organisms. In mice, the disease is characterised by splenic enlargement along with small necrotic foci in both the liver and the spleen and occasionally scattered pneumonic patches in the lung (Wilson & Miles, 1955). The mode of spread and subsequent involvement of the various lesions has been studied in detail by Ørskov & his colleagues (Ørskov, Jensen & Kobayashi, 1928; Ørskov & Moltke, 1928 and Ørskov & Lassen, 1930). Owing to its close similarity to the human infection, it has served as a tool in the hands of the immunologist to study a large number of problems related to the mecha-
nism of immunity in typhoid fever.

Besides this type of inherent insusceptibility to the disease, the rarity of second attacks has been empirically assumed from very early times. It drew comment from Bretonneau in 1629 in his famous paper 'Dothiementerie'. Gendron in 1834 cited numerous instances where individuals and families, though exposed to the acute disease during nursing, were protected from it by having already suffered from a previous attack.

This phenomenon of protection after infection was also observed in experimental animals by Beumer & Peiper (1887). They were able to demonstrate that mice which had recovered from a non-fatal infection with living typhoid bacilli were able to withstand a much larger and fatal dose, the best results being obtained by gradual increase in the successive doses. They further suggested the possibility of immunising human beings by means of sterilized cultures. In the following year, Chantemesse and Widal (1888) by carefully conducted experiments demonstrated that it was possible to immunise mice by inoculating them with killed cultures.

The application of these experimental findings to human beings is associated with two groups of workers (Wright, 1896; Pfeiffer & Kolle, 1896 & Wright & Semple, 1897). These formed the groundwork for vaccination against typhoid fever and subsequently ingenious methods have been devised for the preparation of vaccines, all aimed at producing maximum immunity.

It was soon realised, however, that like other allied biological
cal phenomena, the protection acquired is relative and not absolute.

Typhoid fever does not leave behind a high degree of immunity. The occurrence of second attacks though rare, has nevertheless been reported from early times (Piedvache, 1850 & Budd, 1873). In subsequent years frequency figures varying between 0.75% to 4.2% have been quoted (Gay, 1918). In more recent times, Marmion, Stewart and Baylor (1953) examining records of two outbreaks of typhoid fever at Abyad, found that of 54 men who had contracted the disease during the first epidemic, no less than 20.4% suffered from it again 3 months later when there was a second outbreak.

In immunised subjects although a certain degree of immunity can be established, it is in no way absolute or solid and can be overcome by a massive dose of the virulent bacilli. Numerous outbreaks of enteric fever have in fact been recorded in well-vaccinated communities (Hiller et al., 1951).

The explanation for this breakdown of the immunity is not known and only reiterates the fact that, as in other bacterial infections, the mechanism of production of immunity in typhoid fever is not very well understood. The concepts of the virulence of a parasite and of the phenomenon of immunity have undergone a striking evolution and the emphasis shifted first from the disease to the etiological agent and finally to the host-parasite relationship. This changed outlook has encompassed a wider perspective of the complex nature of immunity. But despite this great advancement, knowledge on the subject is still far from complete.
With the recognition of the complexity of the antigenic structure of bacteria, attempts have been made to determine the role of these factors in the virulence of the microbe and the formation of protective antibodies. In recent years voluminous work has been done on the analysis, chemical and otherwise, of the antigenic mosaic and these have been dealt with in a previous chapter.

Much of the knowledge about the nature of virulence has been derived from the variations which an organism undergoes. In the earlier days of the history of immunisation a general belief was prevalent that the possession of virulence by an organism enhanced the protective value of the vaccine prepared from it. Definite experimental proof of this superiority of virulent strains was not available although Pfeiffer and Kolle in 1896 had reported that in their experiments on guinea-pigs, a virulent strain of B. typhosus required far more immune serum to lyse it than did a strain of low virulence.

Following the demonstration of the flagellar and somatic antigens (Smith & Reagh, 1903 & Weil & Felix 1917, 1920), the existence of differences in the immunising value of these antigens was for the first time explicitly and clearly stated by Felix (1924). Their contention was that flagellar antigens and their corresponding antibodies play little if any part in the specific antibacterial immunity, whereas the heat-stable somatic antigen is all important. This belief was more than borne out by the result of subsequent work (Arkwright, 1927 & Topley, 1929).
At about the same time as the recognition of the flagellar and somatic antigens in typhoid bacilli, Arkwright (1921) described the S-R variation, which has contributed greatly to the study of virulence and to the correlation between morphological and biological characters. It was found that the S-forms were responsible for the natural infection and the S-R variation was associated with a loss of virulence. The cellular modification associated with the variation is the loss of the specific somatic antigen (White, 1926). It is, therefore, reasonable to suppose that this antigenic component plays an important part in virulence. Indeed the complete 'O'antigen isolated in solution from the bacterial cells, on injection into normal animals causes rapid leucopenia (Morgan, 1941). This author also noted that it exerts a repellent action on leucocytes and thus interferes with phagocytosis. It is also known to inhibit the bactericidal activity of serum against homologous organisms. The 'O' antigens therefore, protect the parasite from the defence mechanism of the host and thereby contribute to the property of virulence.

The other antigen which is reputed to play an important part in the virulence of the organism is the 'Vi' antigen. The chemically isolated substance has been found to be less toxic to animals than the 'O' antigen (Henderson & Morgan, 1938). High virulence of an organism requires the concurrence of both 'Vi' and 'O' antigens (Felix & Pitt, 1951). The effect of 'Vi' antigen on virulence is brought about by interference with the reaction between the 'O' antigen and antibody (Wilson & Miles, 1955).

Owing to the association of the smooth phase with the virulence
of the organism, it was hoped that a simple explanation for the problem of immunity would be forthcoming. In fact the antibodies corresponding to these type specific antigens have both been shown to sensitize the organism to the lethal action of complement and to phagocytosis.

Topley and his colleagues (1937) prepared two types of antigens, one containing both the 'O' and 'Vi' fractions and the other with 'O' alone. On injecting into mice, both could confer immunity against a 'Vi' containing strain. But examination of the purer forms of the antigen has made certain immunological differences apparent. Pure 'O' and pure 'Vi' sera specifically neutralize the effect of the corresponding antigen and exhibit cross-protection against strains containing 'Vi' and 'O' antigens (Henderson & Morgan, 1930). But Henderson (1939) has shown that when infection is due to pure 'Vi' and pure 'O' strains, only the specific antisera are protective. Both the 'Vi' and 'O' antibodies have been demonstrated to possess phagocytosis-promoting power (Bhatnagar, 1935 and Felix & Bhatnagar, 1935).

Despite these evidences, the fact that successful immunisation can be achieved with living attenuated strains as well as with killed virulent strains suggests the existence of other antigenic components which participate in immunity.

The host factors which are responsible for eliminating the organism, are both cellular and humoral and the specific immunity to typhoid is undoubtedly related to the specific antibodies. Whether these act mainly by opsonisation or by sensitising the organism to the action of the complement or by both is still debatable.

Gay (1918), basing his conclusions on the disappearance of the agglutinating antibodies soon after recovery from the disease, suggested that the protection was cellular rather than humoral.
During the incubation period of typhoid fever, the organisms are engulfed by the fixed phagocytes of the liver, spleen and bone-marrow and continue to proliferate in these cells (Goodpasture, 1937 and Adams, 1939). This shows that at least in a certain stage of the disease the phagocytic R.E. cells instead of destroying the organisms protect them. Based on this, Florey (1958) has suggested that this specific immunity in typhoid probably depends on sensitisation to the action of complement rather to opsonisation. It is, however, not possible to say that phagocytes play no part in the immunity. The existence of these intracellular forms in the absence of bacteraemia, suggests the possibility of a more intimate host-parasite relationship.

Apart from the gaps in the knowledge about the mechanism by which immunity is brought about, a quantitative assessment of its degree has yet to be established. This is beset with the fallacies and experimental difficulties which are inevitable in such a procedure. The bacteriological and serological data available with regard to the parasite and the host are inadequate to describe the total immunising efficiency. For example the 'Vi' antigen of the S. typhi when subjected to heat, changes in its ability to give rise to anti-Vi protective antibodies in the rabbit but not in mice (Spaun, 1957). The ability of the 'Vi' antigen to combine with the specific antiserum, however, remains intact (Spaun, 1957). The problem is made more difficult by the complex nature of virulence which is dependent on the summation of a large number of attributes of both the parasite and the host.
Analysis of the phenomenon associated with recovery from infectious disease and also those following active immunisation with the attenuated organism during the Pasteurian period, revealed in the serum a variety of antibodies causing agglutination, precipitation or phagocytosis of bacteria. It was also early recognised that heat killed bacteria are as readily agglutinated by antiserum as the living organisms and that they are also capable of producing specific antibodies. Pfeiffer & Kolle (1896) demonstrated that injection of killed bacteria was followed by a rise in the lytic antibodies in serum. This correlation and confusion between the development of resistance and the production of antibodies gradually led to the belief that protective immunity can be measured by estimating the agglutinins, precipitins, bacteriolysins and bacteriotropins. The Vidal test, which made use of the agglutination reaction, was introduced as a diagnostic procedure but imperceptibly took on a quantitative function and weighty inferences were sometimes drawn from the agglutination titre. The employment of the agglutination titre as the criterion for the overall immunological response may largely have been aided by the simplicity and quickness of the technique. This would also explain the tendency among clinicians to base prognosis upon the '0' titre.

That this attitude was not wholly unjustified is proved by subsequent observations. Felix (1924), demonstrated the association of the small granular type of agglutination produced by somatic antigen, with the production of immunity. Subsequently, the '0' antigen has been identified as the endotoxin and been isolated.
in a chemically pure form. It has also been conclusively proved that it gives rise to a specific antibody which counteracts the toxin (Morgan, 1941 and Morgan & Partridge, 1942). The value of the anti-0-antibody in protection has been adequately substantiated by the results of animal experiments (Schutz, 1930) and extensive epidemiological analyses (Callender and Jaippold, 1943).

But in the light of more recent knowledge, it is strange that the mere development of antibodies as a physiological response to an antigen, should have been regarded as practically synonymous with a state of protection. It is now being increasingly appreciated that the agglutination titre does not form a faithful index of the degree of resistance. The Widal titre is known to decline after recovery. Aldershoff (1916) has estimated that 63% of typhoid recoveries, lose the agglutinins by the 7th month. The immunity acquired, however, persists for much longer periods as is evidenced by the low frequency of second attacks. Similarly, healthy carriers who give a negative Widal reaction are on record.

These facts suggest the existence of some hitherto undetected mechanism by which the animal is protected in the absence of detectable antibodies. The limitations of the available techniques in immunology, have permitted its development along somewhat narrow channels. But with the recognition of the chemical nature of different antigenic components, quantitative measurements have become more feasible. At the present state of knowledge, the development of solid immunity can best be measured by protection tests in animals. The variability of individual animals does complicate the evaluation of the results but this fallacy can be eliminated by the use of larger numbers of animals.