PART FOUR

SUMMARY AND CONCLUSIONS.
The occurrence of relapse, and persistence of carrier-state in spite of adequate therapy with chloramphenicol, remain the two outstanding problems in relation to typhoid fever. With this disease persisting as endemic foci in many urban areas in this country and with seasonal increase to epidemic proportions, these still remain important therapeutic and epidemiological problems.

The present investigations were undertaken to study some of the probable mechanisms involved in these seemingly inter-related problems of persistence of viable organisms in one or more foci. There were practical difficulties of studying adequate numbers of human cases from the early stages of the disease. The number of bacteriologically proved cases which could be studied longitudinally early from the onset of the disease, was unfortunately limited. It was, therefore, of necessity rather than of choice, that the major part of the investigations were undertaken in experimental animals.

The bulk of the study deals with different aspects of immunity in experimental animals and efficacy of chloramphenicol in eradicating organisms from the tissues together with an analysis of brief clinico-pathological data from 23 bacteriologically proved cases of typhoid fever.

Two locally isolated strains of S. typhi known to produce high agglutination titres in patients have been employed for most of the experiments dealing with the development of agglutinins in rabbits. For protection tests, a strain of S. typhi-murium (H.T.C.C.) has been
used for the production of immune sera in rabbits and for the tests in mice.

Both heat-killed and live antigens have been used for immunisation in different dosage schedules. The 'H' and 'O' titres were tested by means of formolised and alcoholised suspensions respectively and the 'Vi' titre with a live suspension.

Experiments were devised to test the effect of chloramphenicol on the antibody-formation by administering the drug before, during and after the immunising doses.

With the three-injection schedule of immunisation using killed vaccine, chloramphenicol administered at different stages, did not significantly alter the development of 'H' and 'O' antibodies.

When a single injection of the heat-killed organisms was given after priming the animals with chloramphenicol or administering it subsequent to the injection, the antibody titres were generally lower and recorded a rapid decline when compared to the previous experiment. However, the results within the group did not indicate any significant effect of chloramphenicol on the formation of antibody compared with the control.

Using live organisms in the three injection schedule, administration of chloramphenicol from the very first day did not materially affect the antibody titres against 'H', 'O' and 'Vi' antigens, when compared with the control groups. However, when smaller numbers of live organisms were injected for immunisation, and chloram-
phenicol was administered from the first day, there was almost complete suppression of agglutinating antibodies. The findings of those groups of experiments have been discussed.

A wide variability of the titre of agglutinins was recorded in the individual animals within the various groups.

The data from bacteriologically proved cases of typhoid fever treated with chloramphenicol indicated that in spite of considerable individual variation, chloramphenicol administered from the 3rd, 6th, 7th, 8th, 10th and 11th days of the disease, did not influence the development of 'H', 'O' and 'Vi' agglutinins to any significant degree. In fact, the agglutinin titre in some cases treated early was quite high. On the other hand, some of the individual cases showed a persistently low titre irrespective of the day when therapy was started.

The effect of chloramphenicol on total immunity was studied by active protection tests. Chloramphenicol was administered on different days to groups of mice injected with a live culture of S. typhi-murium. On challenging these groups with a subsequent dose of the live organisms after immunisation, mortality was highest in the group in which the drug was administered from the first day of the immunising dose. The death rate was comparable in the other groups with that of the control group.

When sera from rabbits immunised with three injections of heat-killed organisms with or without simultaneous chloramphenicol admi-
nistration were tested for their efficiency in protecting mice after a challenge dose of S. typhi-murium, it was observed that both sera were equally protective. But, when pooled serum from animals immunised with a single small dose of live organisms and treated with chloramphenicol was used, it exhibited no protective power.

Experiments to study the effect of the drug on antibodies themselves both in-vitro and in-vivo were also undertaken.

When immune sera were incubated at 37°C with chloramphenicol for variable periods, no difference in the agglutinin titres could be detected in the control and test samples of as many as 15 sera.

After the agglutinin titre of two groups of immunised rabbits was ascertained, chloramphenicol was given for 7 days in one of the groups. The sera were subsequently tested in the two groups and the fall of titre was found to be comparable. It was, therefore, concluded that chloramphenicol did not have any effect upon the agglutinating antibodies. Other theoretical objections to this probability have also been recorded.

The possibilities of alterations of the bacterial characters such as their morphology, biochemical behaviour and, more important still, their antigenic components under the influence of chemotherapy were investigated. When the organisms were brought into temporary contact with effective concentrations of the drug for variable periods, certain morphological changes were observed. The antigenic properties, however, did not manifest any alteration.

When the organisms were kept in more prolonged contact by training them to grow in ascending concentrations of the drug incorporated in the growth medium, the 'Vi' agglutinability was condo-
rably diminished without any change in the 'H' or 'O' agglutinability. Antigenically these induced variants behaved in the same way as their predecessors. There was no alteration in the morphological or biochemical behaviour.

In the absence of any evidence that introduction of the drug altered the host-parasite relationship to the disadvantage of the host in the development of immunity, except when the drug can be given very early in the course of infection, an attempt has been made to study the probable cause of persistence of infection in certain foci in spite of chemotherapy.

By intravenous administration of S. typhi it was possible to establish persistent localisation in the gall-bladder in a large percentage of injected rabbits.

Attempt at isolation of the organism from the spleen at the time when they could be freely isolated from the gall-bladder gave negative results.

Large doses of chloramphenicol administered for 10 days failed to clear the gall-bladder of the organisms.

In the subsequent experiments two possibilities for the persistence of the organisms in the gall-bladder in spite of adequate chemotherapy were explored, i.e. that the bile inhibited the biological activity of the drug or else that the drug is present in the gall-bladder in an inactive state.

In vitro studies in which different concentrations of the drug were incorporated in a bile medium and subsequently tested for their bacteriostatic activity revealed that, apart from slight reduction in the diffusibility which could be attributed to the viscosity,
bile per se did not interfere with the biological activity of the
drug.

The chloramphenicol content of the bile was estimated by both
chemical and microbiological methods at intervals of 1, 2 and 4 hours
after administration of the drug to several groups of rabbits. The
results indicated that although some proportion of the chemically es-
timated drug was present in an inactive form, an inhibitory concen-
tration of biologically active form was present in the gall-bladder.

In another group of experiments it was possible to demonstrate
viable organisms in the bile of animals containing chloramphenicol
in significant concentrations estimated by the chemical method. Ac-
tual microbiological assay was not feasible in the presence of viable
organism in the bile but from an analogy of the previous experiment,
it was presumed that adequate concentration of biologically active
form was present.

These results suggested that the persistence of the organisms
in the gall-bladder in presence of biologically active form of the
drug was attributable to an inherent short-coming of a primarily
bacteriostatic drug.

This was to some extent corroborated by the results of an in-
vitro study when the growth pattern in the presence of a bacterios-
tatic and a bactericidal antibiotic was compared in the presence of
bile. While there was rapid eradication of S. typhi from bile in
the presence of penicillin the bile becoming sterile in 24 hours, the
organisms incubated in bile with and without chloramphenicol could be recovered even at the end of three weeks.

From the results of this study it is possible to draw some definite and other tentative conclusions and also to suggest some possibilities where limitations in the existing methodology did not permit a more positive approach.

There is no evidence to indicate that chloramphenicol in adequate therapeutic concentrations interferes with the production of demonstrable antibodies, in the presence of adequate antigenic stimuli. The wide individual variation in the titres of antibodies in response to S. typhi antigen is regarded as a normal biological phenomenon. Chloramphenicol does not appear to have any significant effect on the immune bodies themselves so as to alter their properties of agglutinability. When organisms are kept in a chloramphenicol milieu, apart from morphological changes after a temporary contact and decrease in the 'Vi' agglutinability after growth in a chloramphenicol medium, the drug is unable to effect any biochemical or antigenic alterations.

When administered early, the drug is able to suppress antigenic stimuli by limiting the bacterial population. Such suppression of bacterial multiplication may seriously affect the overall total immunity as has been demonstrated by the active protection test. In the complex drug-host-parasite relationship it seems possible that with a proper time-dose manipulation the drug is able either to sup-
press the infection without adversely affecting host reaction or, along with suppression of the bacterial population, to inhibit the immunological response of the host. Whether it is possible in natural infection for such a situation to occur in which the drug is administered so early in the course of infection as to effectively reduce the bacterial population to a degree where adequate antibody stimulation is inhibited is debatable. The natural history of typhoid infection does not suggest such a possibility. On the other hand in an inherently poor reacting host such a suppressive effect at any stage of the clinical disease, especially during the early stages, may serve to inhibit adequate immunity response and ultimately lead to the persistence of organisms in one or more focal inspite of adequate treatment with an antibiotic which is essentially bacteriostatic.

It has been possible to produce persistent gall-bladder carriers in rabbits injected intravenously. While sterilisation of the blood stream and also the spleen has been successfully achieved with chloramphenicol, prolonged and high doses of the antibiotic failed to clear the gall-bladder.

Adequate concentrations of biologically active form of the drug were shown to be present in the gall-bladder. There was no evidence to suggest that the biological effectiveness of the drug was being interfered with in any way by the physico-chemical properties of the bile itself. Some possible causes of the persistence of the organisms in the presence of active concentrations of the drug can only be postulated.
The persistence of organisms in spite of adequate concentrations of the drug may be due to either drug resistance, or the parasites being inaccessible to the drug or their being in a state of low metabolic activity in which they are physiologically insusceptible to the drug. Also, as in the case of a bacteriostatic drug, the natural immunological processes may fail to eradicate the infection.

In the absence of any demonstrable drug resistance of such organisms, it is possible that under the impact of chemotherapy the organisms are reduced to a state of altered metabolic activity. In the absence of adequate interplay of cellulo-immoral defence mechanisms, the organisms persist in this state. They may resume as soon as the drug is withdrawn. Structurally and functionally the gall-bladder cannot be regarded as a suitable site for the action of immunological processes essential to eradicate the infection even when the multiplication of the organisms is effectively reduced by a bacteriostatic drug. Within the animal body it is by no means certain that microbial units are incapable of surviving for long periods without multiplication. When such a survival without multiplication occurs it may be possible that the host fails to eradicate such microbial units. There are reasons also to believe that certain organisms especially in the intracellular situation have this capacity of persisting over long periods within the host without multiplication.

Thus the problems of relapse and a persistent carrier-state in typhoid fever must be intimately related to the immunity mechanism.
of the host. A real solution of the fundamental problems relating to host-parasite relationship cannot be achieved till such time as knowledge and technical resources permit a better and more objective assessment of the nature and type of immunological processes associated with the development of resistance in different organs and tissues of the host.

The results of this study tend to suggest that failure of chloramphenicol in preventing relapse and carrier-state cannot be attributed in most cases to its unfavourable effects on the development of immunity in typhoid fever. It is true that it can inhibit immunological response by suppressing the bacterial population to an effective degree. With the available evidence, it is unlikely that such a situation can occur in the treatment of the majority of clinical cases of typhoid fever. Relapse and/or carrier-state probably occur in individuals who are biologically poor reactors to specific immunological stimuli. Chloramphenicol offers a chance of recovery to the majority of such individuals with inadequate immunity. They are more likely to fall victims again to relapse as soon as the drug is withdrawn, or become temporary or permanent carriers. The inadequacy of an essentially bacteriostatic drug to completely eradicate infection under such circumstances has to be recognized. The in-vitro comparative studies with penicillin, strongly suggest such limitations of a purely bacteriostatic drug. Unfortunately neither penicillin nor other known bactericidal drugs have proved successful in combating infection in the clinical trials.
These observations, therefore, suggest that relapse and carrier state can only be effectively prevented either by developing means to increase the antibacterial immunity in the host and making the factors responsible for such immunity available in all the tissues, or by employing bactericidal drugs which even in-vivo would invariably carry the drug-parasite relationship to completion and lead to eradication of the organisms from all foci.