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BACTERIOPHAGE-TYPING AND EPIDEMIOLOGICAL PROBLEMS*

BY

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The name of Edward Jenner is so closely associated with smallpox and cowpox and calf lymph, with dramatic episodes such as the successful vaccination of James Phipps, and with the long and acrimonious arguments which since that day have ranged round the subject of vaccination in general, that one is apt to feel a memorial lecture which is unrelated to these matters fails in its purpose. However, this is far from being the case. It is by no means improbable that this versatile scientist and naturalist, whose activities covered a wide range of subjects, was more interested in his ornithological observations than in his excursions into the realms of virus disease, and had more inner satisfaction in being the first to observe the young cuckoo "murderously evict the rightful tenants of the nest" than in his discovery of how to prevent smallpox. We can be sure that a curious and questing mind such as his would have found more than a little to interest him in the subject I have chosen for my lecture.

One of the principal tasks of an epidemiologist entrusted with the investigation of an outbreak of infectious disease is to locate its source and to plot its spread, so that effective steps can be taken to arrest its progress. In the days of clinical diagnosis this was a haphazard process except when the signs and symptoms of the disease were so striking that no mistake could be made. Inevitably, the symptomless carrier was missed. The advent of methods which enabled the causative organism to be identified in cases of bacterial disease added greatly to the precision of such investigations, though even at this stage the position was far from satisfactory. In typhoid fever, for instance, the evidence linking a remote carrier with an outbreak was often tenuous, and frequently it was impossible to say with certainty whether any particular carrier was or was not responsible. The position has been completely changed by the discovery and application of bacteriophage-typing, which enables a number of distinct "lines" or "races" or, as they are usually called, "types" of otherwise indistinguishable typhoid bacilli to be identified with certainty. The number of types of *Salmonella typhi* now described is 29, and the test by which they can be recognized is one of great delicacy and accuracy. The ability to identify these types goes far to narrow down an investigation and makes it possible to reach conclusions which previously were quite unjustifiable.

This point is well illustrated by an interesting example which was, as it happens, the first practical application of phage-typing in this country. A supply of the very limited range of type phages then available had been sent to me by Dr. Craigie, and the technique of using them had just been mastered, when a naval colleague who, like myself, was working with typhoid bacilli came to me in some distress because his technician had developed a severe attack of typhoid fever which he feared might be a laboratory infection. The man's movements in the previous few weeks introduced various complications. He came from Edinburgh to join the staff of the Royal Naval Medical School on December 7, 1938. When in Edinburgh he had been working in a laboratory which at that time was investigating typhoid bacilli isolated from an outbreak at Hawick. He returned to Edinburgh for his Christmas vacation from December 22 until January 3, 1939. The first symptoms appeared on January 14. Where had he picked up his infection? By a happy chance, an answer to this query was found when the various strains of *Salm. typhi* were typed. The Hawick organism had peculiar characters and was at the time regarded by Craigie as a variant of Type A. The only three cultures kept in the naval laboratory proved to be Types A, E, and F. The strain recovered from the patient was Type C. He had therefore acquired his infection privately, presumably when on holiday.

This incident occurred early in 1939. A great expansion in the scope and in the application of this technique has taken place since then. Many new types of typhoid bacilli have been discovered, and typing schemes have been evolved for several other bacteria.

LABORATORY ASPECTS OF PHAGE-TYPING

Phage-typing and Antigenic Structure

All "smooth" bacteria have an O, or somatic, antigen which is of considerable stability, being resistant, within limits, to the action of heat, alcohol, and acid. Certain members of the *Salmonella* group have a second antigen, best exemplified in the Vi antigen of *Salm. typhi*. This is altered by heat and by alcohol, and destroyed by a concentration of acid which does not affect the O antigen (Felix and Pitt, 1936). An identical Vi antigen is present in *Salm. paratyphi* C and in certain members of the coli group. Felix and Pitt describe a similar or at least a related type of antigen in *Salm. paratyphi* A, *Salm. paratyphi* B, and *Salm. typhi-murium*.

The relationship of certain phages and O antigens has long been recognized. When the Vi antigen of *Salm. typhi* was identified by Felix and Pitt (1934) various workers began to study the reaction of phages against bacteria endowed with this antigen. The most fruitful

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researches were those of Craigie and Brandon (1936), who isolated four different phages active against Vi-containing *Salm. typhi* but inactive against strains containing only O antigen (the so-called W strains). They thus established the existence of Vi phages as distinct from O phages. Felix and Callow (1943) found a Vi phage active against *Salm. paratyphi* B and *Salm. typhi-murium.*

Two systems of phage-typing have been built round these two groups of phages. Vi-typing, mainly clear-cut and precise, is applicable to bacteria which possess Vi antigen. O-typing, less specific and less precise, is used in the case of organisms which have O but not Vi antigen.

Preparation of Typing Phages

Lysate containing phage for typing can be prepared in several ways. The simplest is to inoculate an actively growing broth culture of the organism with the phage in question and incubate until clearing takes place—usually within two hours. Any surviving bacteria are then either killed by heating or removed by filtration. The resulting clear lysate, which contains a high concentration of phage particles, is titrated by placing standard drops of a series of decimal dilutions on a plate of suitable nutrient agar previously sown with the homologous organism, and incubating overnight. The appearance of the resultant patches varies from confluent lysis, caused by fusion of many plaques, to a few scattered discrete plaques taking origin from single phage particles. For typing purposes the phage is used at the dilution which just gives confluent lysis (the so-called critical test concentration). This is likely to be somewhere between two of the decimal dilutions in the original titration. The making of pure phages for typing is a very tricky business, not to be undertaken lightly. For reliable results it is essential that they should be prepared by an experienced worker in a central laboratory specializing in this subject, and that each batch should be subjected to exhaustive tests to ensure its purity.

Vi-phage-typing

Conditioned or Adapted Phages.—Craigie and Yen (1938) were the first to observe the phenomenon which led to the development of Vi-phage-typing. They noticed that one of the four types which they isolated—Type II—was active against some strains of *Salm. typhi* but not against others. However, when this phage was tested in strong concentration against one of these recalcitrant strains sown on a solid medium, a few plaques developed. If the phage from one of these plaques was further propagated on the resistant strain it reproduced itself in such a way that a lysate with a high titre for this erstwhile resistant strain was evolved after a few passages. This new phage had little or no action on the original bacterial strains against which the parent phage was active. In this way a conditioned or adapted phage was produced, and by the selective action of this and of the parent phage it became possible to identify these two races of *Salm. typhi.* By a wide and general application of this principle an increasing number of phage types of *Salm. typhi* have been identified, and, as already mentioned, some 29 are now described, each of which can be recognized with a high degree of certainty by the use of a conditioned or adapted variant of Craigie's Vi phage Type II. Within the total of 29 are certain small groups of related types, one of five, one of three, and three of two, but these subtypes are easily distinguished from each other.

Phage-typing an Unknown Strain of Salm. typhi.—To type an unknown strain a plate of nutrient agar (the composition of the medium is of some importance) is sown with a suspension of the organism and allowed to dry. This is then spotted on marked sites with drops of a series of phage preparations, each diluted to its critical test concentration. It is time-saving to have separate phage lysates for the common bacterial types and one or more mixtures of lysates of the remaining rarer types. It is well, too, to include a patch with Craigie's Type I Vi phage, which is active against all Vi strains and hence can be used to detect W strains. The plate is incubated overnight, and next morning examined for patches of lysis. If confluent lysis is produced by one of the monovalent phages there is no need to proceed further, as this reveals the phage type of the organism; if produced by one of the multivalent mixtures it will be necessary to repeat the test with the monovalent phages of which this is made up, to identify the exact type causing the lysis. Type A is lysed by all the adapted phages. "Untypable" strains are lysed by Phage I only. W strains show no lysis at all.

Untypable Bacterial Strains.—In the early days of phage-typing significant numbers of strains containing Vi antigen were found to be untypable. Many of these have now been identified as new types, but some cannot be accounted for in this way. Inhibition of phage action in such cases is apparently due to interference by latent or symbiotic phage present in the bacillus. Certain anomalous subtypes are believed to owe their origin to the same cause. Although this is a complicated problem it affects only a minority of strains and does not detract from the general usefulness of the method.

Persistence of Phage-type Characters.—Experience has shown that, with minor exceptions, these phage types of *Salm. typhi* breed true, so that a bacterial strain maintains its type indefinitely throughout succeeding generations. It is this continuity which gives to these characters their epidemiological significance.

Typing of Salm. paratyphi A, *Salm. paratyphi* B, *and Salm. typhi-murium.*—In India, Dhayagude and Banker (1952) have isolated a phage which has a specific action on *Salm. paratyphi* A. No results have yet been published to show whether this phage is adaptable or if a typing system has been evolved. Felix and Callow (1943) have developed a method of Vi-phage-typing, similar to that used for *Salm. typhi,* which can be applied to *Salm. paratyphi* B and *Salm. typhi-murium.* Ten phage types of *Salm. paratyphi* B are now recognized (Felix and Callow, 1951). However, the interaction of these typing phages with the different strains of *Salm. paratyphi* B does not give the clear-cut and specific results which are found in typing *Salm. typhi* with adaptations of Craigie's Type II phage. Instead of being picked out by one specific phage, most of the bacterial types give a pattern of reactions with the different typing phages. Further, variations occur in the reaction pattern of several bacterial types in such a way that the picture is suggestive of the results given with O phages, which are described later. The authors of this method point out that 2 of the 10 typing phages are not adapted from the same original phage as are the others, and that the 10 phages fall into three different antigenic groups. These facts suggest that contamination of the original phage has taken place. Felix and Callow attribute these "irregularities" to the presence of "natural" (symbiotic) phages in the different strains of *Salm. paratyphi* B, which not only affect the resistance of the bacteria to different phages but render it difficult if not impossible to make pure phage preparations for typing. Despite these shortcomings the scheme is reported to work well in practice, and between 1942 and 1950 90% of the strains of *Salm. paratyphi* B isolated in Britain were successfully typed in the Enteric Reference Laboratory. No details have been published regarding the typing of *Salm. typhi-murium.*

O-phage-typing

Many unsuccessful attempts have been made to adapt O phages to different strains of an organism in the same way as Vi phage Type II has been adapted to *Salm. typhi.* O-phage-typing is carried out by using a series of phages derived from different bacterial strains. Some of these pick

out specific types of the organism. Thus, Wilson and Atkinson (1945), who evolved the scheme which is still used for typing staphylococci, found that 11 of the 21 phages they prepared were monovalent in that they lysed only one type of staphylococcus. More commonly these O phages act on several bacterial types but not on others. When cultures are tested with a series of phage preparations different patterns of reaction are given by which the bacterial types can be recognized. Such patterns of reaction are to be seen in the first three groups of "subtypes" described by Wilson and Atkinson, but are perhaps best illustrated by Lilleengen's scheme (1948) for *Salm. typhi-murium*. Hammarström (1949) has developed a similar but very complicated method for *Shigella sonnei*, and schemes have also been put forward for *Pseudomonas pyocyanea* (Warner, 1950) and certain *Salmonella* strains.

The typing of staphylococci by O phages, although it has its shortcomings, has proved to be of great practical value. Certain types—and these the common ones—are relatively constant in the pattern they give. Others are more variable, and it may be difficult to ascribe them to a definite type. Nevertheless, in any one outbreak of infection the pattern of lysis in the pathogenic strain is constant, so that it is possible in this way to link up cases and carriers.

Underlying Cause of the Phage-type Phenomenon

It has long been recognized that the phenomenon of the phage type is in some way connected with the presence in the bacteria which are being examined of phages of the kind variously known as "natural" or "latent" or, as I prefer to call them, "symbiotic." This was pointed out by Craigie (1942), who showed that "imperfect" or "untypable" Vi strains owe their peculiar characters to the presence of such a phage. Recently Felix and Anderson (1951) have found that the presence of symbiotic phage in *Salm. typhi* Type F2 is responsible for the difference between this type and F1. Further, they have isolated symbiotic phages from 11 of the 29 established types of *Salm. typhi*, and have shown that by grafting certain of these on to *Salm. typhi* Type A (which is apparently free from symbiotic phage) they were able to convert Type A into types either identical with or closely related to those from which the symbiotic phage was isolated. Nicolle and his colleagues (1951) have effected similar transformations of type in *Salm. paratyphi* B by means of phage from lysogenic strains. There can be little doubt that the phage type of an organism is bound up with some specific immunity or resistance which, in some cases at least, it derives from the symbiotic phage it carries. Typing-phages presumably function because they are endowed with the capacity of overcoming this resistance.

A Direct Method of Phage-typing

If symbiotic phage is responsible for the phage type of the organism which carries it, then typing methods which function by detecting the changes produced in the organism by the symbiotic phage must be regarded as indirect. Further, such methods may be incapable of giving a clear-cut answer when the organism contains more than one type of symbiotic phage—a state of affairs which is by no means uncommon. On the other hand, a direct approach to the problem can be made by isolating and identifying the phage or phages with which the organism is infected. This method was suggested some years ago by an unexpected finding during an investigation of the symbiotic phages of *Salm. typhi-murium*. Two cultures, each labelled *Salm. typhi-murium* Copenhagen and believed to be alike, although they were received in the laboratory at different times, were found to contain different varieties of symbiotic phage. As infection with symbiotic phage is, with rare exceptions, permanent and not subject to change, this finding was rather unexpected. Inquiry revealed that the two strains were in reality of different origin. This observation focused attention on the possibility of using the symbiotic phage as an additional characteristic or "mark" by which the bacteria could be "typed": in other words, of identifying strains of bacteria in terms of the symbiotic bacteriophage which they carry. Such a method is feasible only when an exact classification of the symbiotic phages found in a strain of bacteria (*Salm. typhi, Salm. paratyphi* B, *Salm. typhi-murium*, etc.) has been worked out and when the characters of each symbiotic phage have been defined in such a way that it can be identified with certainty and without undue delay and difficulty.

This has now been done in the case of *Salm. typhi-murium* (Boyd, 1950). For identifying symbiotic phage it is necessary to have one or more indicator strains—that is, cultures of the organism which are either free from symbiotic phage or contain one so weak that it does not produce resistance to, or interfere with the action of, the phages which are found in other strains of the organism. Two such indicator strains have been used in the work on *Salm. typhi-murium*. On these the different symbiotic phages are propagated and can be made to reveal their characteristic plaque characters. The identity of the symbiotic phages, which fall into two groups, can in most cases be established by the appearance of the plaques and can be confirmed, where necessary, by serological methods, by the thermal death-point, by reaction on different indicator strains, and by a few special tests which need not here be described. Twelve different symbiotic phages for *Salm. typhi-murium* are now recognized, and, as certain of them may occur in combination, a wide range of "marks" is provided.

With the co-operation of Dr. Parker and Dr. Mair, of the Monsall Hospital, Manchester, this method of typing has now been given a two-year trial, some 446 strains having been examined. The findings have corresponded closely with epidemiological expectations, and the method has proved to be one of great accuracy. Nevertheless it has its shortcomings. It is more laborious than the Vi-phage-typing method, involves the use of more culture media, and takes longer to put through. At best, results are not available for 48 hours, and may take three to four days. The method has been criticized on the grounds that symbiotic phages are so numerous and diverse that it is not practicable to identify them by this technique. In investigations which have now gone on for over four years, during which time bacterial strains have been received from all parts of the country, this has not proved to be a difficulty.

This method will never rival or replace the simple and exact technique used for the Vi-phage-typing of *Salm. typhi*. It is possible, though on the whole unlikely, that it may one day replace the method now in use for *Salm. paratyphi* B, which is becoming rather involved. It may well prove in the long run to be the best way of typing bacteria which do not possess Vi antigen and which are at present typed, if at all, by O phages.

EPIDEMIOLOGY

Soon after its introduction phage-typing became established as a procedure capable of affording much assistance to the epidemiologist. In 1940 the Emergency Public Health Laboratory Service adopted it as a routine method of investigation, and since then there has been a steady build-up of the organization. Subsequently, the International Association of Microbiologists has interested itself, and arrangements now exist for international co-operation. This aims at integrating the work in different countries and ensuring that comparable results are obtained. In time this plan will enable a world-wide survey to be made, from which much valuable information is likely to accrue.

Typhoid Fever

Various reports have been published of outbreaks of typhoid fever where phage-typing has helped to solve a problem which otherwise would have remained a mystery. Perhaps the best example is to be found in the account given by Bradley (1943) of a series of sporadic cases which occurred in 1941 and 1942. This is not a new story, but it will bear retelling.

The first case was notified from Hertfordshire, and attracted particular attention because the organism isolated proved to be

Type D4, a variety not previously found in this country. In a little under two years a further 22 cases occurred—18 in Buckinghamshire, two in London, one in Kent, and one in Wiltshire. In the series there were a few small groups of associated cases, but the majority of infections were sporadic and apparently unrelated. All, however, were caused by Type D4, and there could be no reasonable doubt that they originated from a common source. A careful investigation showed that the only common factor was the milk supply. This was traced through the Hertfordshire Milk Distributing Depot to a group of 13 farms in Wiltshire which sent milk to this depot. All these farms were inspected, and four were investigated in detail with negative results. The clue which led to the solution of the problem was provided by the sixteenth case of the series, which occurred in Wiltshire. When this patient was questioned it transpired that at the time of his infection he had been working in one of the suspect Wiltshire farms. Attention was at once directed to this farm, and very soon it was discovered that the farmer himself was a chronic carrier of D4.

But for the precise information afforded by phage-typing, which might be compared to the finger-prints of the criminal, suspicion would never have been focused on this particular farm, and the carrier might still have been the cause of recurring cases of infection, perhaps even of an explosive epidemic. Not the least interesting part of this story, as Bradley points out, is the revelation that milk-borne infection does not necessarily conform to the pattern of the classical explosive outbreak.

Similar, if less dramatic, accounts are to be found in the literature of different countries throughout the world, and they leave no doubt that phage-typing in typhoid fever has established its claim as a method which can be of unique value in revealing the chronic carrier who constitutes the reservoir of infection.

Wartime Experience in the Middle East

Most published accounts are concerned with the detection of typhoid fever in a stationary population. It was my good fortune to have the opportunity of using it in following the ebb and flow of typhoid infection in what was undoubtedly one of the most fluid populations which have ever existed—that of the Middle East during the three years when it was a theatre of active operations in the last war. I was armed with the limited number of type phages then available (some with a different designation from that now used), all of which had been given to me by Dr. Craigie. The bacterial strains for typing came from a wide variety of sources. The majority were from the laboratories of the hospitals of the British and Commonwealth Forces—but these were scattered throughout Egypt, Libya, Cyrenaica, Tripolitania, Malta, Cyprus, Syria, Palestine, Iraq, Iran, Sudan, and Eritrea. Other cultures came from the civilian public health laboratories in Cairo and Palestine, some from the American University of Beirut. The patients from whom the strains were isolated were members of the Allied Fighting Forces, prisoners of war, refugees, and the normal civilian inhabitants of the Middle East countries. The cultures examined were in some instances a limited selection derived from a large number of related cases. In all, 1,386 strains were typed.

These observations began at the end of 1940, and the first batch of cultures came from Italian prisoners of war, of whom we captured some 120,000 in December, 1940, and January, 1941. These men had to be hurriedly accommodated in camps in the Suez Canal Zone. Their numbers were both unexpected and embarrassing, as we were unprepared for so many involuntary guests. At first sanitary arrangements in general were unavoidably primitive, and in the circumstances we feared there might be major epidemics of infectious disease, but by good fortune these fears did not materialize. There was, however, a small outbreak of typhoid fever which developed from infection present among the prisoners when they were captured. This lasted for about two months. Without exception all cultures from this outbreak were Type G. Although this type caused no further significant outbreaks, it continued to haunt us during the next three years, and appeared sporadically in military personnel and prisoners of war. Curiously enough, it did not occur among cultures from civilian sources in Egypt, of which 50, supplied at random from the public health laboratory, belonged to Types A, B4, C, H, L, and M.

Further Outbreaks

After a free interval of about three months cases of typhoid infection again began to appear in another group of prisoner-of-war camps, and by June a major outbreak was in progress which reached its peak in July, declined in August, and came to an end in September. Two phage types, B4* and C, were responsible. Simultaneously with these typhoid cases, an outbreak of infection with *Salm. paratyphi* A started in May and continued until October, reaching a higher level than did the typhoid epidemic.

Although not strictly relevant, it might be of interest to record the inoculation history of these prisoners. Stocks of British Army T.A.B. vaccine were too low to allow it to be issued for their use at the time of their capture, nor could it be procured immediately, for at that period of the war supplies took from six to nine months from the date of requisition to get to Egypt from the United Kingdom. However, ample stocks of vaccine of Italian origin had fallen into our hands, and steps were taken to ensure that all prisoners were inoculated with this. During the summer of 1941 adequate supplies of our own vaccine were received, and thereupon the prisoners were promptly reinoculated. Whether *post hoc* or *propter hoc* it is impossible to say, but no further outbreak of enteric group fever of any significance occurred among these men, and there was a period of calm in the camps until the end of October, 1942, when a further series of prisoners arrived from Alamein.

In this interval, however, a storm blew up from another quarter. In the early months of 1942 some 80,000 to 100,000 Poles of both sexes and all ages were transferred from Russia to the care of the Western Allies. This move was carried out before adequate preparations could be made to receive the immigrants. They were shipped to Pahlevi, a Persian port on the Caspian Sea, and handed over to us there, whence they were taken to the cities of Kazvin and Teheran. Many of them were extremely ill, and some presented the unmistakable symptoms of old-world typhus fever, a fact which caused us very grave concern. It transpired, however, that these were late cases in an epidemic which was all but burnt out, and the application of the usual measures of disinfestation, although far from easy in the circumstances, soon brought it to an end. To our great relief no outbreak of typhus occurred among the civilian population. From the first there was considerable doubt whether all the cases were typhus fever, and when adequate means of laboratory diagnosis became available there was no difficulty in demonstrating that many were in fact typhoid fever. The causative organism was Type C. After a period of rehabilitation in Persia these Poles were transferred westwards, mainly to Palestine.

In the summer months of 1942 an outbreak of typhoid fever of some magnitude spread through Palestine and Syria. From the Public Health Laboratories, Jerusalem, representative batches of cultures emanating from hospitals in Haifa, the Tel-Aviv/Jaffa area, and Jerusalem itself were sent for typing. Without exception all were Type C. Another batch, which also proved to be Type C, was received from the Bacteriology Department of the American University at Beirut. It is doubtful if this particular strain ever made its way across the Sinai desert. Although sporadic cases caused by Type C occurred among the troops in Egypt, and one large outbreak took place in an R.A.F. unit, it is more likely that these were related to the prisoner-of-war outbreak already mentioned.

For the first time Type D became comparatively common in Egypt. Early in 1941 a small group of four cases occurred in an R.A.F. unit, and later two cases were found

*Not now listed under this designation.

in a New Zealand regiment. The majority of, though not all, the Type D infections which appeared subsequently were either in the R.A.F. or in New Zealand troops.

The Final Phase

The next and final phase followed the Battle of Alamein. As our armies moved forward and Mersa Matruh was recaptured, we found that the Axis troops had used the Egyptian barracks there as a hospital, and that in this hospital were a large number of seriously ill men, both German and Italian, most of whom were suffering from typhoid fever. The organism with which they were infected was Type A, and from these patients and subsequent cases which occurred among the prisoners captured at the time a large number of cultures of this type reached me. This outbreak was soon checked and did not spread in the prisoner-of-war camps, among our troops, or in the civilian population. I might say that, with our previous experience in mind, all these new prisoners were promptly vaccinated with British Army vaccine.

It would be a mistake to imply that the information gained from typing these strains was of much practical value to the epidemiologists. The steady inflow of unseasoned and inexperienced troops into the Force, the constant movement of troops within the area, and the need to make the utmost use of man-power, which led to the employment of prisoners of war and civilians in duties connected with the handling of food, made the tracking down and eliminating of carriers a task of impossible magnitude. The entire available laboratory resources, directed to this subject alone, would have done little more than touch the fringe of it. The problem had to be tackled in other ways, and the fact that the incidence of enteric group fevers in the British Army in the Middle East for the years 1942 and 1943 was 0.8 per 1,000, a figure which compares favourably with the pre-war level, is evidence that these measures were reasonably successful. Nevertheless, the information which accrued from the typing results was of general interest and gave very clear evidence of the usefulness of this method in maintaining a check on the spread of infection.

An Unsolved Problem

A brief account of a problem which was revealed, though not solved, by phage-typing will round off this tale. In 1943 occasional sporadic cases of typhoid fever occurred among R.A.F. personnel—the figures are lost, but my recollection is that there were between 12 and 20. Without exception these were Type G, which at that time was not much in evidence elsewhere. The curious feature of this observation was that these cases were widely scattered throughout the command, one coming from as far afield as Malta. A special effort was made to discover the source of this infection, and the surprising fact emerged that, except for odd groups of two or three, these cases had no apparent association with each other, and were separated by distances which excluded all the more familiar channels of infection. The problem, so far as I am aware, remains unsolved. Our guess was that infection might be coming from a carrier at home who was engaged in making up and dispatching parcels of "comforts," but this was never confirmed. There can be little doubt that these cases did have a common source, which would never have been suspected had it not been for the phage-typing results. In less difficult circumstances it is possible that the carrier would have been tracked down and the danger of further infection from this source eliminated.

Paratyphoid Fever

Fever caused by *Salm. paratyphi* B is a serious problem in this and certain other countries, being much commoner than typhoid fever. The application of phage-typing follows the same lines and may provide an extremely useful label by which the relationship of cases and carriers can be checked.

A good example is furnished by an outbreak which occurred in Wootton, Isle of Wight, and has been reported by Wallace and Mackenzie (1947). In all, 62 persons were affected, including members of the family of the farmer who supplied milk to the village. However, although the infection was undoubtedly milk-borne, the farmer's relatives were fellow sufferers and not the cause of the trouble. All the organisms isolated were of Vi phage Type I. The source was ultimately traced to two carriers in a distant house, whence the infection passed via a cesspool (flooded by heavy rain) and a stream to a muddy field on which the cows grazed. Conditions in the farm were somewhat primitive, and it appears that the milk became contaminated from infected mud on the cows. *Salm. paratyphi* B, Vi phage Type I, was isolated from the carriers and from various points along the channel of infection—the cesspool, the stream at various points, and the mud. It is true that most epidemiologists would regard the isolation of the causative organism at these points as sufficient evidence, but the confirmation given by phage-typing clinched the matter in a very convincing way.

Salmonella Typhi-murium

Up to date little has been done in the way of typing *Salm. typhi-murium*, although there are instances on record where, in the case of an outbreak of food-poisoning, the test performed by the Vi-phage method of Felix has been useful in confirming that the strains isolated from the patients and the suspected food were identical. It has been stated that the possible sources of infection with this organism are so numerous and heterogeneous that typing does not offer much assistance to the epidemiologist. It is doubtful if this is a valid criticism.

During the last two years most of the cultures isolated in the Manchester area have been typed by the direct method which has already been described. Now that the reliability of this method has been established it is possible to draw conclusions which would not previously have been justifiable. In one hospital ward sporadic infection without an explosive outbreak has been occurring for over a year. Sixty-two strains were isolated in 1951, and all were of the same "mark." The majority were from infants or young children in hospital or recently discharged, but some were from secondary cases among associates outside hospital, and eight were excreters among home contacts. The typing results were 100% consistent. It was felt by those concerned that without this evidence it would have been difficult to accept this large and disjointed series of infections as associated cases. The origin of the outbreak is shrouded in mystery, but the subsequent transmission seems to have been by a chain of cross-infections from convalescents and excreters kept in hospital for the treatment of their primary disease. Over 30 further cases have occurred in 1952.

An outbreak due to a different "mark" occurred and died out in this same hospital in 1950, but not before infection had been transferred to another hospital. In 1951 10 cross-infections with this "mark" occurred in a cubicle ward in the second hospital.

During a period of four weeks a total of 14 sporadic cases, three fatal, due to yet another "mark," occurred in the general population of the city. No connexion was established between these cases, but there is little reasonable doubt that the infection came from a common source.

The epidemiological picture in *Salm. typhi-murium* infection is of course complicated by the fact that this organism has a wide host range, being found in many animals, such as cattle, horses, dogs, rats and mice, hens, turkeys, and ducks. It can occur in a variety of foodstuffs, and it is possible that a comprehensive investigation might reveal an association between phage types and foods which would give some direction to epidemiological investigations. It seems probable that the typing of *Salm. typhi-murium* will assume greater importance in the future than it has in the past.

Other Salmonella Infections

Other varieties of *Salmonella* have been typed by O phages. *Salm. thompson*, for example, has been split up in this way into 11 types (Williams Smith, 1951). A large number—perhaps all—of the strains of this organism are lysogenic, and it seems likely that they could be accurately typed by the direct method—that is, by the identification of the symbiotic phage. *Salm. dublin* and *Salm. enteritidis* have been divided into six and eight types respectively (Lilleengen, 1950). There can be no doubt that, should occasion arise, any strain of *Salmonella* which became of economic importance could be typed by either O phages or by the direct method of symbiotic phage identification. These organisms are, of course, chiefly of interest to the veterinarian.

Dysentery

Some years ago an attempt was made to classify the mannitol-fermenting dysentery bacilli by means of phage-typing. This did not materialize as a practical routine procedure, mainly because it offered no advantage over the established serological method of identifying these organisms by their antigenic structure. It is doubtful if the epidemiological problems presented by infection with these organisms would be simplified by the additional breakdown which phage-typing might provide.

These remarks do not apply to *Shigella sonnei*, which has been a bugbear to the public health authorities in this country for many years. Various attempts have been made to work out a typing scheme for this organism. Hammarström (1949) has given this subject much attention and has produced a tentative scheme in which he uses 11 phages and recognizes 31 types of bacteria: actually he found 68, but regards 37 of these as unverified. It is clear from his reported results that there is a good deal of instability in the phage strains he used, and the very large number of types which he has found casts some doubt on the value of the method. Attempts at typing in this country have not progressed far. Cooper and Mayr-Harting (1951) found seven types in Bristol. They point out that the "multiplicity of types and the smallness of the epidemiological unit will make it possible to trace some of the strands in the tangle of the pathways of infection." Nevertheless, they express caution regarding the stability of the types. Much work is still needed on this subject, and so far little of practical value has emerged.

Cholera

Although a great deal of work was done in India on the bacteriophages of the cholera vibrio, no phage-typing scheme has been worked out in conformity with modern ideas. Bruce White (1937) observed that a particular "lysogenic" phage is present in most Indian strains of vibrio. Japanese and Chinese strains do not contain this phage but are lysed by it. El Tor and other non-pathogenic vibrios neither contain this phage nor are lysed by it.

Staphylococcal Infection

In staphylococcal infections, typing has proved of much assistance in tracking down carriers. It has been particularly useful in maternity wards, where, for example, outbreaks of pemphigus among newly born children may cause trouble. Several instances are now on record where, by typing the staphylococci recovered from the infants and those from nasal swabs taken from the staff of the institution, the carrier has been located and the outbreak terminated. Similar helpful results have been obtained in investigating outbreaks of mammary abscess. Staphylococci can, of course, be recovered from most nasal swabs. Without the more precise information given by phage-typing it would be impossible to pick out the carrier of the pathogenic strain from among those harbouring non-virulent cocci.

Cows often suffer from staphylococcal mastitis. Williams Smith (1948) made an extensive survey of this subject from the veterinary angle and found that 93.3% of the strains which he isolated could be typed by Wilson's phages. Although his findings did not enable him to differentiate strains which were either pathogenic or harmless so far as the cow was concerned, he made the important observation that types which have been shown to be accountable for human mastitis and food-poisoning have been found as causes of bovine mastitis, and can maintain themselves in the cow's udder for as long as six weeks. Raw milk from an infected but symptomless cow is therefore a potential source of human disease. Macdonald (1946) found *Staph. aureus* in more than 50% of samples of accredited milk, the majority belonging to a phage type commonly found in human infections. Strangely enough, practically all staphylococci isolated from the udders of sheep are different from those found in human beings.

Phage-typing has proved a useful tool for investigating outbreaks of staphylococcal food-poisoning and serves to link up the strains found in the patients, the contaminated food, and the source. A small outbreak reported by Gillespie (1947) is a typical example. A specific phage type of *Staph. aureus* was isolated from the faeces of five out of six cases of gastro-enteritis. The same type was *not* isolated from the noses of the six patients or from two food-handlers, but was found in the nose of the chef. Unfortunately, none of the trifle believed to have been the vehicle of transmission was available for culture.

Miscellaneous

Although, as already mentioned, a scheme has been suggested for typing *Pseudomonas pyocyanea*, nothing of any practical application has so far been published thereon. No workable scheme for typing streptococci has yet been devised. Unsuccessful attempts have been made by Williams Smith (1949) to isolate phages from brucella.

It is strange that no serious attempt has yet been made to evolve a scheme for typing diphtheria bacilli. Keogh, Simmons, and Anderson (1938) have pointed out that phage can be used as an auxiliary means of classification of strains of diphtheria bacilli, and have been able to show differences in gravis, mitis, and intermediate strains by this method. Recent work in Canada by Toshach (1950) suggests that adapted strains of phage for the diphtheria bacillus can be produced. A remarkable observation which is not altogether irrelevant is reported by Freeman (1951), who found that with a certain phage he could produce from non-virulent strains of *Corynebacterium diphtheriae* both a lysate containing potent toxin and symbiotically infected bacilli which were highly virulent and multiplied in this state indefinitely. The problems of the diphtheria bacillus and its related phages are without doubt ripe for careful investigation.

Conclusion

I have said enough to show that phage-typing has placed at the disposal of the epidemiologist a new and promising means of solving many of his problems, and that results of much importance and interest have already emerged from its use. I mean no disparagement of the work which has already been done when I say that a great deal has still to be learnt, both about the principles and the technology of typing and about the different ways in which it can be applied to the solution of epidemiological conundrums. This is a field of research which offers a very promising prospect.

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SAFETY-BELTS ARE NOT DANGEROUS

BY

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For the past ten years Crash Injury Research at Cornell Medical College, in New York City, has been analysing the causes of injuries in aeroplane accidents. Under the direction of Hugh De Haven, particular attention is paid to seat belts, and medical data from hundreds of crashes show that injury from safety-belts is rare. As a result seats and belts have been strengthened in military and civilian planes. The use of shoulder harness is advocated, but when this is not available pilots and passengers are encouraged to fasten their seat belts tightly. The Cornell project has been in close touch with the authorities in Great Britain, and its work has been well summarized in a recent article by Walter Tye (1952).

The U.S. programme was going well until the appearance in this *Journal* of a report on the Viking crash by Dr. Donald Teare (1951), who stated as one of his conclusions, "The immediate cause of death in more than half of the victims was acute flexion of the body over the safety-belt." The acceptance of this conclusion was immediate and its effects were widespread, at least in America. Several journals gave it prominence. The *Scientific American* in December, 1951, headed its abstract "The Dangerous Safety-Belt." Thousands of airline passengers became apprehensive and fastened their belts loosely if at all.

Results of Research

When Cornell's Crash Injury Research was started in 1942 it was popularly believed that 1,000-lb. (454-kg.) safety-belts caused internal injuries, and even cut people in two. Since that time a careful analysis of accident details and medical data in 858 crashes has shown that there is no foundation for such beliefs. The portions of the body adjacent to and supported by the safety-belt sustain less injury than any other body area. Also, the concept of acute flexion of the spine has proved to be untenable. When the body, held at the hips by a properly applied belt, is suddenly stopped there is immediate acute pivoting at the hips. The torso and the legs hinge on the safety-belt and jack-knife forward.

The 1,000-lb. belts formerly used in United States civil aircraft often broke, and now the minimum requirement for civil aircraft demands safety-belts with a holding capacity of 3,000 lb. (1,360 kg.). Careful analyses of several recent severe accidents in civil transports have shown bruises on the hips, but no evidence of serious flexion or belt injury.

The effect of seat belts was carefully studied in Germany during the war by Ruff (1941). With seat belts properly applied, just as they are now in British and American planes, he tested men in a swing that was stopped abruptly. The speed was increased gradually until the braking produced a maximum snubbing effect of 1,000 kg. (2,204 lb., about 14 G). He found that when the brake is applied the body goes through a pivoting movement with the belt as an axis. The body and head fly forward and downward, the legs forward and up. Ruff says, "The demands on the tissues and organs in the region of the belt were found to be entirely tolerable; in Test 7 a slight nausea occurred which lasted about an hour."

Cornell's Crash Injury Research has recently made a survey of 800 survivors in serious crashes in which the occupants were wearing seat belts. The belts were stronger than those in the Viking crash, most having a holding capacity of 2,000 lb. (907 kg.). There were 704 survivors with injuries in the head, 641 with injuries in the legs, and 250 with injuries in the combined areas of the lumbar spine, abdomen, hips, pelvis, sacrum, and perineum. However, injuries in the region of the belt (usually bruises) were sustained by a mere 32 survivors, and evidence of intra-abdominal injury was found among only 23 of the 800 survivors. Injuries from belts played a minor part in these crashes, in which exposure to belt injury was great because the belts were strong. It is not the belt that causes injury if the body jerks forward and strikes dangerous objects in the plane. Little evidence regarding belt injury can be obtained in severe crashes when the belt or its attachments break, allowing the victim to shoot forward into a mass of wreckage.

Analysis of the Viking Crash

With this background we are in a position to study the evidence presented in the Viking crash. Dr. Teare's article is important, as it represents one of the most comprehensive and careful studies of pathological findings in a severe accident. In his brief report he could not make the type of analysis that is employed by the Crash Injury Research investigators, and it is doubtful if he could anticipate the effect of his conclusions. Fortunately, analysis is now possible, since we have received a map of the landing-field showing the location of parts of the plane, photographs of the wreckage, and details of the necropsies of the 28 victims. We hope that Dr. Teare will forgive us if we differ from his conclusions.

The basic facts of the case are derived from evidence on the ground and in the wreckage. On October 31, 1950, the Viking aircraft crashed in a thick fog at London Airport. There were no witnesses; the stewardess and the one passenger who survived have no accurate recollections. Both were seated in the rear of the cabin. There were marks at the edge of the runway indicating that the craft hit the ground at an angle of about 20 degrees and a speed of 120 knots as judged from scars left by the propellers, though some estimates are as low as 80 knots. The plane bounced and then was airborne for about half a mile (800 metres), possibly out of control, before it struck the edge of a concrete runway with the right wing while flying in a right-wing-low attitude. The right wing was crushed and sheared off just outboard of the engine. The right tail plane and elevator (stabilizer) were also found in this vicinity. The ship cartwheeled, bounced, or slid 350 ft. (107 metres), hit a pile of "asbestos type" water-pipes, and came to a sliding stop. The aircraft caught fire; structures and bodies were badly burned and much evidence was destroyed.

The nature of the crash has an important bearing on the causes of the injuries. The first glancing impact could have caused little strain on the belts. The second and principal crash impact on the right wing must have thrown the