TRANSLATION

PHAGE TYPING OF BACTERIA

By

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# TABLE OF CONTENTS

Preface ............................................................................................................. 1

Introduction ..................................................................................................... 3
  Species Specificity of Phages ........................................................................ 4
  Type Specificity of Phages ........................................................................... 7

Chapter 1. Principles of Phage Typing of Bacteria ........................................ 11

Chapter 2. Phage Typing of Typhoid Bacteria ................................................ 17
  Vi- and O-Phages ....................................................................................... 17
  Typing Phages and Strains ......................................................................... 19
  Degraded and Non-Typable Strains ............................................................ 22
  Stability and Variability of Phagotypes ....................................................... 25
  On the Mechanism of Specificity of Phagotypes ........................................ 26
  The Process of Adaptation of Vi-II Phages to Heterologous Phagotypes .... 32
  The Practical Significance of Phage Typing of Typhoid Cultures ............... 40
  Geographical Distribution of Phagotypes .................................................. 47
  Additional Subdivision of Frequently Encountered Phagotypes ............... 52
  Method ....................................................................................................... 60

Chapter 3. Phage Typing of Paratyphoid A and B Bacteria ........................... 80
  Bacteria of Paratyphoid A .......................................................................... 80
  Bacteria of Paratyphoid B .......................................................................... 81
  Scheme of Felix and Callow ....................................................................... 82
  Typing Phages and Strains ......................................................................... 82
  Causes of the Specificity of Phagotypes .................................................... 85
  Application of the Method in Epidemiological Investigation ..................... 87
  Method ....................................................................................................... 89
  The Natural Scheme of Scholtens ............................................................. 90
  Mild Phages of Paratyphoid B Bacteria ...................................................... 91
  Typing Phages and Strains ......................................................................... 92
  Comparison of the System of Felix and Callow with the Natural System .... 98
  Method ....................................................................................................... 103

Chapter 4. Phage Typing of Other Kinds of Salmonella ................................. 109
  Phagotype Determination of Salm. Breslau .............................................. 110
  Typing According to the Principle of Craigie and Ian ................................ 110
  Direct Typing Method ................................................................................ 114
  Phagotype Determination on Other Species of Salmonella ....................... 118
The current state of the problem of the application of bacteriophages for the intraspecies identification of bacteria is presented in this monograph. The methods of phage typing of the typhoid, paratyphoid B, Salmonella Breslau, staphylococci, dysentery and enteropathogenic coliform bacteria are described in detail. The theoretical aspects of the method of phage identification (specificity of phage type, adaptation mechanisms of phages etc.) are discussed.

The book is destined for scientific workers in the field of microbiology, epidemiology and for practical physicians.
Dedicated to the Respectful Memory of
Professor
Lyudmila Yakovlevna
Kats-Chernokhvostova
PREFACE

Phage typing is a method of differentiating bacteria within a species or serotype by means of a bacteriophage.

This method has assumed the greatest importance for improving the accuracy of diagnosing infectious diseases, and the improvement of the quality of epidemiological investigations and epidemiological analysis. By determining the phagotype of the bacteria which cause the disease, it is not only possible to recognize the true source of infection but also to trace sometimes the complex pathway of the pathogenic agent from the infection source to the receiving organism, and to make a profound analysis of the epidemic flareup. Epidemiological practice has accumulated sufficient material illustrating in what manner the determination of the phagotype of the pathogenic agent was confirmed by information, obtained by the conventional method, concerning the infection source and the pathways of its transmission or how it helped to remove contradictions in the epidemiological conclusions during investigation of flareups, foci and sporadic illnesses.

It should be pointed out that phage typing does not in any way replace the method of epidemiological examination: it has the purpose of improving and supplementing the epidemiological study of diseases. Phage typing makes it possible to improve the accuracy of bacteriological diagnosis and the same time to shorten the time required for the investigation.

About 25 years have passed since it became possible for the first time to subdivide typhoid bacteria by means of specific bacteriophages.
Since that time, the sphere of application of the method of phage typing has included a large number of bacteria. At the present time, typhoid bacteria, paratyphoid A and B, Salmonella Breslau and other types of Salmonella, the plasmacoagulating types of Staphylococcus aureus, the enteropathogenic serotypes of Escherichia coli, dysentery bacteria and other microorganisms are identified by means of phages. The methods of phage typing of typhoid bacteria, paratyphoid A and of staphylococcus aureus have been standardized by the International Association of Microbiologists.

Up to the present time, numerous data have been accumulated with regard to the theory and practice of phage typing of pathogenic bacteria, which required correlation and systematization. Monographs on this problem do not exist in the Soviet Union or abroad. The absence of a systematized résumé of the data on phage typing has a negative effect on the level of scientific investigations concerning these problems in the USSR and on the successful practical application of the method in laboratories. The aim of the present monograph is to fill in, even partly, this gap and to aid the practical and scientific workers in mastering this valuable method.
INTRODUCTION

Among the methods of bacteriological diagnostics those in which intra-species typing of microbes, isolated from patients and carriers, from the environment etc., is carried out, assume evergrowing importance at the present time. The need for differentiating cultures of the same species or serotype is dictated above all by epidemiological aims. In epidemiologically connected cases, the bacteria should belong to the same types.

As we know, a species of bacteria is by no means homogeneous in its properties. The intraspecies nonuniformity may be manifested in biological, biochemical or antigen properties, in the sensitivity to sulfanilamides, antibiotica and bacteriophanges. Corresponding to this, biotypes, serotypes, etc. are distinguished within a species of bacteria. In some species of microorganisms, the number of such types may be fairly large and serve as a means of additional differentiation of cultures of different origin. For example, several tens of serotypes of hemolytic streptococci of group A are distinguished. In other species there are only a few antigenic and biochemical variants. Among the typhoid bacteria, for example, the serological types are limited to variations in the quantitative content of Vi-, O- and H-antigens, which give in all several rarely found and not always stable serotypes. According to the biochemical characteristics (behavior to xylose and arabinose) only the 4 Kristensen types (I, II, III, IV) are distinguished, with the overwhelming number of cultures belonging to the biochemical types I and II. Thus, Chomiczewski (1961a) detected
among 2002 typhoid bacteria cultures, isolated in Poland, type I in 60.8%, type II in 29%, type III in 0.7% and type IV in 0.5%. It is obvious that such a differentiation is of little use in an epidemiological sense. This is why in recent years an ever growing number of investigators turned to the phage method of subdividing bacteria.

The above indicated methods differentiate the microbes into a fairly large number of practically stable types, they are technically simple and at the same time give precise results, which harmonize with the data of the epidemiological investigation. The methods in which phages are used for differentiation of a species or serotype, have been designated in the French and German literature by the term "lyso-typing of bacteria", while in the Russian and English literature, the term "phage typing of bacteria" which we shall also use in the following, has taken root.

SPECIES SPECIFICITY OF PHAGES

The possibility of using a phage for differentiation of bacteria is primarily due to the specificity of its lytic action. Even at the time of discovery of the bacteriophage phenomenon, N.F. Gamaleya (1899) observed the specificity of action of the lytic agent and attributed considerable significance to this. The subsequent study of this phenomenon led to a practical classification of the lytic agent. At the present time, phages are known for the following genera of bacteria: Pseudomonas, Xanthomonas, Vibrio, Rhizobium, Chromobacterium, Micrococcus, Gaffkya, Neisseria, Streptococcus, Lactobacillus, Corynebacterium, all genera of the family Enterobacteriaceae, Brucella, Pasteurella, Bacillus, Clostridium, Mycobacterium, Streptomyces, Nocardia (M. Adams, 1961). Only spirochetae and pneumococci seem to be free of phages.
As we know, the lytic effect of the phage begins with the adsorption of its particles on the surface of the microbe cell. Then follows the penetration of the deoxyribonucleic acid (DNK) of the phage into the cell, the phase of latent proliferation (latent period) and the phase of the escape of the particles from the cell.

Even d'Erelles already demonstrated that bacteria incapable of adsorbing phages cannot serve as a host for its reproduction. The adsorption of the phage on the bacteria depends on the chemical and physical properties of the medium, the nature of the phage, the physiological state of the bacterial cell, but mainly on its antigen structure. The range of hosts for a phage is mostly limited to one bacterial species or type although exceptions from this rule are frequent. Once again the lytic activity of the phages with regard to other species and types of bacteria is correlated primarily with their antigen structure. Thus, in the experiments of Burnet (1930) the same phage lysed Salmonella with general O-antigens. The loss of O-antigen during the transition from the smooth to the rough from was accompanied by a loss of sensitivity of the Salmonella to this phage.

The connection between the specific effect of the bacteriophage with the antigen structure of the lysed bacteria is also confirmed by immunochemical data. As we know, the specific polysaccharide of the microbe extracts is capable of inhibiting the lysing activity of a homologous phage (Levine, Frisch, 1934).

Phages whose action could be entirely connected with the peculiarities of the antigen apparatus of the cell, found application for the more detailed study of the antigen structure of some related groups of microbes. Thus, Levine and Frisch (1936) through testing the phage sensitivity of strains of Salmonella cholerae suis discovered antigen differences between them. These differences were confirmed by antigen
analysis and helped to detect the $6_1$ and $6_2$ somatic antigens in the C-group of Salmonella.

Phages which specifically lyse a certain species of microorganisms, are most frequently employed in practice for diagnosis. Even Sonnenschein (1925, 1928) already made an attempt to utilized the species specificity of phages of different groups of microbes for differentiating one serotype of Salmonella from another (in particular, Salmonella Breslau from paratyphoid B and typhoid bacteria). Later on, the D phages of Sonnenschein were used with the same aim by other authors (Marcuse 1934, a, b). The concentration of the efforts of researchers in the search for highly specific diagnostic phages led to the discovery of the O-phage, which is active for bacteria of the genus Salmonella (Felix, Callow, 1943), (Cherry, Davis, Edwards, Hogan, 1954), (Wassermann, Saphra, 1955). The same diagnostically effective Salmonella O-phage was obtained in the USSR by V.A. Kilesso (1960) from polyvalent Salmonella Breslau bacteriophage of the Gor'kiy Institute of Vaccines and Sera. Both diagnostic O-phages have a wide range of action on Salmonella and are practically inactive with regard to nonpathogenic representatives of enteric bacteria. At the present time the reactions with these phages are used as a simple auxiliary method of identifying cultures of the coliform group.

V.D. Timakov and D.M. Gol'dfarb (1955, 1962) worked out an original and specific method of phage diagnostics using the reaction of increase in the titer of the phage. This method is now widely used for diagnosis of typhoid, dysentery, plague, cholera, brucillosis etc.

The advantage of phage diagnosis consists in the fact that it requires several days less than the conventional biochemical and serological methods and does not always require the isolation of a pure culture of the bacteria and, finally, is technically very simple and
practicable. It must be pointed out that even if highly specific phages are used, phage diagnostics cannot completely replace the biochemical and serological methods of culture study because the specificity of the phage is not absolute.

TYPE SPECIFICITY OF PHAGES

In addition to observations of a close interrelation between antigen structure and phage sensitivity of bacteria, data have been accumulated, indicating that these characteristics do not always fully coincide. In experiments of Burnet (1930), for example, serologically identical smooth forms of some type of Salmonella differed in their phage sensitivity. These differences were also retained during the transition of the cultures to the rough form. The author also observed, that the appearance of phage resistance did not correspond to changes in the serological properties in all cultures. The absence of a connection between phage sensitivity and serological characteristics was demonstrated on the Flexner dysentery bacteria by A.G. Leonova (1947), on typhoid bacteria by Craigie, Ian (1938), on paratyphoid B bacteria (Felix and Callow, 1943) and others. All these facts gave a new direction to the research concepts. The study of phages, whose activity with respect to bacteria identical with regard to antigen characteristics, was different, led to the elaboration of a new method of intraspecies typing of bacteria - the method of phage typing.

The method of phage typing arose as a purely empirical method, and its theoretical basis was given only later. The nature of the phenomenon of type specificity proved to be different from the species specificity. The latter is based mainly on the specific tropism of the phage to strains with a certain antigen structure and on its capacity to be adsorbed on certain receptor regions of the host cell. In most cases the bacterial antigens are either identical with these regions
(in the phage sensitive species) or, conversely, prevent the adsorption of phage on them purely mechanically (in the phage resistant species). Adsorption is essential in the phenomenon of type specificity but is not the sole condition of interaction between the lytic agent and the bacteria. Cultures which adsorb a phage, do not always reproduce it (Craigie, 1939).

Two causes of the appearance of resistance to phages in bacteria can be distinguished: mutation and lysogenization (M. Adams, 1961). As we know, mutation is a hereditary change in some properties of a cell. Mutations often occur spontaneously. The phage resistant mutants of bacteria are easily detected when a culture is inoculated on agar with an excess of phage. The phage plays the role of a selective agent, by annihilating the sensitive species. The surviving specimens multiply and give colonies of phage-resistant mutants. Such variability can in some instances be accounted for by a loss of the receptor (of an antigen or nonantigen type) which adsorbs the phage, in other cases by the appearance of a substance which envelops the receptor and prevents phage adsorption. Sometimes the mutants lose the capacity of synthesizing the adsorption stimulators (cofactors), for example, tryptophan (Anderson, 1946; quoted according to M. Adams, 1961) or proline (Volman, 1947; quoted according to M. Adams, 1961). Mutations may lead to a transformation of phage-resistant forms to phage-sensitive ones. Such mutations have been studied less intensively owing to the difficulty of isolating them (absence of selective agents).

Another way for a culture to acquire phage resistance is a transition to a lysogenic state. By a state of lysogenicity must be understood the presence of mild phages in the inactive form, which has been termed prophage. The lysogenicity is detected on the basis of the capacity of the microbes to form so-called mild phages, which in contrast
to the virulent types can impart lysogenicity to the cell. This process is termed lysogenization. During lysogenization, the nucleic acid of a mild phage penetrates into the cell and without causing it any harm, goes over into the prophage state. The prophage is bound to the genetic material of the cell and is transmitted to its progenies. Hence, every cell of a lysogenic culture carries, in the form of non-infectious units (prophage) the genetic information essential for phage formation. It is assumed that the prophage is localized in a certain point of the bacterial chromosome and is the sole determinant of lysogenicity. The phage, isolated from lysogenized bacteria, is identical to the phage which was used for its lysogenization.

Under normal conditions, a lysogenic bacterium is protected against the lytic action of external phages, which are serologically identical with or related to its prophage, although it also may adsorb them. This phenomenon has been termed the incompatibility phenomenon or phenomenon of immunity of the lysogenic bacterium. Not being lysed by related phages, such a bacterium react to infection with a heterogeneous mild phage like a nonlysogenic bacterium. In other cases, lysogenization is accompanied by resistance to certain heterogeneous mild phages. This phenomenon has been termed prophage interference. Thus, Bertani (1953) lysogenized a culture of Sh. dysenteriae with mild E. coli phage P2, which prior to lysogenization had been sensitive to the phage P2 and to 7 types of T-coliphages. Following lysogenization with P2 phage, the culture acquired resistance not only to the latter, but also to 3 coliphages (T2, T4 and T5).

The mechanism of the appearance of resistance in a culture under the influence of lysogenization is not yet clear, but it is specific, because cells, which are immune to some phages, continue to be lysed by some others. After lysogenization the bacterial cells can retain
the ability of adsorbing mild phage which has penetrated into them, although this ability is sometimes lost (Uetake and others, 1955).

It must be supposed that under natural conditions of formation of the resistance and sensitivity of cultures, lysogenization as well as the selection of mutants and changes in the antigen structure are important. The leading role, however, is played by lysogenization.

Lysogenicity is an extraordinarily widespread state in the world of microbes. Thus, for example, the number of mild phages for Salmonella is counted in tens and even hundreds and a single bacterium can separate several different types of phage. A constant exchange of mild phages takes place between lysogenic bacteria. As a final result, the cells can acquire multiple lysogenicity, replace one phage by another and free themselves of lysogenicity (Boyd, 1956). Hence it is completely comprehensible that there is an unusual diversity of reactions of Salmonella with phages, and such an abundance of phagotypes within the serotypes of this genus of coliform bacteria. Conversely, in other species of bacteria, for example cholera vibrios, the number of mild phages is small: Mukerjee and others (1959) classified several hundred types of cholera phages into 4 main groups. This is the reason why typing this species of microorganisms is less promising than that of Salmonella.
Chapter 1

PRINCIPLES OF PHAGE TYPING OF BACTERIA

There are two possible approaches to the phage typing of microorganisms. In the first case, the microbes are classified into phage-types on the basis of the qualities of the mild phages isolated from them, in the second, on the basis of the sensitivity to specific (standard) bacteriophages in a certain dilution.

The first method of typing is based on the unusually widespread occurrence of lysogenicity among pathogenic bacteria. The mild phages are detected on sensitive cultures which are called indicator cultures. Investigations showed that in presence of the necessary test series of indicator strains it is possible to detect lysogenicity in 80-100% of Salmonella, coliform bacteria and staphylococci. Burnet (1932) and independently, Boyd (1950) found that mild phages of strains of Salmonella Breslau of different origin differed qualitatively from each other with regard to the range of lytic activity, thermal stability and serological characteristics. The prophages are transmitted by heredity from the parent to the daughter cells and thus, serve as "indicators" for the bacterium. Boyd proposed to use this fact for typing. The author termed this method the direct method of phage typing, because by its means the direct identification of the phage which exists in the cell in the form of prophage is achieved. Determination of the peculiarities of the phage "indicator" make it possible to recognize epidemiologically related strains of bacteria, and to trace the origin and distribution of infection flareups, because cultures from connected
cases of illness and bacillus carriers produce the same mild phage. The direct method of typing bacteria and its modifications proved convenient for the subdivision of Salmonella, some serotypes of enteropathogenic coliform bacteria etc. As will be pointed out later on, however, this method is laborious and slow which limits its practical applicability to a certain extent.

For the majority of microorganisms, the second method is used—the differentiation of cultures on the basis of their phage sensitivity. The Canadian microbiologists Craigie and Ian reported in 1938, that serologically identical typhoid cultures were not uniform with regard to their reactions with Vi-phages (phages which lyse cultures containing Vi-antigen) (see further on). For subdividing the cultures, the authors utilized the capacity of one of the Vi-phages (Vi-II) to adapt easily to a resistant Vi-strain and thus to lose instantly the activity to the original Vi-strain. All the phages derived from the Vi-II strain in the corresponding dilution lysed, as a rule, only strains of homologous type (homologous cultures are termed those, on which the given phage had been bred; heterological are cultures of other types).

The methods of Craigie and Ian were used for typing many microorganisms of paratyphoid A and B bacteria, some species of Salmonella, plasmacoagulating staphylococci, corynebacteria etc.). The Vi-II phage proved to be unique in its unusually great adaptive capacity, which possibly is innate only in Vi-phages and is unattainable by O-phage, differing in their serological characteristics and the spectrum of lytic activity are used for typing the above-enumerated species of bacteria. Such phage may lyse several phage types. The phagotypes differ from each other in these schemes by the combinations of the phages which lyse them.
The selection of phages is of great importance for the successful typing of microbes. For the purposes of phage diagnostics, phages equally active on all representatives of the species or even of an entire genus of bacteria are necessary. These requirements are met by the highly virulent races of phages. They are normally isolated from the feces of patients and reconvalescents, from sewerage effluents and from pseudolysogenic cultures. The virulent phages are not very suitable for typing owing to their wide range of lytic activity. By means of the previously mentioned phages, the strains are differentiated into a relatively small number of phagotypes which lowers the epidemiological value of this subdivision. Thus, Marcuse (1934, a, b) differentiated by means of virulent Sonnenschein phages only 6 phagotypes of typhoid bacteria.

Most useful for intraspecies typing proved to be the mild phages, isolated from true lysogenic cultures. These phages show a high specificity in their lytic effect, which helps to make apparent a fairly large number of phagotypes within a species or serotype of bacteria. In some typing schemes, such specificity is explained by the interference of the prophage, in others by the immunity of the cell to the related prophage. As mentioned previously, different prophages provide the microbes with unique "indicators", owing to which a multiplicity of phagotypes is revealed within the limits of the species or serotype. Thus, the modern scheme of phage typing of typhoid bacteria, where all the typing phages have their origin in the mild phage Vi-II, counts up to 72 phagotypes, and the scheme of typing of Salmonella Breslau, about 90 phagotypes.

Because the method of Craigie and Ian reveals the differentiating effect of the prophage indirectly, in the reactions of the cell with the typing phages, it may be termed indirect typing (Boyd, 1958).
Mild phages are not free of disadvantages. Their activity is normally not very high and is difficult to increase by passing through a sensitive culture. In the end, even on a homologous type, some preparations develop a weak lysis and their sterile spots are fairly rapidly overgrown by secondary growth. This makes the recording of results difficult and can lead to diagnostic errors. This is why in some typing schemes, where mild phages are used, the results are recorded through a magnifying glass at a magnification of 10-20x or even by means of the microscope. Anderson (1961) recommends to select, if possible, the most virulent races of mild phages for the preparation of typing phage.

The typing phages in the typing systems for microorganisms according to the method of Craigie and Ian are used in critical test dilutions (KTR). This is at most a tenfold dilution of the phage which gives a confluent lysis of a homologous strain on a solid medium. The necessity for diluting the phages is due to the fact that phages in high concentration on a solid medium can lyse heterologous phagotype nonspecifically (without phage reproduction). Such a type of lysis has been observed under conditions of high numbers of phage (up to 200 phage particles per bacterium) (Delbrück, 1940 a, b). This reaction is not connected with the function of proliferation of the phage and is evidently caused by the activity of the tail parts of the protein envelopes of the phage (D.M. Gol'dfarb, 1961). Dilution reduces the numerical preponderance of the phage and the nonspecific lytic effect disappears.

A quite separate method is that of typing bacteria on the basis of the lytic properties of bacteriocines which are substances of the antibiotic type, formed by some microorganisms. The bacteriocines were discovered by the French researchers Gracia and Frederic in the family
of coliform bacteria and were thus termed colicines (Jakob and Wolman, 1961). Later on they were detected in other species of bacteria: the pyocines in strains of pseudomonas aeruginosa, the megacines in cultures of Bacillus megatherium, the pesticines in P. pestis. The bacteriocines are not particulate and are, apparently, a lipo-carbohydrate protein complex. At the same time, many properties of the bacteriocines, in particular the specificity of their lytic action, are reminiscent of the bacteriophage, although direct indications of any relationship between these agents are lacking.

The phenomenon of colicinogenicity is extraordinarily similar to the phenomenon of lysogenicity. The capacity for the formation of colicine is a hereditary property of the bacterial strain. As in the cases of formation of mild phage, the synthesis of colicine causes the death of reproductive cells. Similar to mild phages, the colicines are detected by the formation of spots of lysis when applied the culture medium on the agar surface, inoculated with the sensitive (indicator) culture. In contrast to mild phages, an inhibition of growth is observed during the titration with colicines in dilution, but not isolated plaques. The colicines do not proliferate on a sensitive culture and their action is of a bactericidal type.

As in phages, the action of the colicines depends on the presence or absence of specific receptors on the bacteria. These can be the same for both lytic agents. The action of colicines is specific. They act primarily only on some members of the family Enterobacteriaceae. Within this family there exist colicines which act only on their own group of bacteria (for example, on Shigella). Finally, every colicine has its own typical spectrum of lytic effect on strains of the same serotype of different origin. This property is also used for epidemiological purposes.
For the differentiation of cultures, the lytic spectrum of the colicines produced by the bacteria or their sensitivity to a test series of colicines may be studied. The bacteria of Sonne's dysentery, entropathogenic coliform bacteria and other microorganisms are currently typed by means of bacteriocines.

For some species of bacteria, 2-3 typing schemes have been proposed, based sometimes on different principles. Not all typing systems are perfected to the same degree and convenient for practical application. Most widespread recognition received the methods of phage typing of the microbes of typhoid, paratyphoid B and of the plasm-coagulating staphylococci. These have been standardized by the International Association of Microbiologists.
Chapter 2

PHAGE TYPING OF TYPHOID BACTERIA

Of great importance for the decrease in the morbidity rate of typhoid and paratyphoid infections is the method of phage typing which makes it possible to improve the epidemiological analysis. The method of phage typing, permitting the subdivision of species into several tens of phagotypes, helps to trace, confirm or reject epidemiological connections in a flareup, to differentiate one flareup from another and to connect separate sporadic illnesses. A knowledge of the phagotype population provides better information on the typhoid epidemiology in a republic district or town and helps to distinguish the local cases from those brought in from other parts of the country or from abroad.

The first attempt at phage typing of typhoid bacteria was undertaken by Marcuse (1934, a, b). The Marcuse phages have not found practical application. Only a detailed study of the antigen receptors and the phage typing of typhoid bacteria.

VI- AND O-PHAGES

The investigations of Felix and Olitzky (1929) showed that the typhoid microbes possess two antigens: a somatic heatresistant O-antigen and a flagellated thermolabile H-antigen, each of which, as was found later, in turn represents a complex of antigens. Felix and Pitte in 1934 discovered that strains, freshly isolated from the organism, contain Vi-antigen, to whose presence the resistance to O-serum is due, and, as they originally proposed, also the high virulence of these strains. Kauffman (1935) introduced the terms V-, VWM and W-forms for
designating strains, containing different quantities of Vi-antigen and those which so not contain it.

The Vi-antigen is extraordinarily widespread in typhoid cultures. In the organism of the patient or bacteria carrier the typhoid microbe is nearly always present in the form with the highest Vi-antigen content (R.V. Gordina and V.N. Kuznetsova, 1950, and others).

When Salm. typhi is cultured on laboratory media, the Vi-antigen content decreases owing to the fact that the microbes which gradually acquire the ability to agglutinate with O-serum, an go over via the VW form into the W-form, which does not contain any Vi-antigen.

A study of the peculiarities of the antigen structure of typhoid bacteria revealed the nature of the specificity of action of some typhoid phages. The first phage which is specific for Salm. typhi was isolated in 1932 (Sertic and Boulgacow). The works of three groups of authors were published simultaneously in 1936, who laid the basis of the study of specific Vi-phages for typhoid. The phages of Sertic and Boulgacow (1936) were divided into 2 groups according to their lytic activity. The first were strictly specific for typhoid bacteria, the second were not specific. In the experiments of Scholtens (1936) the cultures lost the Vi-antigen following treatment with specific phages. The nonspecific phages which lyse, in addition to Salm. typhi, cultures of Salm. Gärtneri and Salm. gallinarum, which have the same O-antigen as Salm. typhi, were termed O-phages. Craigie and Brandon (1936) demonstrated convincingly the complete parallelism between the Vi-agglutinability of the culture and its sensitivity to a specific phage, which they termed Vi-phage. The fact of existence of phages, specific for the Vi-antigen of the typhoid microbe was confirmed in subsequent investigations. It was found that the specific action of the Vi-phages is connected with their capacity of selective absorption.
on cultures containing the Vi-antigen.

Craigie and Ian (1937) detected 4 serological types among the Vi-phages isolated by them and other authors from cultures of Salm. typhi: Vi-I, Vi-II, Vi-III, Vi-IV. These differed from each other in their lytic capacity with regard to typhoid strains, containing Vi-antigen, in the morphology of the sterile spots and the point of thermal inactivation. Phages of the serotypes Vi-V and Vi-VI were described later (Desranleau, Martin, 1950), Vi-VII (Brandis, 1955 c) and others.

Concentrated Vi-I phage showed activity with regard to nearly all typhoid cultures, containing Vi-antigen. The sensitivity to this phage could be taken as a criterion for the presence of Vi-antigen in the culture and, owing to the high specificity of the latter, also of the typhoid nature of the strain. This opened up prospects of using this phage diagnostically (Craigie and Brandon, 1936). It is true, though, that cultures of E. freundii and Bethesda Ballerup were discovered recently which interact also with Vi-I phage (Cherry, Davis, Edwards, 1954). Because these bacteria do not contain Vi-antigen, the authors propose the presence of some substance in them, which adsorbs Vi-phages in the same way as Vi-antigen. Such cultures are rarely found in the intestine of patients and carriers and thus the Vi-I phage retains its importance as an auxiliary diagnostic test.

**Typing Phages and Strains**

In the experiments of Craigie and Ian, the range of hosts for the phage of serotype II comprised only a few cultures. At the same time, the phage displayed extraordinarily great flexibility in its lytic properties during passage through resistant cultures. By adapting to them, the phage often ceased to dissolve the former primary substrate strain. By changing the substrate strain, the type of the original phage could be transformed. By studying a large number of phages and
strains, Craigie and Ian arrived at the conclusion that types of typhoid bacteria exist which differ from each other in their behavior to the derivatives of the Vi-II phage.

These researchers provided the basis for the practical application of phages for intraspecies typing of bacteria. In 1938 Craigie and Ian described 11 types and subtypes of Salm. typhi and obtained, by adaptation of the original phage of serotype II to them, an equal number of corresponding typing phages. The phages and strains were designated by the letters of the Latin alphabet from A to J. The phage-type of the strain was established on the basis of lysis by the corresponding typing phage in a critical test dilution. Craigie and Ian demonstrated that phage differentiation of microbes isolated from patients and carriers, helped in tracing the connections between them and to eliminate the contradictions in the epidemiological conclusions.

The work of the Canadian scientists attracted the attention of some microbiologists in the USSR, Great Britain, France, Japan and Belgium. The possibility of additional subdivision of typhoid microbes seemed tempting from the point of view of an extension of epidemiological research, the perfection of phage diagnostics and the phage therapy of typhoid. The researchers became convinced of the valuable features of the typing scheme of Craigie and Ian. The simplicity of the method in combination with its reliability and the reproducibility of results earned it international recognition.

At the present time, the standard scheme of phage typing of Salm. typhi as worked out by Craigie and Felix in 1947 has been adopted. It has been adopted by the International Committee for Phage Typing of Bacteria of the Coliform Group, formed at the Copenhagen Congress of Microbiologists. The standard phages are prepared and distributed by the International Typing Center in London. The technique of typing has
been standardized. The typing of cultures of Salm. typhi is now carried out practically in all countries of the world. Differences in the result of typing in different countries are very rare.

The list of typing phages and strains increases from year to year. Twenty-four phagotypes and as many adapted phages of serotype II (Craigie and Felix, 1947) were known in 1947. In 1960, the International Center for Phage Typing prepared 72 typing phages. The reactions of the standard Vi-phagotypes of Salm. typhi with these phages are presented in Table 1 (Data of the Central Coliform Bacteria Laboratory at London, from October-November 1960).

All phages in the scheme are serologically identical, being variants of the phage Vi-II of type A.

The combination of the subtypes into groups B, C, D, E, F etc. is based on the fact that the strains with ordinal number 1 (E1, D1, C1 etc.) in critical test dilution (by confluent lysis) are efficiently dissolved by the homologous phage and also by the phages of all other subtypes of the given group. The strains of all the other subtypes of this group, as a rule, are lysed only by the homologous phages. Exceptions from this rule are rare. Thus, for example, the type C3 in critical test dilution is efficiently lysed not only by the homologous phage but also by phage C2, and the type D5 by the phages D6, D8 and D9 etc.

The phagotype A interacts with all Vi-II phages. The phagotypes B1, B2, C1 and 41 have a similar sensitivity spectrum as the type A, but are lysed distinctly and constantly only by the homologous and group phages and are therefore classified as an independent phagotype. The phage 28 was obtained from T-phage and lyses both phagotypes (T and 28).

Completing the description of the typing phages and strains it
should be pointed out that the phage Vi-II is not unique in its flexibility. Scholtens, in 1950, isolated 4 Vi-phages from water wells which had the same adaptive qualities as the phage Vi-II. Anderson (1961) reported on a similar Vi-phage. Such flexibility is not known in O-phages.

DEGRADATED AND NON-TYPABLE STRAINS

The perfection of the method of typing and discovering new phagotypes involved a continuous increase in the number of different strains. At the present time up to 80-100% of typhoid cultures are typed with standard phages. The cultures whose type could not be established, can be separated into 3 groups.

The first group are strains devoid of Vi-antigen (they are not lysed by Vi-phage and are not agglutinated by Vi-serum) and were termed the W-form. The average frequency of these strains is 5%. Nearly half the W-forms are isolated from bacillus carriers (Brandis, 1955 c). The W-forms are normally absent from test cultures. As an exception we can quote the somewhat unique water-borne typhoid flareup, described by A.Ye. Mkrtchan (1961) in Armenia, where all the cultures from the patients were isolated only in the pure W-form.

The second group are cultures containing Vi-antigen, which are resistant to all Vi-II phage preparations tried on them. They are also termed "nontypable Vi-strains".

Part of these cultures cannot be differentiated owing to the absence of a typing phage homologous to them among the selection of diagnostic phages. Completion of the missing phages makes it possible to subdivide cultures of this type successfully.

In other cases, the nontypable strains can be new, hitherto unknown phagotypes. In these cases one tries to obtain a diagnostic
phage by adapting Vi-phages of serotype II (type A etc.) to the nontypable culture. The successful attempt often leads to the discovery of a new phagotype. In other instances, the proportion of untypable Vi-strains is considerably reduced by this method. Thus, in Cambodia, 17.61% of all the cultures, isolated during the years 1957-1960 could not be typed by the 56 standard phages. Following the discovery of 4 new phagotypes there (E10, J3, M3, 37) this percentage dropped to 7.04 (Nikolle, Diverneau and others, 1960).

Finally, there are strains to which the phages of serotype II are not adapted even after repeated passage. Such types of culture are termed true imperfect forms, gamma forms, or group VI-I - VI-IV (the cultures are lysed efficiently by Vi-phages of serotype I and IV). This category of strains accounted for 5% of all cultures typed in 32 countries of the world in 1948-1958 (Nikolle, Diverneau, du Plessis, 1959).

The third group are polylysable Vi-strains, which show high sensitivity to several typing phages. When kept for long periods, they are often transformed into type A, which is lysed by all typing phages. Cultures of this kind are often termed degraded Vi-phagotypes. In the literature, dealing with the problems of phage typing the term "degradation" of strains, by the way, is used in two senses: 1) degradation as a gradual loss of Vi-antigen by the strain and transformation into the W-form via the VW-form (according to Kauffman) and 2) degradation in the sense of a loss of the specificity of the strain and its transformation into a polylysable culture.

Nicolle, Hamon and Edlinger (quoted according to Rische, 1961) recommend terming the degraded Vi cultures minus-variants of phagotype A. For example, a degraded Vi culture, which is resistant to the phages B1 and D6 is termed A-minus-B1, D6-variant. Rische claimed
TABLE 2

Standard Phagotypes of Typhoid Bacteria

<table>
<thead>
<tr>
<th>Фигурин</th>
<th>Персональное обозначение</th>
<th>Год и место выделения</th>
<th>Источник</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A Balston</td>
<td>1937, Канада 1</td>
<td>Кредки и Иен, 1938 22</td>
</tr>
<tr>
<td>B1</td>
<td>1937</td>
<td></td>
<td>H (1,3)</td>
</tr>
<tr>
<td>B2</td>
<td>1937</td>
<td></td>
<td>1937, Канада 22</td>
</tr>
<tr>
<td>В3</td>
<td>1938, Шотландия</td>
<td></td>
<td>Н (1,3)</td>
</tr>
<tr>
<td>С1</td>
<td>1937, Канада 1</td>
<td></td>
<td>1937, Канада 22</td>
</tr>
<tr>
<td>С2-33</td>
<td>T5061</td>
<td>1946</td>
<td>K</td>
</tr>
<tr>
<td>С3-30</td>
<td>T4677</td>
<td>1951, Япония 3</td>
<td>L</td>
</tr>
<tr>
<td>С4</td>
<td>1954, США 4</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>С5</td>
<td>4392</td>
<td></td>
<td>E-58-1092</td>
</tr>
<tr>
<td>С7</td>
<td>T230-55</td>
<td>1961</td>
<td>N</td>
</tr>
<tr>
<td>С9</td>
<td>2668-57</td>
<td>1967</td>
<td>T</td>
</tr>
<tr>
<td>Д1</td>
<td>T273-51</td>
<td>1961</td>
<td>T879</td>
</tr>
<tr>
<td>Д3</td>
<td>1937, Канада 1</td>
<td></td>
<td>1948, Канада 25</td>
</tr>
<tr>
<td>Д4</td>
<td>1936, Англия 3</td>
<td></td>
<td>1948, Канада 26</td>
</tr>
<tr>
<td>E1</td>
<td>1917, Россия 6</td>
<td></td>
<td>1948, Канада 27</td>
</tr>
<tr>
<td>E2</td>
<td>1940, Англия 3</td>
<td></td>
<td>1950, США 4</td>
</tr>
<tr>
<td>E3</td>
<td>1945, Канада 1</td>
<td></td>
<td>1950, Канада 29</td>
</tr>
<tr>
<td>E4</td>
<td>1944</td>
<td>1950</td>
<td>1953, Канада 26</td>
</tr>
<tr>
<td>E5</td>
<td>1954, Канада 1</td>
<td></td>
<td>1954, Канада 30</td>
</tr>
<tr>
<td>E6</td>
<td>1954</td>
<td></td>
<td>1954, Канада 33</td>
</tr>
<tr>
<td>E7</td>
<td>1954</td>
<td>1955, Канада 35</td>
<td>1955, США 4</td>
</tr>
<tr>
<td>E10</td>
<td>1960, Канада 1</td>
<td></td>
<td>1957, США 4</td>
</tr>
<tr>
<td>F1</td>
<td>1900, Англия 3</td>
<td></td>
<td>E-58-1092</td>
</tr>
<tr>
<td>F2</td>
<td>1938, Канада 1</td>
<td></td>
<td>Н (1,3)</td>
</tr>
<tr>
<td>F3</td>
<td>США 4</td>
<td></td>
<td>1938, Канада 22</td>
</tr>
<tr>
<td>F4</td>
<td>1937, Канада 1</td>
<td></td>
<td>1938, Канада 22</td>
</tr>
</tbody>
</table>

that the same A-minus variants are isolated from epidemiologically
connected cases. At the same time, the isolation of different A-minus variants does not always reliably indicate the absence of epidemiological connections between cases. Detection and identification of mild phages may also be useful for the epidemiological characterization of degraded Vi cultures.

In some instances it is very important to determine the original phagotype of a polylysable degraded Vi strain, but this is normally not possible. Anderson and Felix (1953a) tried to distinguish the phagotypes of 5 polylysable cultures, by passing through them phage A and 10 other typing phages. The authors were of the opinion that the phages undergo an evolution into a type which is homologous to the polylysable type of the precursor strain. All the phages which had been used, were either transformed into phage of type A or did not change. The researchers were convinced that for successful typing of degraded strains it is best to send no less than 12 colonies to the laboratory, because in several cases part of them retains the characteristics of the original phagotype.

STABILITY AND VARIABILITY OF PHAGOTYPES

The stability of the phagotypes during the period of flareups was confirmed by all investigators. In the course of many years the phagotype of the cultures did not change either during storage and reinoculation or in the organism of the bacteria carriers (L.Ya. Kats-Chernokhvostova, et al. 1947; N.I. Fedorova-Talashenko, 1951; Craigie, 1940; Foley, 1942), (Henderson, Ferguson, 1949, and others).

The list of observations which attest to the great stability of the typhoid phagotypes could be considerably prolonged, but there is no need for this. The method of phage typing, based on this property of the abovementioned microbes, proved satisfactory in the epidemiological investigations.
Thus, the phagotype characteristics are a persistent property of the microbe. Exceptions from this rule are possible. The variability is most frequently manifested in an increase in the range of sensitivity of the bacterium to heterological phages, including its transformation into the type A, which interacts with all Vi-phages of serotype II. The reverse process has also been described: a transformation of the phagotype A into a more specific state, as reflected by a loss of sensitivity towards individual phages (C, D1, D2, D5, F2, G, O) (T.Ye. Perkaleva-Klyuchareva et al., 1957) and also to a nontypable form (Eors1, 1956).

A simultaneous presence of phagotypes D1 and the variant of type D4 in the intestine of a carrier has been described by Henderson and Ferguson (1949). During a flareup, caused by salad, infected by the carrier, both kinds of cultures were isolated from the infection cases.

A milk-borne flareup of typhoid is known in which a female carrier of type E4 infected through milk several persons, all cultures from whom belonged to the type E1 (Desranleau and Martin, 1950). One of these patients infected her sister in whom the type E4 was found. It was not possible to produce in-vitro E1 → E4 → E1 transformations.

Epidemiological situations similar to those described above, are not often observed. Carriers of mixed types are a rare phenomenon. The instability of the cultures in vitro is observed only during aging of species on nutrient media or during artificial creation of special conditions. During flareups, lasting for several months and longer, the phagotypes, as a rule, are stable.

ON THE MECHANISM OF SPECIFICITY OF PHAGOTYPES

It is now known that the specificity of the phagotypes of typhoid microbes is mainly connected with the presence of type-determining prophages in their cells. From 18 phagotypes 18 mild phages were isolated
on the indicator strain of type A and others. They were given the de-
signations corresponding to the names of the phagotypes, by lower-case
Latin letters or figures with a stroke, for example b3, d1, d4, 25',
26', etc. (Anderson and Felix, 1953b; Ferguson et al., 1955). All phages
clearly lysed Salm. typhi O-901, while some of them (phages f3, 29, d1,
d4, k) lysed Salm. paratyphi C and Salm. gallinarum and, thus were O-
phages. They were differentiated according to their serological charac-
teristics into 5 separate groups, which did not have anything in common
with the Vi-phages (Ferguson and others, 1955).

First group: b3, 28'.
Second group: a) d1, d4, d5; b) r; c) b2.
Third group: a) d6, f2, 29', 33'; b) 30'; c) 31', 26'; d) 25'.
Fourth group: a) t; b) k.
Fifth group: a) a-phi; b) a-TH25'.

On typhoid phagotypes, serologically identical phages, as a rule,
had the same spectrum of lysing activity and never lysed the strains,
from which they originated, or strains, where phages were detected
which are serologically related to them. The limit of lysing activity
of the phages was 25-45⁰, and the point of thermal death in the range
of 64-76⁰. Under the electron microscope the phages d4, and k had
a short thick tail and head dimensions of 214-226 x 80-92 μm.

The type-determining function of individual phages were demonstra-
ted in artificial lysogenization experiments. As a result of inocula-
tion with mild phage the cell assumed the phagotype identical with the
type of the infecting phage. For example, lysogenization of a F1 cul-
ture with phage f2 transformed it into the type F2. Conversely, when
the mild phage was lost by the strain F2, it reverted to strain F1.

Noteworthy is the fact that in the artificial phagotypes, the sen-
sitivity to the Vi-1 phage did not change but that to O-phages decreas-
ed. One of the phages (k) imparted an absolute resistance to O-phage to the culture. The sensitivity spectrum to typing phages, as a rule, became more narrow. Thus, the mild phages transformed the polylysable type A into the types D6, 19, 25, 26, T and D1, which are lysed at most by 2-3 phages; the type C was transformed into type 30, the type Fl into F2, which interact only with homologous phages, etc. The artificial phagotypes were identical in all their properties with the natural ones and did not change on Dorset's medium in the course of 18 months.

The phage 31' transformed into the gamma form, which is resistant to all phages of serotype II, the types G, H, L, M and O, while phage 33' transformed them into the types Bl, D4, I and L1. The artificial gamma forms proved to be less stable than the natural ones. Loss of the mild phages frequently transformed the strain into a polylysable culture.

Altogether, a type-determining function in the bacterial cell was demonstrated for 13 mild phages; 5 phages (b2, a-TH25', b3, k and 28') proved to be inert in this sense.

Type determining phages were subsequently also found in other phagotypes, as a rule, in newly discovered ones. The presence of the mild phage of a certain serotype is a property which is extraordinarily typical for strains of the same phagotype. For example, all 120 strains of phagotype D1 from different countries proved to be homogeneous with respect to the mild type-determining phage dl (Cefalu, Fichera, 1961).

The testing of lysogenicity became an obligatory test, which determined in new phagotypes their individual nature or the fact that they belonged to a certain subgroup. Thus, for example, from a newly discovered phagotype, Nicolle, Diverneau and others (1960) isolated a phage, and using this, transformed the phagotype M1 into a new one.
This confirmed the fact that the new type belonged to group M. It was termed type M3.

A study of the typedetermining phages revealed the close relationship of the subtypes of several groups and caused doubt in some cases whether individual subtypes belonged to their group. Scholtens (1955a) isolated the phage e7 from a culture of the new phagotype E7, which cannot be distinguished on the basis of its serological characteristics from the phages d6, f2, 29', and 30'. This phage transformed the type A into 29 a the type El into a type similar to, but not identical with, E7. The properties of the phage e7 confirmed the surmise on the close genetic relationship of type 29 with group E. Later on, the same author (Scholtens, 1956b) demonstrated by means of mild phages, a connection between the types El, E3 and Dl. In an experiment the phage dl transformed the type El into E3. The appearance of type E3 is observed during simultaneous incubation of cultures D1 and El (probably also as a result of the activity of the phage dl). Moreover, it became known that the artificial and natural strains E3 contain phage dl. This combination of data proved the close relationship between the three above-mentioned phagotypes.

Hence, not all mild phages, isolated from typhoid phagotypes and capable of transforming them determine the type. Very often the same mild phage is responsible for different phagotypes. For example, in the above-described experiments of Anderson and Felix the phage dl transformed the type A into Dl. Scholtens (1956b) observed, how the same phage transformed the type El into type E3. It is obvious that during the formation of a phagotype by a mild phage, the final result is also determined by the quality of the phagotype precursor. It is believed that the specificity of the phagotypes is created, not by one, but several mild phages, whose combination can be very different.
The type-determining role of individual mild phages is not undisputed. The fact that they were not obtained from all phagotypes does not disprove their importance. This depends to a considerable extent on the imperfection of the methods of induction of these phages in general (difficulties in the selection of the indicator strains, low phage yield, etc.).

Thus, the capacity of different phagotypes of typhoid bacteria to be lysed only by strictly determined phages is due above all to the lysogenicity of these cultures and represents a particular case of lysogenic conversion.

As we know, lysogenic conversion is a change in the hereditary properties of bacteria during lysogenization by several mild phages (D. M. Gol'dfarb, 1961). As a result of the lysogenic conversion, the bacterium acquires the capacity of forming a full-fledged phage and to retain a hereditary determinant in the form of the prophage. The virulent, serological and culturing properties and also the sensitivity to unrelated phages is sometimes altered in such a bacterium. The latter phenomenon, which has been termed prophage interference, evidently plays a certain part in the nonuniform relationships of typhoid cultures with the derivative Vi-II phages.

As we know, all phagotypes of typhoid bacteria adsorb equally successfully the homo- and heterologous Vi-II phages, but are lysed only by the homologous phage (Craigie, 1939). This is also understandable, considering that the fixation of the phage on the bacterium is primarily correlated with the antigen structure of the cell and therefore all strains which contain Vi-antigen, should adsorb any Vi-phages. It must be assumed that the action of the prophage is realized during the subsequent stages of interaction between the cell and the phage. It is not likely that the invasion of phages into the cell is impeded in
heterological phagotypes. It is known that in some cases, concentrated heterological Vi-II phages can produce a lethal effect, which is not accompanied by phage reproduction (M.D. Krylova, 1951). Hence it follows that the Vi-II phage is capable of dissolving the cell membrane of a heterologous phagotype and of "injecting" nucleic acid through it. It must be assumed that the prophage impedes the reproduction of the heterologous phage. In most typhoid phagotypes the action of the prophages is not very strong. In 65% of typing phages the blocked proliferation starts again after 1-2 passages through the given phagotype (M.D. Krylova, 1951).

Phagotype A probably does not contain any prophages which interfere with the adapted Vi-II phages. The specific Vi-Phagotypes appear as a result of its lysogenization by different mild phages, whose determinants (prophages) can interfere with the typing phages.

The appearance of the gamma forms possibly takes place during the lysogenization of cultures by a certain combination of mild phages. Not to be excluded is the possibility that the gamma forms appear in some cases during infection of the phagotypes with latent phages, which are serologically related to the Vi-II phage. It is known that such phages are found in cultures of Salm. typhi (Craigie and Ian, 1937, 1938; Desranleau and Martin, 1950). In such a case we may be dealing with an immunity of the lysogenic bacterium to phages which are serologically related to its prophage.

The Vi-degradation of the phagotype, as manifested by the fact that it begins to be lysed by a large number of typing phages, may be accounted for by a loss of prophage as a result of some disharmony between the state of the host cell and the prophage and also the replacement of one prophage by an other.
THE PROCESS OF ADAPTATION OF VI-II PHAGES TO HETEROLOGOUS PHAGOTYPES

Investigations of the role of mild phages in the specificity of the phagotypes threw new light on the phenomenon of adaptation of the Vi-phage of serotype II to typhoid cultures. As we know, Craigie and Ian (1938) explained this adaptation by a selection of mutants pre-existing in the original phage preparation (type A). Such an interpretation, however, is only partially correct (Felix, 1949; M.D. Krylova, 1954). It was found that the phage itself can produce hereditarily modified forms, the direction and stability of these changes being determined by some qualities of the host strain.

How profoundly is the phage A altered in the process of adaptation to the type strain? Attempting to answer this question, Anderson and Felix (1952) passed 10 typing phages through different cultures of phagotype A. As a result, 5 phages (C, El, Fl, L1 and T) reverted to phage A; 4 phages (D5, D6, O and N) retained their type unchanged. The ease of reversal of the first 5 phages to the original phage revealed the superficial nature of the changes of phage A on the phagotypes C1, El, etc. Noteworthy is the fact that most typing phages are easily adapted to these phagotypes (M.D. Krylova, 1951). These changes do not concern the genotype of the phage. They relate to modifications controlled by the host cell. It may be assumed that the superficial nature of the changes in phage A in this case is connected with the absence of type-determining prophages in the above-mentioned cultures (Anderson and Felix, 1953). The absence of certain prophages in the culture must be assumed to greatly simplify the process of proliferation of the typing phages, requiring only negligible modification of the latter.

The phages D5, D6, O and N when subinoculated on type A, do not revert to the original phage. Anderson (1962) proposed that phage A
during its passage through these strains changes its genotype and therefore the corresponding phages retain their properties so persistently. The strains D5 and D6 contain the type-determining prophages d5 and d6. It is not impossible that this circumstance is the one controlling the degree of variability of phage A on these types. Noteworthy is the fact that most typing phages do not multiply on some lysogenic types (M.D. Krylova, 1951). All these facts attest that the mild phages of the substrate strain determine the result of adaptation as well as the intensity of the changes in the heredity of the phage which has passed through this strain.

Based on experiments, involving artificial creation of phagotypes, Anderson (1955) established structural formulae for several lysogenic phagotypes (Table 3). The phagotypes are represented in them by two letters, of which the first (Capital) designates the basic strain (non-lysogenic precursor of this phagotype) and the second, the type determining phage, which transforms the basic type into the given phagotype during the experiment. For example, for the phagotype F2 the author proposes the formula F1 (f2), because the phagotype F2 contains phage f2 and is easily produced artificially by lysogenization of strain F1 by the phage f2.

The structural formulae of Anderson helped to predict the lytic properties of the phages produced in the adaptation process, and explain the origin of the reactions of group lysis. The mild phage and the precursor strain (the nonlysogenic basic strain) of the phagotype determine the spectrum of the lysing activity of the adapted phage. The new phage will lyse cultures with the same basic strain or with the same type-determining phage, as in the new host phagotype. In a certain number of cases the new phage can interact with strains, containing serologically related type-determining phages, but the lysogenic
TABLE 3

Structural Formulae of Phagotypes, Containing Type-Determining Phages (Anderson, 1955)

<table>
<thead>
<tr>
<th>Оригинальное обозначение</th>
<th>Тип-определяющий фаг, несомый фаготипом</th>
<th>Структурная формула фаготипа</th>
<th>Фаготипы, лизирующие фаготипы, несомые фаготипом в критическом тесте разведения</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>d1</td>
<td>A(d1)</td>
<td>A, D1</td>
</tr>
<tr>
<td>D4</td>
<td>d1</td>
<td>A(d1)†</td>
<td>A, D4, D6, D9</td>
</tr>
<tr>
<td>D6</td>
<td>d6</td>
<td>A(d6)</td>
<td>A, D6, 29</td>
</tr>
<tr>
<td>F3</td>
<td>f2</td>
<td>F1(f2)</td>
<td>A, F1, F2, F9</td>
</tr>
<tr>
<td>F1</td>
<td>f1</td>
<td>A(f1)</td>
<td>A, F1</td>
</tr>
<tr>
<td>T25</td>
<td>25</td>
<td>A(25')</td>
<td>A, 25</td>
</tr>
<tr>
<td>T26</td>
<td>26</td>
<td>A(26')</td>
<td>A, 26</td>
</tr>
<tr>
<td>T29</td>
<td>12 или 30'</td>
<td>A(12) или A(30')†††</td>
<td>A, 29</td>
</tr>
<tr>
<td>T30</td>
<td>12 или 30'</td>
<td>C(12) или C(30')†††</td>
<td>A, C, 29, 30</td>
</tr>
<tr>
<td>T31</td>
<td>c26'</td>
<td>E1(c26')</td>
<td>A, E1, 26, 31</td>
</tr>
<tr>
<td>T33</td>
<td>d6</td>
<td>C(d6)</td>
<td>A, C, D6, D9, 29, 30</td>
</tr>
</tbody>
</table>

*The nonlysogenic precursor of phagotype D4 is not known.
**The phages d6, f2 and 30' belong to the same serological group. Their type-determining properties are also closely connected.

a) Original designation of the phagotypes; b) type-determining phage, carried by the phagotype; c) structural formula of the phagotype; d) phagotypes, lysed by the homologous Vi-II phage in critical test dilution; e) or.

basis of these strains should be the same as in the host phagotype or phagotype A. Let us illustrate the above by means of an example. The typing phage 33 was obtained on a strain with the formula C (d6) (see Table 3). This phage should lyse the phagotype 30 (lysogenic basis of this type – phagotype C), phagotype D6 (contains prophage d6), and phagotype 29 (carrying on phagotype A the prophages f2 and 10' serologically related to phage d6). The experiments, as a rule, confirm these conceptions. Moreover, phage 33 lyses the type C1 (type 33 is now related to group C and is termed C2).

The structural formulae make rational selection of the original phage for producing a new typing phage for nontypable strains possible when phage A does not multiply on them. A mild phage is then isolated from such a strain and its serotype determined. Through the nontypable
strain is passed a typing phage from a phagotype with the same type-
determining prophage, which was detected in the untypable culture
(Anderson, 1956). It is possible that the variability of the phage
which has been passed through the culture in this case is limited by
the modification of the lytic spectrum.

Using structural formulae, Anderson and Frazer (1955) subdivided
the preparations of Vi-II phage on the basis of the intensity of the
changes in the original phage into 3 groups.

Among the first group are counted the phages, obtained by adapta-
tion of phage A on strains which do not contain type-determining pro-
phages (C, E1, E2, F1, G, H, I, L1, L2, M, T, 27, 32). During reverse
passage through the strain A, these phages rapidly lose the acquired
lytic properties and revert to the original phage A. The authors term
them nonhereditarily (phenotypically) modified variants of phage A.

To the second group belong the preparations, obtained by adapta-
tion of phage A to strains containing the type-determining phages
(B1, D1, D5, D6, K, 0, N, 25, 26, 28, 29). During reverse passage
through a strain of type A, the lytic properties of the preparations
remain unchanged. The authors consider these phages to be hereditari-
ly (genotypically) modified mutants.

The third group contains the phages D2, D4, F2, 30, 31, and 33.
Like the phages of the second group, they are obtained by adaptation
of phage A to lysogenic strains (exception — phage D2, which was cul-
tured on the nonlysogenic type D2). During their passage through a
culture of type A, the phages of the third group were modified, but
did not revert to type A, but acquired the lytic properties of a new
phage, which was analogous to the phage, adapted to a culture with
identical prophage. Let us illustrate by an example. The phage F2, af-
ter passing through a culture A was transformed into phage 29 (the
phagotype 29 contains the determinant of phage f2). Noteworthy is the fact that the phagotypes corresponding to the new phages, could be obtained artificially by inoculating A cultures with the latent phages of the strains D4, F2, 30, 31, 33. The authors term the phages of the third group phenotypically modified mutants.

It is obvious from the above that the nature of the variability of phage A in the course of adaptation to the typhoid phagotype is decided by the specific nature of the structural formula of the latter. On the phagotypes, which contain type-determining prophages, phage A is subjected to considerable reorganization, which concerns the hereditary apparatus and which excludes reversion to the original state. The less specific phagotypes which are free of type-determining prophages, impart to phage A superficial, easily eliminated changes (Anderson, 1962).

The mechanism of the variability of phage A is as yet very far from being understood. Equally nebulous is the nature of the processes which take place during passage of typing phages through phagotype A, as a result of which part of the phages revert to type A, another part to other types, and some remain unchanged.

It is possible to assume that the phages of the second and third group are homologous to the phagotypes, which carry type-determining prophages, and that they contain an admixture of the active form of these prophages. The latter enter the preparation from the culture, on which the phages had been bred (M.D. Krylova, 1951).

From this point of view, one could imagine the phage D1 in the second group, for example, as a mixture of two phages: D1 + D1, the phage D6 as D6 + d6, etc. In the experiments in which artificial phagotypes were produced, all the known mild phages of the phagotypes corresponding to the second group — d1, d6, 25', 29' — transformed the
type A into the types D1, D6, 25, 26 and 29, respectively. Now let us imagine that some typing phage of the second group, for example D1 (containing an admixture of phage dl) passes through type A. The possibility cannot be excluded that individual bacterial cells, before the DNK of phage D1 penetrates into them, are lysogenized by the mild phage dl and are transformed into a phagotype with the structural formula A (dl), i.e., into Dl. Naturally, these bacteria, being homologous with respect to phage Dl, are primarily the substrate for its proliferation. On these, phage D1 is not modified. Exactly the same assumptions can also be made with regard to the other phages of the second group.

In the third group, the variability of the phages during their passage through type A takes a different direction, but the nature of the process is possibly the same. The phagotypes of this group (F2, 30, 31, 33) also contain type-determining phages (f2, 30', 26', d6) with which the typing phages can be impregnated. In contrast to the second group, the mild phages f2, 30, etc., transform the bacteria of type A during the experiment into phagotypes different from those, from which these phages had been isolated (Table 3). For example, the phage f2 transforms the phagotype A into a type with the structural formula A (f2), i.e., into phagotype 29. The same transformation may be imagined to take place during the passage of phage F2 (F2 + f2) through phagotype A.

The situation is somewhat simpler in the first group of phages, seeing that they do not contain admixtures of type-determining phages. During the reverse passage through strains of type A, the phages of this group do not lysogenize it and revert rapidly to the original phage A.

We ought to mention that this interpretation of the experiments on the variability of typing phages is highly hypothetical. Its weight-
iest indication are the results of the experiments in which the phages were passed through A cultures. The above expressed hypotheses positively require detailed experimental verification: proof of the presence of admixtures of mild phages in the typing phages, of the possibility of lysogenization of typing cultures which is simultaneous with the processes of adaptation of the typing phages, etc.

The structural formulae enabled Anderson and Frazer (1955) to combine the lysogenic phagotypes into 4 groups (Table 4).

The nonlysogenic precursors of the phagotypes were used as the basis of this classification. They were placed ahead of the group. This arrangement revealed latent connections between typhoid bacteria, which at first glance appeared to be different. This connection, the common phagotype precursor, confirmed the single origin of the subtypes of the same group of Craigie and Felix (the types D1 and D6, and the types F1 and F2 coincided).

The similarity of the phagotypes stipulated by this classification was confirmed in the investigations of other scientists. The types 30 and 33 in group C proved to be similar with regard to their sensitivity to the adapted Vi-phages and were renamed types C3 and C2, respectively. In the group E, the type 31 was later designated as E8. In two types of this group, E1 (d1) and E1 (f2), obtained artificially at the laboratory, natural analogs were found. The experiments on the artificial production of types predicted their existence, as it were. One of these, the type E7, produced the mild phage e7, capable of transforming the type E1 into a type, similar to E7 and the artificial type E1 (f2) (Scholtens, 1955a). Another type, E3, carried the phage d1 and had the formula E1 (d1), i.e., it was identical with the artificial type E1 (d1) (Scholtens, 1956b).

The above-described classification of types was not only interest-
ing from a theoretical point of view. In epidemiological investigations of flareups, when at the same time two and more types are isolated from carriers and patients, it is very important to know the degree of relatedness of these cultures.

This helps in the correct interpretation of the results of phage typing and in correlating them intelligently with the epidemiological data. This problem will be considered in greater detail in the following section.

TABLE 4
Classification of Vi-Types on the Basis of Their Structural Formula (Anderson, Frazer, 1955)
Using also Data from Other Authors

<table>
<thead>
<tr>
<th>Группа</th>
<th>Оригинальное обозначение</th>
<th>Формула</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>A (d1)</td>
</tr>
<tr>
<td></td>
<td>D1</td>
<td>A (d6)</td>
</tr>
<tr>
<td></td>
<td>D8 (Вильсон и Эдвардс)</td>
<td>A (d8)</td>
</tr>
<tr>
<td></td>
<td>29 (Борман)2</td>
<td>A (72) или A(30')</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>A (i)</td>
</tr>
<tr>
<td></td>
<td>25 (Ли Кiang Дэо)</td>
<td>A (25')</td>
</tr>
<tr>
<td></td>
<td>26 (Кларк)4</td>
<td>A (26')</td>
</tr>
<tr>
<td>C</td>
<td>C1</td>
<td>Cl(46)</td>
</tr>
<tr>
<td></td>
<td>C2-33 (Дерано)5</td>
<td>C1(46)</td>
</tr>
<tr>
<td></td>
<td>C8-30 (Андерсон)6</td>
<td>C1(22) или C1(30')</td>
</tr>
<tr>
<td></td>
<td>C8 (Вильсон и Эдвардс)</td>
<td>C1(25')</td>
</tr>
<tr>
<td></td>
<td>C9 (Вильсон и Эдвардс)</td>
<td>C1(29)</td>
</tr>
<tr>
<td>E</td>
<td>E1</td>
<td>E1</td>
</tr>
<tr>
<td></td>
<td>E2-4904 (Вильсон и Эдвардс)</td>
<td>E1 (d6)</td>
</tr>
<tr>
<td></td>
<td>E2-31</td>
<td>E1 (26')</td>
</tr>
<tr>
<td></td>
<td>Искусственно полученные типы</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>E1 (d1)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>E1 (i)</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>F1 (22)</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>F1(22) или F1(30')</td>
</tr>
</tbody>
</table>

- 39 -

a) Group; b) original designation; c) formula. 1) Wilson and Edwards; 2) Borman; 3) Li Kiang Dzho; 4) Clarke; 5) Desranleau; 6) Anderson; 7) artificially produced types; 8) or.
THE PRACTICAL SIGNIFICANCE OF PHAGE TYPING OF TYPHOID CULTURES

The epidemiological value of the method of phage typing of Salm. typhi is based primarily on the practical stability of the phagotype. As mentioned previously, the changes in the phagotypes, which are easily produced artificially under special experimental conditions, rarely take place during typhoid epidemics.

The Soviet and foreign literature is extraordinarily rich in descriptions of epidemic flareups, which demonstrate how the isolation of identical phagotypes from patients, connected epidemiologically, confirmed the data of the conventional research methods, while isolation of different types compelled a search for other sources of infection, thus refuting the epidemiological common origin of these cases. Epidemiological practice has accumulated numerous data proving that group infections, connected by a single source of infection, are characterized by uniformity in the isolated phagotypes.

Phage typing often helps to reveal the common source in cases of widely scattered cases, which at first glance do not appear to be connected. Thus, Foley (1942) described a flareup, in which, on the basis of the discovery of a common phagotype for 40 cases, the investigators concluded that there must be a single course of infection, which was later confirmed by the results of epidemiological research.

Numerous investigations showed that for water-borne flareups, whose cause was the use of untreated water by the affected patients, contaminated by sewer effluents, a preponderance of multiple types is typical. If the water-borne flareup takes place in a relatively closed locality, where the monotypical microbe population is due to negligible migration processes, the same phagotype of the microbe can be isolated from all patients. Thus, M.P. Mevzos et al. (1962) described a flareup of typhoid in a Young Pioneer Camp where 66 children became
ill within 20 days. All the cultures from the patients belonged to phagotype A. The common phagotype pointed to a single infection source. Epidemiological investigation showed that the infection of the children could have been caused by bathing in a small stagnant pond. Even prior to the flareup, typhoid bacilli could be isolated from the irrigation channel which supplies the pond. Typhoid cases had occurred among persons, living on sections adjacent to this irrigation ditch. Remarkable is the fact that cultures of phagotype A, i.e., of the same phagotype as in the Young Pioneer Camp, were isolated from all patients.

All these investigations afford convincing proof of the great practical value of the method of Craigie and Ian. The phage subdivision of typhoid cultures is an aid in research on typhoid, removes contradictions in epidemiological conclusions and makes the epidemiological study of typhoid more profound and accurate. One could quote numerous examples from practice to illustrate these conceptions, but we take the liberty of referring the reader to the corresponding reports (L. Ya. Kats-Chernokhvostova, 1947; A. Ye. Mkrtchan, 1957, 1961; V. A. Kilessso, 1954; S. F. Bubes, 1953; M. D. Drylova, 1961; Raska K. and others, 1950; Eorsi, 1957, and others).

The method of phage typing assumes special significance for the recognition of the role of chronic carriers in the incidence of typhoid. Registration of the phagotypes isolated from carriers is now carried out in many countries, which helps to reveal their true role in the appearance of sporadic diseases and typhoid flareups. Keeping records of reconvalescents and carriers often enables the source of infection to be traced in situations where the epidemiological data do not give reliable indications.

The introduction of a new research method into the daily practice of the bacteriological laboratories of necessity enriches the science...
with casuistics. This was also the case with the method of phage typing. In proportion to the accumulation of practical data, situations were revealed in which typing did not completely agree with the epidemiological findings and, moreover, even made the investigation more difficult. True, such flareups were recorded extremely rarely, and they did not in any way discredit the method as a whole. However, once they have been shown to exist, it would be useful to dwell on them in greater detail.

Situations are possible when the epidemiological findings irrefutably indicate a connection between cases of disease (single infection source, contact, etc.), while different phagotypes are detected in the focus. A.Ye. Mkrtchan (1961) came across such a discrepancy during a study of family flareups at Yerevan. Here is a description of one of them.

On the 1st August 1955, a girl fell ill. She was treated at home without a clear diagnosis until the 7th August. On the 10th August her brother fell ill with typhoid. The intervals between the beginning of the cases and the entire epidemiological situation attested to contact infection of the brother by the sister or a single infection source.

Contrary to expectations, the type A was isolated from the sister and type D1 from the brother. Types E1 and A were isolated in a similar situation in another flareup. The infection source in both flareups could not be detected.

Scholtens (1950) reported a flareup, during which phagotype A was isolated from some patients and type D6 from others. Nontypical or Vi-degraded cultures are sometimes isolated in epidemiological single foci in addition to specific phagotypes.

Naturally, a correct interpretation of the epidemiological and bacteriological findings is of great importance in all the above-indi-
cated cases. Several different causes can be imagined which are often very difficult to distinguish.

It must be pointed out in the first place, that the discrepancies under discussion should be an incentive to a more careful search for the source of infection. This is particularly justified when the intervals between cases put the simultaneous infection of the patients into doubt. It may happen that repeated, faultless study of a flareup fails to reveal the existence of other sources of infection.

The isolation of different phagotypes within a family is often observed when water from open water reservoirs is consumed against the background of a water-borne flareup of typhoid, which, as we know, is frequently characterized by the presence of a monotypical microbe population.

Finally, the existence of two different phagotypes in an infection source can be assumed. In the abovementioned flareup of Scholtens (1950) the source proved to be a carrier who gave off two phagotypes D6 and A. Anderson encountered such a case (1951) during an investigation of a water-borne typhoid flareup at Kilkregan (Scotland). Most patients were infected by the consumption of water from a small stream into which sewer effluents from a cottage were discharged, whose owner proved to be a carrier. The phagotype F1 was found in her urine and feces. In the patients, however, two types were isolated: F1 and F2. Enormously careful investigations did not reveal any other sources of infected water, which led to another investigation of the cottage owner. Finally, two types were found in her, F1 and F2.

In flareups, when different phagotypes are isolated from the patients but only one from the supposed source, the combinations of these types are remarkable. The subtypes of the one group were found together: F1 and F2 (Anderson, 1951), D1 and D4 (Henderson and Ferguson, 1949),
El and E4 (Desranleau and Martin, 1950), the types A and E in company with the specific phagotypes, for example: A + D1 (A.Ye. Mkrtchan, 1961), A + D6 (Scholtens, 1950).

An other fact is also remarkable: in such flareups, as a rule, the infection source proves to be a carrier. These two circumstances are not fortuitous. Many investigators found two phagotypes in the urine and feces of carriers. Thus, Eorsi (1956) observed in 25 out of 825 carriers the simultaneous presence of two phagotypes or a change of types in the combinations: A + D6, A + D1, F1 + F2, D1 + D6. The combinations of phagotypes in the carriers are normally the same as in the flareups.

The appearance of subtypes of the same group is obviously connected with the variability of the cultures in the organism of the carrier. The structural formulae attest to the close relationship of type F2 with type F1. The same can be said of the types D1 and D4, E1 and E2. A degradation of the above types during the freeing of the cultures of the type-determining prophages may be assumed under conditions of the living organism. As a result, type F2 is transformed into F1, or D6 into D1, etc. This same process may lead to the transformation of specific phagotypes into type A or Vi-degraded cultures.

The reverse process is also possible – the formation of a specific type under the influence of lysogenization of the culture by type-determining phages. The results of this process are easy to predict, starting out with the structural formulae of the phagotypes (see Tables 3, 4). For example, it may be expected that type A evolves into the types D1, D6, T, 25, 29, etc., type E1 into the types E3, E4, E8, type F1 into F2, type C into C2, C3 and others, etc. In any event, such transformations can be realized under conditions of laboratory experiment. Such a process undoubtedly takes place during the circula-
tion of types in nature and also in carriers. An indication of this is the great variety of phagotypes and the simultaneous isolation of these combinations of types from carriers. The type-determining mild phages taking part in this process, in the intestine of the carrier may maintain their existence not only on typhoid cultures. Being O-pha-
ges, they also reproduce well on Salmonella of group D and B, in parti-
cular on Salm. paratyphi C, Salm. gallinarum (Henderson et al., 1955).
In the organism of the patient, where the period of presence of the typhoid culture is short, the possibility of a new phagotype is less probable, although it cannot be excluded.

In view of the above, the detection of the above-mentioned combin-
ations of phagotypes in a single flareup, connected by a single source of infection, should not categorically disprove the data of the epi-
demiological investigation. It is appropriate here to recall that the method of phage typing is not in any way destined to replace the method of epidemiological investigation. Its role remains an auxiliary one, intensifying and guiding the investigation of the flareup.

Certain difficulties are presented by situations, when in two dif-
ferent flareups, not connected at first sight, the same phagotypes are isolated from the patients. Such information certainly impels to an intensified search for the factors which connect these flareups. It may happen, however, that in spite of the data of phage typing, the search for common epidemiological connections between separate cases of infection proves unsuccessful. A knowledge of the local type of microbe pop-
ulation can then be of great assistance. A widely prevalent phagotype (for example A or El), isolated simultaneously in autonomous foci, cannot prove a connection between them. Differences in the biochemical type in cultures of the same phagotype, originating in two foci, confirm their autonomous nature. The same is demonstrated by additional
phage typing (see further on). In some cases, testing of the crossed-over lysogenicity helps to recognize the single or independent origin of cultures of the same phagotype. Mild phage never interact with the culture, from which they had been isolated. For this reason, the lysogenicity of a strain can be manifested only on a culture of different origin.

L.Ya. Kats-Chernokhvostova and co-workers demonstrated the expediency of phage typing of bacteria not only for the improvement of epidemiological investigations but also for the acceleration of the laboratory diagnosis of typhoid. Taking into account the great specificity of the Vi-antigen and its wide distribution among cultures of Salm. typhi, Craigie and Brandon (1936) proposed the use of the phage of serotype Vi-I for the identification of cultures. Z.S. Ostrovskaya and V.N. Papkova (1951) provided authentic proof that by using Vi-II and Vi-I phages it is possible to shorten the time required for laboratory analysis to 30-36 hrs.

As a result, not only is the typhoid nature of the strain established but also its phagotype. Within 30-36 hrs from the moment of culturing of the patient's blood, A.Ye. Mkrtchan (1957) identified 83.6% of blood cultures. The effectiveness of phage identification of a blood culture was also demonstrated in other works (A.G. Matus, 1958, and others), Sechter (1957) used a mixture of the phages D1, E2, F, N, O and T. These phages proliferated in all experiments only in presence of Salm. typhi and never proliferated in their absence. V.D. Timakov and D.M. Gol'dfarb (1962) used the Vi-phage as indicators in the reaction of increase in the phage titer.

No less interesting prospects were opened up by the method of phage typing for the proper solution of the problem of specific phage prophylaxis of typhoid and also the perfection of this method on the
basis of a principally new approach to the development of the phage preparation. The high protective effect of certain Vi-II typing phages with respect to homologous strains and the absence of a protective action with regard to heterological strains was demonstrated by the investigations of numerous Soviet authors (O.A. Gukovskaya, 1949; L.Ya. Kats-Chernokhvostova, 1949; K.Z. Levtova, 1952; M.D. Drylova, 1956; Ye.G. Makashvili, 1957, and others). This confirmed the necessity of including in the bacteriophage preparation, used in foci for prophylaxis, phage types which are homologous to the phagotypes, prevalent in the given region. M.D. Krylova et al. (1958) demonstrated the usefulness of using bacteriophage A. Bred on the polylysable phagotype A, this phage not only has high adaptive capacity to certain heterologic strains in vivo, but also protects against them in minimum doses.

GEOPHAGICAL DISTRIBUTION OF PHAGOTYPES

Prior to the formation of the International Committee on Phage Typing, which carried out measures on the standardization and regulation of the technique of typing and producing of phage preparations, it had not been possible to obtain an adequate idea concerning the worldwide distribution of phagotypes. In addition to the discrepancies of the results of typing in different parts of the world, the true pattern was masked by the incorrect counting principle: the distribution of the types was calculated on the basis of the number of strains (in the optimum case of patients and carriers) detected in the given region.

The rationalization of counting during the determination of the distribution frequency of phagotypes was carried out by Felix (1955). He proposed to count, not the number of cases caused by the phagotype, but the number of foci. With this method it was suggested that every focus or flareup, independently of the number of cases, be counted as
a single case.

The counting according to the method of Felix requires accurate determination of the boundaries of foci which is not always possible. In countries where the incidence of typhoid is of an endemic nature, and where epidemics are extremely rare, it can be recommended to count the individual cases as one unit for the purpose of simplifying the count. When epidemics or large foci appear, all cases recorded in them must also be taken as a single case. With this counting method, the distribution frequency of the phagotypes will be close to the real one. Buczowski, and Lalko (1958) used this principle in Poland in the years 1956-1957, where the strains were typed in 78% of cases. The percentage distribution of the phagotypes, calculated on the basis of the number of foci of cases, did not differ from that calculated from the number of patients. This was explained by the absence of large epidemics during this period (6344 cases and 4986 foci). A.Ye. Mkrtchan (1961) arrived at the same conclusion during a study of the percentage distribution of phagotypes in the Armenian SSR.

A chart of phagotype distributions can be established for countries and regions provided that typing is carried out in no less than 80% of infection foci (Nicolle, Hamon, 1954).

The standardization of the method and the regulation of counting enabled Felix in 1955 to systematize the data on the geographical distribution of the phagotypes of typhoid bacteria over the whole world. These data prove that certain phagotypes (El, A, Cl, D1 and others) are distributed universally, while others (G, H, J, M, 30, 31, 32) constitute the specific microbe population of some countries and are not detected in other countries.

The types El, A, Cl, D1, Fl, N, O, T, 28, 29, I + IV are found in Europe (Nicolle, Vieu, Diverneau, 1956).
The cultures of the Vi-I – Vi-IV group are isolated in Europe in 1-5%, in Africa in 0.05-4%, in the Americas in 0.5%, in some Asian countries, up to 12-19% (Nicolle, Diverneau, 1961).

The changes in the microbe population may be accounted for, one should think, not only by epidemiological causes, but also by the improvement in bacteriological diagnostics in general and also by the more widespread introduction of phage typing in the work of the bacteriological laboratories. As a result, a much greater number of cultures are typed which in turn increases the information on the type population. Besides, new phages appeared during these years in the diagnostic arsenal, which made it possible to detect new phagotypes.

The distribution frequency of typhoid phagotypes may remain fairly stable in the course of several years. In the Federal German Republic, for example, important changes in the phagotype distribution were not found in the course of 7 years (Brandis, 1958).

A knowledge of the phagotype population helps to differentiate local cases from those brought in from other parts of the country as well as from abroad.

As an evident example for this may serve the typhoid flareup in England in 1943, caused by a phagotype unknown at that time. The source of the infection proved to be a chronic carrier, who had suffered from this disease during his sojourn in South Africa. The culture of the new phagotype, termed T and the phage which adapts to it, were sent to South Africa. This made it possible to determine there a considerable distribution of this phagotype (Felix, 1955).

The appearance of new phagotypes helps to recognize typhoid cases brought in from elsewhere. For example, a variety of type C1 was detected at Strasbourg which had up to then only been found in Central Africa (see the next section). Epidemiological searches confirmed the
hypothesis of the foreign origin of the disease: the patient had been sent by air from Banjo for determination of the diagnosis (Nicolle et al., 1960).

Individual phagotypes are found very often within the limits of a single country (A, E1, C). In 1953 the phagotype A occupied first place in Denmark, Italy, Rumania, Yugoslavia, Portugal and in the Malagassian Republic with regard to the frequency of isolation, being responsible for 20 to 30-35% of foci of the disease. The phagotype E1 dominated in England, the GDR, FRG, Denmark, Holland, Ireland and Austria (Felix, 1955).

The International Committee for Phagotyping of Coliform Bacteria carried out a survey of the world distribution of phagotypes also during the following years. These investigations showed that over a period of several years, the phagotypes which predominate in the same country or region, as a rule, remain the same. Thus, in 1953-1957, as in the preceding years, the phagotypes A, E1, C1, F1, D1 and the Vi-degraded strains predominated in Europe (Rische, 1961). Only in Portugal was a preponderance of phagotype B3 noted. It had predominated there also during the preceding years (Felix, 1955). In some countries of Asia and Africa, a replacement of some predominating types took place. Thus, in Turkey the types T, D1, E1, F1 (Felix, 1955) predominated up to 1953, but in the following years, the types A, the Vi-degraded strains and F1 began to dominate; in the Iran, the type G began to dominate instead of the types F1 and A.

Phagotyping of typhoid microbes has been carried out in the USSR by the method of Craigie and Ian since 1945. An important part in the study of the significance of this method and its practical introduction played the works of L.Ya. Kats-Chernokhvostova and her co-workers and also the investigations of Ye.B. Ginzburg-Maslova, V.A. Kilesoo and
From 1947 onwards and up to the present, reports have appeared in the Soviet Press, in which a characterization of the type population of microbes in individual republics and regions of the Soviet Union are given (S.F. Bubes, 1953; L.Ya. Kats-Chernokhvostova, 1957; B.K. Rubashkina, 1951, and others).*

Using 15-18 types of the Craigie and Felix phages, the authors typed an average of 70-70% of the strains. The following phagotypes were detected on the territory of the USSR: A, B2, C, E1, E2, F1, Fa, D1, D2, D4, D5, D6, G, L1, L2, M, O, T, H, N. Different phagotypes predominate in different regions, but most frequently these are A, F2 and E1 and sometimes C and E2.

The phagotype F2 was not found to occur frequently in any country of the world and possibly represents a specific feature of the microbe population of the USSR. Typical for the central regions is a diversity of phagotypes, which is evidently connected with the more intense migration processes. In the border regions, the type population is more uniform, but not everywhere. A certain stability of the microbe type population in different years has been demonstrated (R.I. Zubkova, 1956; A.Ye. Mkrtchar., 1957; T.Ye. Perkaleva-Klyuchareva and others, 1957).

The data on the geographical distribution of the phagotypes in the territories of the Soviet Union must be regarded as preliminary. Firstly the authors used an incomplete test series of Vi-phages for typing and produced these in most cases themselves by passing them through standard phagotypes. In a number of cases, the critical test dilution was determined by titration of typing phages on a homologous strain only. This could have led to the use of insufficiently specific phages, which in critical test dilution lyse not only the homologous strain,
but also some heterologous strains. Nearly all authors (with the exception of A. Ye. Mkrtchan) gave a characterization of the local microbe population on the basis of the distribution of phagotypes among a certain number of strains. With such a method of counting, a domination of some phagotype in a series of cases could be obtained on account of typhoid flareups and also as a result of repeated isolation of several strains of typhoid microbes from individual patients.

The production of typing Vi-II bacteriophages is now standardized in our country. Phages in standard test dilution are supplied by the Tbilisi Scientific Research Institute for Vaccines and Sera (Tbilisi, Saburtalo, Voyenno-Gruzinskaya Road) and the Rostov Scientific Research Institute of Microbiology, Epidemiology and Hygiene (Rostov-on-Don, Gazetnyy pereulok, No. 119). It is to be hoped that the type population of the USSR will be determined with greater accuracy in the next few years.

ADDITIONAL SUBDIVISION OF FREQUENTLY ENCOUNTERED PHAGOTYPES

The practical importance of phage typing for the country depends on the degree of diversity in its types of microbe population. If the overwhelming number of strains consists of one or two phagotypes, the role of phage typing in diagnosis and epidemiology of infections is naturally reduced. Conversely, if a great diversity of phagotypes exists, the importance of this method increases correspondingly.

As stated previously, the phagotypes A, Cl, El predominate over the others not only in the USSR, but also in every country of the world. Naturally, when the same phagotype accounts for 30 to 50% of all cultures, detected in patients and carriers, phage typing has little effectiveness in an epidemiological sense. For this reason, phage typing is used in combination with biochemical methods in localities with a limited number of phagotypes or a preponderance of certain types.
Biochemically, the groups are distinguished on the basis of the enzymatic degradation of xylose and arabinose. Kristensen (1938), using these sugars, subdivided the strains of the typhoid bacillus into 4 biochemical types (I, II, III and IV).

Biochemical typing makes it possible to differentiate some phagotypes frequently encountered in a country into biochemical subtypes, which facilitates the epidemiological analysis. Such double typing was first carried out by Olitzki, Shelubsky, Strauss in 1945 and in Israel in 1948.

Similar investigations were later carried out in the USSR (R.I. Zubkova, 1956; A.M. Slavina, 1953; N.I. Fedorova-Talashenko, 1951), in France (Jude and Nicolle, 1949); Pavlato and Nicolle (1953), in the FRG (Brandis, Mauer, 1954), in Austria (Edlinger and others, 1954), in Poland (Chomiczewski, 1961a) and in other countries. The conception has been evolved on the basis of these works that the biochemical activity of the typhoid microbe and its sensitivity to phage Vi-II preparations are not connected. For example, the type D1, according to the data of Levi (1956), is biochemically uniform, but according to the data of R.I. Zubkova (1956), it is not uniform. The same can be said for nearly all other phagotypes. The type M1 (Nicolle, Vieu, Skalova, Brault, 1956) was found to be an exception. All 163 tested cultures of this phagotype, obtained from remote geographical regions (Canada, Peru, Iran, Vietnam, Japan, Cuba) belonged to type II of Kristensen. The latter discovered subtypes M2 and M3 (Nicolle et al. 1960) were also related to this type. Nicolle et al. (1958) demonstrated on a voluminous material (4663 cultures from 26 countries were studied) that in all cases where the epidemiological focus has been accurately determined, the typhoid bacteria are uniform with regard to phage and biochemical characteristics. If the cultures were isolated in different foci, their
biochemical properties can be nonuniform. Even this makes it possible to subdivide cultures of the same phagotypes.

For a more detailed subdivision of frequently encountered phagotypes, such as A, C, El, into a biochemical series, sodium citrate and sodium and potassium d-tartrate are added in addition to xylose and arabinose. This enabled Olitzki and co-workers (1945, 1948) to determine in the type C, which was dominant in the microbe population of Israël, 5 types of enzymatic degradation. Cambiesco and Meitert (1957) subdivided the type A, isolated in Rumania, into 11 biochemical types and the nontypable Vi-degradated and Vi-negative strains, into 5-7 types.

A certain proportion of the detected phagotype can be differentiated by means of bacteriophages. Two schemes have been proposed for the additional phage typing of type A. Desranleau and Martin (1947, 1950) described two variants of phagotype A, isolated in the province Quebec (Canada) and designated them by the Greek letters \( A^\Psi \) and \( A^\Phi \). Three subtypes are distinguished in type A — the normal, \( A^\Psi \) and \( A^\Phi \). The strains of each group do not give identical reactions with the nonadapted Craigie Vi-phages of the types I, III, IV, or the Desranleau and Martin VI-phages of the types V and VI.

The normal type A interacts with all 5 phages. The subtype \( A^\Psi \) only with the phages of the serotypes I, IV and V, \( A^\Phi \) with the phages of the types III, IV and VI. In the subtypes of A (normal) and \( A^\Phi \), mild phages have not been observed. Conversely, all cultures of the subtype \( A^\Phi \) produce the latent 0-phage. In the laboratory, the above type often evolved into the gamma form, acquiring a resistance to all phages of serotype II. The same transformation was observed in two flareups, connected with the subtype \( A^\Phi \): in one focus, the strains \( A^\Phi \) were isolated from part of the patients and in another, the gamma forms.
The authors demonstrated the epidemiological value of this subdivision of phagotypes. Apparently, however, it is not significant for all countries. Thus, in Yugoslavia Tomasic (1958) studied 239 strains of phagotype A by the above-indicated method but did not detect the subtypes Aψ and Aφ.

The second scheme of additional subdivision of type A was proposed by Nicolle, Pavlato, Diverneau and Brault (1953, 1954, 1958). By means of 8 phages (only two of these were Vi-phages), the authors subdivided over 3000 Vi-strains of phagotype A, obtained from 32 countries, into 9 subtypes, termed according to the locality where they had been isolated (Table 5).

**TABLE 5**
Subdivision of Phagotype A According to Nicolle, Diverneau, Brault (1958)

<table>
<thead>
<tr>
<th>Группа</th>
<th>Подгруппы</th>
<th>Фаги</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>I</td>
<td>Кокилхативиль (Coquilhatville)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td></td>
<td>Монреаль (Montreal)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td>II</td>
<td>Тананариве (Tananaive)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td></td>
<td>Дуала (Douala)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td></td>
<td>Шамбле (Chambles)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td></td>
<td>Велипуй (Welahpool)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td>III</td>
<td>Освестри (Owestry)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td></td>
<td>Леопольдvilla (Leopoldville)</td>
<td>сл</td>
<td>сл</td>
</tr>
<tr>
<td>IV</td>
<td>Маракайбо (Maracaibo)</td>
<td>сл</td>
<td>сл</td>
</tr>
</tbody>
</table>

Symbols: (sl) confluent lysis; (<sl) lysis lower than confluent; (±) designates an inconstant reaction; (-) signifies the absence of reaction.

- a) Group; b) subtypes; c) phages; d) Vi-phage; e) Vi-III Craigie phage; f) sl.
It can be seen from Table 5 that the subtypes can be combined into 4 groups on the basis of their reactions with the phages 1, 2, 3 and 5. For each group there is a "group" phage. Thus, phage 1 is specific for group I (it comprises the subtypes of Coquilhatville and Montreal), phage 2 - for group II (Tananarive, Douala, Chamblee, Welshpool), phage 3 for group III (Oswestry and Leopoldville), phage 5 for group IV, containing a single subtype - Maracaibo. The subtypes within the group differ in the reaction with the 7 "indicator" phages.

The lysogenic state was found in most of the subtypes. Only three, Tananarive, Oswestry and Douala (with exception of one culture) did not produce mild phages. The nonlysogenic subtype Tananarive proved to be a faultless indicator for the mild phages of the 8 subtypes: only the phage from the Coquilhatville cultures proliferated weakly on this phagotype, in consequence of which mild phages from the Coquilhatville culture were isolated on Salm. dublin.

Remarkable is the fact that nearly all the mild phages of identical subtypes obtained from different places of the earth had similar lytic properties. Undoubtedly lysogenic were the cultures Coquilhatville and Maracaibo. In the other subtypes, the lysogenic state was characterized by a certain lability and could often be observed only with difficulty.

As one would have expected, not one of the mild phages lysed the cultures of the subtype, from which they had been isolated. It is noteworthy that the mild phage also failed to react with the nonlysogenic cultures of its subtype. The authors assumed the existence of a "latent" lysogenicity in some cultures of these subtypes. It is entirely possible that in every concrete case this "latent" lysogenicity was due to unsuccessful selection of the indicator strain. On the heterologous subtypes, the range of action of the mild phages of different
subtypes was different. An exception were the phages isolated from the subtypes Leopoldville and Montreal. With respect to their serological characteristics and the range of activity on heterologous strains they proved to be identical with each other and with the phages 2 and 4.

The result of the lysogenicity investigation confirmed the fact of the existence of subtypes among bacteria of type A and showed that the difference between the subtypes depends mainly on their different lysogenicity. This conclusion was illustrated in experiments involving artificial creation of subtypes (Cefalu and Fichera, 1958).

The nonlysogenic subtype Tananarive could be transformed into the subtypes Montreal, Chamblee, Welshpool, Coquilhatville and Maracaibo through lysogenization with the mild phages of these subtypes.

In correspondence with this, structural formulae were proposed for the subtypes (Cefalu and Fichera, 1961). The nonlysogenic subtypes (Tananarive and Oswestry) were designated as A1(−) and A2(−), respectively. The designation A1 and A2 attests to the different sensitivity of the subtypes to mild phages. The lysogenic subtypes were designated by the letter A and the two first letters of the name of the specific mild phage, isolated from the given phagotype, for example: A(mo) for the subtype Montreal, A(du) for the subtype Douala, etc.

The subtypes of phagotype A, which were differentiated by additional typing, proved to be practically stable (Rische, Rohne and others, 1958).

Nicolle and co-workers demonstrated on a number of examples the practical value of the subdivision of type A: strains of the same subtype were isolated, as a rule, from epidemiologically related foci. This was confirmed by investigators in other countries. Tomasic (1958) who typed strains of phagotype A, isolated in Yugoslavia in 1956-1957, by means of the 7 additional phages of Nicolle, observed that the cul-
tures, obtained in a single focus, belonged to the same Nicolle subtype. This confirmed the epidemiological value of the method.

The scheme of additional phage typing of type A according to Nicolle still has many deficiencies: the phages are insufficiently specific and lose their titre fairly rapidly during storage, and unequivocal results cannot always be obtained (Tomasic, 1958). Nonetheless, further improvement made the method useful in countries, where type A dominates.

The phagotype C1 was differentiated into two variants (Nicolle, Van Al, Brault, 1955). One of these is the phagotype C1 normally ubiquitous in Europe, Asia, America and North Africa, which is lysed by 6 phages of this group (C1, C2, C3, C4, C5, C6) and the Vi-V phage. The phages of group D develop clearly discernible and numerous plaques on this variant. The other variant is resistant to the phages C3, C6, Vi-V, and only slightly sensitive to the phages of group D (the plaques are very small or absent); it is found more rarely: in the Central African Republic, in the Republic of the Congo and in the Malagassian Republic. In 1958, the Central Arafican variant of type C1 caused a large typhoid epidemic at Leopoldville (Nicolle and others, 1960).

The phagotype El can be subdivided into two practically stable subtypes: Ela and Elb. Brandis (1955b) achieved this by means of a phage, termed Vi-VII (initially IAr) and which is serologically different from the well-known Vi-phages. The Vi-VII phage was isolated from sewerage effluents. In critical test dilution it lysed only part of the strains of phagotype El (the subtype Ela), while the other part (subtype Elb) did not react with it. The Vi-phages of the serotypes I and IV also lysed only the subtype Ela, while the phages E1 and E2 lysed both subtypes. In Poland the subtype Ela was found in 89% of foci (Oles and others, 1960), in the GDR the subtypes Ela and Elb were found in a ratio of 2:1 (Rische, Schneider, 1959). These did not change in
the carriers over a period of several years and were stable during their passage through the organism of guinea pigs and rabbits. The epidemiological value of this subdivision was demonstrated in 10 epidemics and 99 foci.

The phagotype F1 was subdivided by Chomiczewski (1961b) into two subtypes (Fla and Flb) with a Vi-phage, isolated from sewerage effluents, and provisionally designated as "Lodz – 13." With respect to its serological characteristics this phage differed from the phages of the serotypes I, II, III, IV, and VII. The subtype Fla was sensitive to this phage, while the subtype Flb was not. The phagotype F4, which was later included in the official scheme, could also be subdivided by means of this phage into two analogous subtypes. The subtypes were stable during storage under laboratory conditions.

The nontypable Vi-cultures (group Vi-I – Vi-IV) can be differentiated by means of a test series of O- and Vi-phages, which are used for the subdivision of type A (Nicolle, Pavlato, Diverneau, 1954) and also with the mild phages, isolated from cultures of the groups Vi-I – Vi-IV on the subtypes A (Tananarive) or on Salm. dublin (Nicolle, Diverneau, 1961). By means of 8 of these bacteriophages, 449 strains of group Vi-I – Vi-IV, obtained from different regions and countries, were subdivided into 13 subtypes (Table 6).

Cultures which have lost the Vi-antigen (W-form) are typed at the French center with a series of O-phages. Part of these phages are the same as in the series for type A, and another part were isolated from lysogenic cultures (Nicolle, Diverneau, 1958).

The cultures of identical phagotypes which cannot be Vi-typed, the polylysable and the W-form can also be subdivided by lysogenicity tests. This test is based on the fact that epidemiologically related strains do not manifest lysogenicity with respect to each other. Only cultures
TABLE 6
Subdivision of the Group VI-T - VI-IV
(according to Nicolle and Diverneau, 1961)

<table>
<thead>
<tr>
<th>Plateau</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c</td>
<td>3</td>
<td>3</td>
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<td>3</td>
</tr>
</tbody>
</table>

Symbols: (sl) confluent lysis; (±) inconstant reaction; (−) absence of reaction.

a) Subtype; b) bacteriophages; c) sl.

from a different focus can serve as an indicator of this property. The manifestation of lysogenicity in a pair of cultures of identical phage types attests to their epidemiological difference.

METHOD

The method of typing typhoid bacteria with adapted phages of serotype II was described by Craigie and Ian (1938) and was later improved by Craigie and Felix (1947) and also by other authors (L.Ya. Katschernokhvostova; Anderson and Williams, 1956, and others).

Media. The conventional nutrient media are used for phage typing, only the concentrations of some ingredients being altered. Many researchers recommend standard dry nutrient media, because on these it is easier to obtain comparable typing results.

Liquid media are prepared in accordance with the following formula:

- Dry nutrient broth 20.0 g
- Sodium chloride 8.5 g
- Distilled water 1000 ml
The medium is sterilized at 120° for 25 minutes. Final pH = 6.8. It is not recommended to adjust the pH with alkali. To prepare the solid medium, 1.3% agar of the best quality is added to the liquid medium. When Sovietic powdered nutrient agar is used, according to the instructions of the Tbilisi IVS, 4 g of the powder is to be taken per 100 ml of cold water instead of 5 g, otherwise the method indicated on the label is used.

This medium gives standard typing results, although not always sufficiently clear. A more intense proliferation of the culture and phage is achieved on tryptic digested media, prepared directly at the laboratory. For the tryptic digestion according to Hottinger no less than 500 g of fresh meat for 1 l of water are taken; the pH should be 7.4. The precipitate is removed because an excess of phosphates inhibits lysis. In view of the unstandardized nature of such media, every new portion of agar must be compared with the preceding one by typing the phagotypes N, O and T on it with the whole series of typing phages in critical test dilution. The medium is suitable if the phages develop on these phagotypes distinct sterile spots of the dimensions.

When typing cultures which give dwarf spots, it is useful to add 0.2% sodium sulfate to the agar. The culture grows on such a medium like a normal culture. Addition of 5% glycerol to the medium increases the dimensions of the phage spots but leads at the same time to an increase in the number of crossed-over reactions with heterologous phagotypes and is not recommended for that reason.

Preparation of typing phages and determination of their critical test dilutions (KTR). The typing phages reproduce on the phagotypes which are homologous with them, the other Vi-phages (I, III, IV, V and VI) on any strain of typhoid microbes, rich in Vi-antigen and capable of retaining the latter permanently; the O-phages on the strain O-901,
which is free of Vi-antigen.

The typing phage must first be purified. For this purpose a Petri dish with simple agar is inoculated with 6-7 drops of a day-old broth of the culture from a homologous strain. The culture is distributed over the surface in such a manner that the culture is continuous. After drying slightly, drops of several phage dilutions are applied on it to obtain isolated sterile spots. The culture is maintained for 16-18 hours at 37-38.5°C. The isolated phage colony together with the underlying agar and the surrounding culture is transferred into a test tube with broth, which is incubated for 3-4 hours. The phage is then heated at 57°C for 40 minutes (or filtered) and titrated on a solid medium for the typing strain corresponding to it. The operation is repeated in this order 3-4 times (the isolated plaque from the last dilution is again incubated into the broth, etc.) and a pure line of phage is obtained as a result. The titer of the phage, as a rule, increases during its passage through a sensitive culture.

The preparations of typing phage are prepared immediately in the volume of the required stock solution. The titer of the phage is highest at equal concentration of phage particles and microorganisms. Into 100 ml of a broth previously heated to 37°C, 10^8 bacteria individuals are transferred during their phase of logarithmic growth and approximately the same quantity of particles of the homologous typing phage. The incubation at 38.5°C is continued until the broth has become entirely clear (maximum 7-1/2 hrs). The lysate is heated 40 minutes at 57°C and centrifuged to eliminate the dead bacteria at 3000 rpm for 20-30 minutes. The preliminary critical test dilution is then determined on solid medium. For this purpose, the phage is titrated in tenfold dilution on a homologous strain and on the phagotype A.

If it is not possible to produce a phage with satisfactory criti-
cal test dilution on the liquid medium, the method of agar layers is employed to advantage. On the eve of the experiment, 25-30 ml of 1.5% meat-peptone agar is poured into sterile dishes. The dishes, covered with sterile filter paper, are dried for several hours under a bactericidal lamp, then the dishes are covered and they are left overnight in an inverted position. 3 ml of 0.45% agar is first poured into a test tube, then cooled to 45-46°C. $4 \times 10^8$ microbes are mixed into this agar with typing phage. The phage dose is determined empirically. It should be equal to the smallest number of particles which give confluent lysis with the above-indicated number of microorganisms. These conditions favor a maximum number of cycles of phage proliferation, as a result of which a minimum of bacterial cells remains intact. The mixture of phage and bacteria is poured on the surface of 1.5% agar and left on the table until it solidifies. The culture is grown at 38.5°C for 16-18 hrs. 6 ml of broth are then added to the dish, the surface layer of agar is scraped off, and the mixture of broth and semiliquid agar transferred into a 20 ml flask, heated 40 minutes at 57°C and centrifuged. Thus, a phage suspension containing up to $10^{12}$ phage particles per ml can be obtained (Anderson, Williams, 1956).

As soon as phage of sufficient concentration has been obtained, the final determination of the critical test dilution is carried out. The phage in the preliminary critical test dilution and in two subsequent dilution is tested on all known phagotypes and on the strain O-901 (Vi-negative strain, resistant to all Vi-phages). The last test serves for determining the presence of O-phages in the lysate. Such contamination is rare, but possible because the typing phages are grown on lysogenic strains which contain the determinants of the thermostable O-phages.

The titer of these phages in the lysate is extraordinarily low.
As a rule, a critical test dilution of the typing phage is unattainable for low titers of these phages.

Some difficulties in the determination of the critical test dilution arise with typing phages which produce on the homologous phagotypes fine, and on some heterologous phagotypes, large sterile spots.

As we know, for the formation of a confluent lysis, a drop of phage should give about 100 large spots and more than 1000 small spots (visible only with a magnifying glass). Hence, the fine-spot phage in a dilution, which gives confluent lysis with a homologous culture (critical test dilution) will lyse the heterological phagotypes, in which it gives large sterile spots, in the same manner. In order to avoid this, the critical test dilution of such phages must be such that a drop gives about 100 plaques on a homologous phagotype.

Highly specific typhoid typing phage in critical test dilution should have a range of activity approximately the same as that presented in Table 1. The reservation must be made that in identical phages from different production runs only the reactions of confluent lysis are unchanged. Weaker reactions (from ± to ++++) are not so stable. They cannot be considered as diagnostic reactions: the strains, on which they appear, vary from one series of phage to another.

The phages are stored at 4°. Undiluted preparations do not change in titer for 8-10 years. Critical test dilutions of the phages at the above-indicated temperatures are stable for several months.

**Typing technique.** A laboratory, where phage typing of typhoid cultures is carried out, should dispose of a range of typing Vi-phages of serotype II, Vi-phages of serotypes I, III, IV, V, VI and VII, and typhoid-adsorbing Vi- and O-sera. It is also desirable to have standard typing strains of known phagotypes. The scheme (Table 1) must be used in the establishment of the phagotype of the culture to be tested.
The typing technique is not complex. Into Petri dishes with a diameter of 9 cm, 20 ml agar is poured, and they are dried with covers off for an hour in a thermostat. Squares or sectors denoting the number of the phages used are drawn on the back of the dish with India ink or glass inks. The names of these phages are written on at the same time. It is convenient to mark the dishes by means of a grid on a rubber stamp. During the work, the stamp is moistened and, placed on a surface impregnated with glass ink. Some researchers (V.A. Kilesso) trace small circles on the agar surface with a test tube according to the number of phages used (Fig. 1). 32-34 such circles can be accommodated on a Petri dish. Drops of the culture to be tested are applied to the circle, the excess culture flowing into the furrows. The circles are inscribed and drops of typing phage applied to them. With a certain amount of practice it is possible to dispense entirely with the inscriptions on the bottom of the dish, if the phages are always used in a certain order, once and for all established and put down in writing. A spiral is drawn on the bottom of the dish, in a counterclockwise direction. The straight line which intersects the beginning of the spiral indicates the point of application of the first phage in the series. The possibility of formation of layers of phage drops is excluded because the drops of the preceding phage cannot dry out so quickly as to be invisible at the moment when the next one is applied. When the results are read off, the drops of all phages without exception are distinguished in relief on the dull velvet background of the growing culture.

The phages of the rarely encountered phagotypes are best used in the form of a mixture containing 4-5 phages. During the development of lysis, separate typing is carried out at the point of application of the mixture. The following order of using phages can be recommended:
A, A in KTR \times 20, B1 (B2, B3), C1 (C2, C3, C4, C5), (C6, C7, C8, C9),
D1, Da, D4, D5, D6 (D7, D8, D9, D10, D11), E1, E2 (E3, E4, E5, E6, E7,
E8, E9, E10), F1, F2 (F3, F4, F5), (G, H, J1, J2, J3) (K1, K2) (L1,
L2, M1, M2, M3), N, O, T (25, 26, 27), 28 (29, 32, 34, 35, 36, 37),
(38, 39, 40, 41, 42), (43, 44, 45, 46), V-I + Vi-IV, Vi-VII, 0-phages.
The phages enclosed in parentheses are mixed with each other. The
mixtures are prepared in such a manner that every phage is present in
critical test dilution.

Phage A is the most specific, which lyses in critical test dilu-
tion only the phagotype A. Phagotypes which have lost their specifici-
ty acquire a sensitivity to this phage. Phage A is used in critical
test dilution and in a 20 fold greater concentration in order to reveal
such degradation of cultures (Anderson and Williams, 1956).

Supplementary to the Vi-II phages, the Vi-phages can give clearly
defined reactions with Vi- and 0-phages and can be differentiated in
this manner. Secondly, the lysis of a culture by Vi-phages in critical
test dilution is specific for Salm. typhi and reveals its typhoid na-
ture. Thirdly, the Vi-phages help additionally to subdivide the phago-
types A, E1 and C1 and others, which dominate in the microbe popula-
tion of a number of countries. It is recommended to use also undiluted
Vi-I phage as in indicator of the presence of Vi-antigen in the strain.

A fresh broth culture is most easily typed because it contains the
largest quantity of Vi-antigen and hence adsorbs Vi-phages strongly.
The strain to be tested is introduced into 2 ml of undiluted tryptic
Hottinger digestion or Martin peptone in a quantity which gives an on-
ly just visible turbidity. The culture is kept in the thermostat at
38.5^\circ until it becomes cloudy, corresponding approximately to 5 \times 10^8
microbes per ml, which develops within 2-2 1/2 hours. The agar surface
is then inoculated with 7-8 drops of the typing culture which is dis-
tributed evenly over the whole dish by slight mutually perpendicular
movements of a spatula and dried. 1-2 ml of broth culture can be poured
on the agar and the excess sucked off with a pipette. Some researchers,
as has been pointed out earlier, apply the drops of the culture to
separate circles on the agar. Experience showed that the dishes with
the culture can be dried with the covers off without any harm. Drops
of the diagnostic phages are then applied to the surface of the culture.
Most convenient for this purpose is the use of Pasteur pipettes (L.Ya.
Kats-Chernokhvostova, 1949, and others), or syringes (Nicolle, 1957).
A separate pipette is taken for each phage. To avoid spilling of drops
from the pipette, a small quantity of phage should be taken (only in
the capillary) and the phage applied by lightly touching the agar
surface with the drop.

The culture can be inoculated with a standard platinum loop with
a diameter of 3 mm (classical method). The volume of the loop is approx-
imately 0.01 ml. The drop is spread by a circular motion until it has
a diameter up to 1 cm. The number of such drops is equal to the number
of phages used. To the dried culture drops, the drops of phage are ap-
plied with the same loop.

Almost identical results are obtained with all methods but the
first method is simpler and faster (Nicolle, 1957). Following the dry-
ing of the phage drops, the covered dishes are turned upside down and
incubated at 38.5°. This temperature is optimum for typing. The lower
limit of the temperature optimum is 38°. If the temperature is lower,
a tendency to an increase in the number of crossed-over reactions is
observed.

Evaluation and interpretation of the typing results. The first re-
cording of the results is best done after 6-8 hours of growth. In ur-
gent cases this makes it possible to report on the results of typing.
on the day on which the cultures were received. The second and final estimate of the data is carried out after 16-24 hours.

The lysis reactions are detected with the unaided eye, using indirect or direct illumination and shielding with the hand held between the dish and the light source and slowly moved. The results are easiest to evaluate in daylight. All doubtful or negative phenomena are checked by means of a magnifying glass with 7-10x magnification. Anderson and Williams (1956) recommend the following conventional symbols for the recording of the results:

<table>
<thead>
<tr>
<th>Dimensions of plaques</th>
<th>Number of plaques</th>
<th>Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>B large</td>
<td>± 0 to 5 spots</td>
<td>psl semi confluent lysis</td>
</tr>
<tr>
<td>N normal</td>
<td>± 5 - 20 spots</td>
<td>psl confluent lysis</td>
</tr>
<tr>
<td>M fine</td>
<td>± 21- 40 spots</td>
<td></td>
</tr>
<tr>
<td>OM visible only with</td>
<td>± 41-60 spots</td>
<td>psl &lt; sl intermediate degree of lysis</td>
</tr>
<tr>
<td>magnifying glass</td>
<td>+ 61-80 spots</td>
<td>ml turbid confluent lysis (turbidity caused by secondary growth)</td>
</tr>
<tr>
<td>μ microspots</td>
<td>+ + 81-120 spots</td>
<td></td>
</tr>
</tbody>
</table>

With a normal state of the strain, the determination of its phagotype does not offer any difficulty, provided the critical test dilution of the phages is correct and their activity corresponds to that in Table 1. Examples of the typical appearance of positive typing results are presented in Fig. 1.

Anomalous typing results belong among the following basic groups.

I. The culture is not lysed by the adapted Vi-II phage but is sensitive to the Vi-phages of other serotypes. Such a type of reaction is shown in Fig. 2a. This situation can be due to the following causes.

1. The culture is a new type. In this case it must be assayed with phages of rare types or Vi-II phage must be adapted to it. Phage A is used, as a rule, as the starting phage. Several methods of adapting phages of serotype II are known.
a) Several tenfold dilutions of the original phage are prepared in 5 or 10 ml of broth (Craigie, Ian, 1938). 3-4 hours old broth culture of the unknown type is introduced into all test tubes, diluted in such a manner that 1000 microbes cells are present in each test tube (with the aim of reducing the possibility of secondary growth to a minimum). The test tubes are placed into a thermostat overnight. The last test tube which shows a clearing-up, is heated 44 minutes at 57°C. A new series of tenfold dilutions is prepared from this and this is repeated several times until the titer of the new phage stops increasing.

b) The fresh broth culture of the strain to be tested is inoculated on the gason of dishes with 1.5% agar. Drops of the tenfold dilution of the phage to be adapted are applied to the dried culture. The dishes are incubated overnight at 38.5°C. In the morning the separate plaque with the culture under it is transferred into a test tube with 2 ml of broth and incubated at 38.5°C until the initial growth has disappeared. The lysate is then heated to 57°C and kept at this temperature for 40 minutes and then centrifuged for removing the dead bacteria. This procedure is repeated several times with the aim of obtaining a highly specific phage (until its titer on the strain to be tested stops increasing).

The newly produced phage in critical test dilution is checked with all standard phagotypes. Selective lysis with the culture, through which the phage has been passed, and the absence of lysis with the standard strains confirms that a new phagotype has been isolated. In the rare cases, when the Vi-II phage of Craigie and Ian (type A) does not reproduce on the untypable Vi-strain, it is recommended to test this capacity on other phages of the test series. For example, only T-phage could be adapted in the experiments of Scholtens (1950) to the new phagotype with identical type-determining phage (see section
"Adaptation"). Through the new phagotype T 4904, containing phage d6, the author successfully passed D6 phage (structural formula of type D6-A(dg)). Finally, Desranleau and Martin (1950) isolated the original Vi-phages of the serotype II from the types A, Cl, El and Fl (the Vi-II phage of Craigie was isolated from a culture of type A). The flexibility of these phages was not equal. The authors could not adapt the Vi-II phage of Craigie to even a single one of the 234 cultures of type E4. The Vi-II phage isolated from phagotype El did adapt to them. This was also found by other researchers (Scholtens, 1956b).

II. The culture in the gamma or Imperfect-form is stable to phages of serotype II, and does not allow them to proliferate during adaptation. Such cultures can be subdivided on the basis of their reactions with unadapted Vi- and O-phages, and the phages of Nicolle and Diverneau (1961) in lysogenicity tests.

III. The culture is destroyed by several adapted Vi-II phages, by confluence or semiconfluent lysis (polylysable or Vi-degradated strain). Figure 2 shows an example of the reactions of such a strain. The culture is clearly lysed by phage of type A, which indicates its degradation. Under such circumstances it is very difficult to determine the phagotype of the strain but it is possible, on the basis of the pattern of reactions with Vi-II phages which is normally constant, to discern and combine epidemiologically connected strains. It is permissible to term these cultures as phagotype A indicating the gaps in the lytic reactions.

Among the polylysable cultures the strains which interact with phage N and with individual phages of group D (the so-called phagotype D3 or N + D1) must be specially separated. Several strains of phagotype N can give such reactions, when typing is carried out at low temperature (36-37.5°). Incubation of the dishes at 38.5° decreases
the nonsepecific reactions with D phages and the strains are classified as normal type N.

IV. The culture is weakly lysed by several typing phages, is lysed efficiently by the unadapted Vi-phages and is insensitive to phage A. The cause of this may be a decrease in the dimensions of the phage plaques after passing through an atypical variant of the phagotype. The homologous phage in critical test dilution gives confluence lysis under conditions of normal dimensions of the sterile spots. As a result of the decrease in the dimensions of the plaques, the lysis becomes semiconfluent, sometimes in the form of isolated plaques. In such situations it is recommended to repeat the typing with phages in a 10 times greater concentration than the critical test dilution.

Fig. 1. Reactions of phagotype A and E4 of typhoid bacteria with adapted Vi-II phages in critical test dilution. a) Type A; b) type E4; (in the center — lysis by Vi-I, 0- and Vi-VII phages [according to V.A. Kilesso]).

In the extreme case, when a strain is not clearly differentiated even by a single one of the standard phages, so-called indirect typing is justified.

Vi-II phage is adapted to the strain to be typed. The new phage is tested on all standard typing strains. The strain to be tested is considered to be identical with the phagotype with which the new phage preparation clearly interacts in critical test dilution. The lytic
Fig. 2. Nontypable Vi-culture (lysis with a mixture of Vi-I and Vi-IV phages can be seen); b) degenerate Vi-culture (lysis with phage A in 20 x KTR can be seen) (acc. to Anderson and Williams, 1956).

inertia of the new preparation with regard to the standard phagotypes attests to the isolation of a phagotype, whose typing phage is absent in the test series.

V. The cultures is not lysed by any of the Vi-phages, used in critical test dilution, but is sensitive to concentrated Vi-phage of serotype I. The cause may be a weak development of Vi-antigen in the culture, which inhibits the adsorption of the typing phages on it. One can try to type such a culture with a more concentrated phage (one dilution less than the critical test dilution).

In order to increase the Vi-antigen concentration in the culture, the following methods are known:

a) Inoculation of the strain on 1.3-1.5% agar with subsequent selection of the colonies which are turbid in transmitted light and which are strongly agglutinated on a glass slide by adsorbed typhoid Vi-serum. Up to 40-50 separate colonies of different subcultures must be checked.

b) Passage through broth containing 4% typhoid O-serum for 6-18 hours in a thermostat, followed by inoculation of the culture from the upper part of the broth on agar dishes and selection of colonies by
means of Vi-serum. After having been kept in the thermostat, the culture can be centrifuged and then inoculated from the supernatant liquid.

c) Passages through the organism of white mice. A day-old culture, grown on normal broth, is diluted in a ratio of 1:20. 0.5 ml of the dilution is injected into the mice intraperitoneally. 60 hours later the mice are killed with chloroform. The blood from the heart and the exudate from the abdominal cavity, washed off with physiological NaCl solution is inoculated on normal weakly alkaline agar. In the tentative agglutination tests, the Vi-antigen content is checked in no fewer than 20 colonies.

All methods of transforming Vi-negative cultures into Vi-positive cultures are only successful if the strain is present in the VW-form, i.e., if it has lost only part of the Vi-antigen. Such cultures are normally not agglutinated by Vi-serum, their lysis with a mixture of Vi-phages of the serotypes I and IV in critical test dilution is inconstant and absent in individual cases. The strains interact more regularly with concentrated Vi-phages of these types.

VI. The culture is not destroyed by even one of the Vi-phages in critical test dilution, including the undiluted Vi-I phage. This indicates a complete absence of Vi-antigen in the culture or even excludes the typhoid strains. In cultures present in the pure VW-form, as a rule, the Vi-antigen is not restored. If this is necessary, they can be typed by means of O-phages or biochemically.

It must be pointed out that clones of the same culture may differ in the quantity of Vi-antigen content and in the degree of their specificity. Hence, when the reactions are not clear, it is useful to repeat the typing with other clones. In order to do this, it is best to send for typing a mixture of 10-12 colonies of the original culture particu-
larly if it has been isolated from enrichment media. Sending only one colony always entails the risk that the clone will be polylysable or present in the pure VW-form which cannot be differentiated with Vi-phages.

Cultures which are constantly used in the work should preferably be kept on a Dorset egg medium in the dark at 4° after minimum incubation at 37-38°. A tendency to lose part of the Vi-antigen is present in some strains, hence it is useful, when renewing the culture to incubate it on agar and select by the above-described method those clones which are rich in Vi-antigen. Colonies which give a clear Vi-agglutination, are transferred to Dorset medium and stored as usual. The lyophilization preserves the cultures for longer periods.

Isolation of mild phages from cultures of Salm. typhi. The isolation of mild phages from cultures of typhoid bacteria is difficult to achieve: the phage yield is low and their titers increase only slightly. Cultures of phagotypes A, El, the strain 0-901, Salm. gallinarum, Salm. paratyphi C in the logarithmic phase are used as indicators. The mild phages of subtype A are isolated on the strains Tananarive and Salm. dublin.

Isolation of mild phages. Several methods of freeing mild phages have been described (Anderson, Felix, 1953; W. Ferguson, Junkes, R. Ferguson, 1955; M.D. Krylova, 1951; Nicolle, Diverneau and Brault, 1958).

1) To 2-3 ml of undiluted broth (Difko [?], Martin or Hottinger) a small quantity of the strain to be tested for lysogenicity is added. The culture is grown at 38.5° for 8-16 hours and centrifuged at 3000 rpm for 10-20 minutes. The supernatant liquid is divided into two parts. One part is heated to 57° and kept at this temperature for 40 minutes while the other one is not heated. Drops of the heated and unheated
liquid are applied to the gason of the indicator culture. Following overnight incubation at 38.5°C, the presence of phage plaques is checked. The plaque with underlying culture is transferred into broth.

2) To 20 ml of the heated undiluted broth add $10^8$ microbes of the strain to be tested and of the indicator strains. The mixture is grown at 38.5°C for 8 hours and checked for the presence of phage on the indicator culture by the above-described method. During growing of the strain to be tested together with the indicator strains, the mild phages are isolated with greater success, than in a pure culture.

3) To 15-50 ml of an infusion of calf broth add $10^5$ of the microbes to be tested (with poor phage yield the strain to be tested is inoculated together with $10^5$ individuals of the indicator strain). After overnight incubation at 37.5°C the culture is mixed and aerated for 3 hours at the same temperature. The content of the flask is then centrifuged at 3000 rpm for 10-20 minutes and checked for the presence of phages as indicated above. The supernatant liquid can be filtered off.

4) The method of agar layers according to Gracia. On the surface of well dried 1.5% meat-peptone agar the culture to be tested for lysogenicity (donor culture) is applied in streaks. 0.7% agar in a quantity of 2.5 ml which has been previously poured into a test tube, is melted and cooled to 45°C. 0.1 ml of the indicator culture is then added and quickly mixed with the agar. The mixture is poured on the surface of 1.5% agar and left to cool for 30 minutes, after which it is placed into the thermostat at 37°C and left for 18-20 hours. If the culture is lysogenic, plaques are formed along the streaks; these are isolated, and purified by inoculation on an indicator culture.

5) The strain to be tested is lysed by Vi-phage of serotype I. The lysate is passed through the strain 0-901 until a constant liter is obtained (M.D. Krylova, 1953).
Increase in the titers of mild phages. The maximum titer of mild phages is not very high: $10^7 - 10^8$ particles per ml. Ferguson and co-authors (1955) obtained phages with such concentration in the following manner. Into 20-50 ml of the medium they inoculated $10^6$ microbes of the indicator strain. After aeration for an hour at 37-38°C, they added $10^5 - 10^6$ particles of mild phage and continued to aerate the mixture for another 2-2 1/2 hours. The suspension was centrifuged and the supernatant liquid filtered off.

Anderson and Felix (1953) found that the highest phage titers develop at an optimum ratio of the phage and the indicator culture, which is individual for each system. If an excess of microbes is present, the mild phage is adsorbed on the cells. As a result, the titer drops quickly. This is why during minimum secondary growth, the maximum phage titers are obtained. Into a flask containing 20 ml of Difko broth, 2 x $10^8$ microbes of the indicator culture are placed and 1 ml of primary mild phage added. The mixture of the culture and phage is incubated at 38.5°C for 8 hours. Its turbidity is checked and compared with that in the control flask (without phage), which had been inoculated with only $10^8$ microbes. At the end of 8 hours, the two flasks are transferred to a refrigerator (4°C) and left overnight. In the morning, 0.1 ml from each flask is transferred into 20 ml of preheated Difko broth and again incubated for 8 hours at 38.5°C after which they are placed in the refrigerator overnight. These manipulations are repeated daily and the phage titer is checked daily in the test flask. All flasks are kept at 4°C. After several passages, the phage titer increases, which is manifested by a clearing up of the medium in the test flask. This attests to a high phage titer. With this passage the operation is terminated because the next transfer is usually accompanied by a rapid increase in turbidity and a decrease in the phage titer in the test flask.
flask preceding the one, in which the clearing up had been observed, and the control flask, 0.75 ml is transferred into 150 ml of fresh Dif-ko broth. Both flasks, the test flask and the control flask, are incubated at 38° until complete clearing up of the test flask. The phage preparation is freed of microbes. Anderson and Felix (1953) recommend 3 methods for this separation.

1. Heating to 57° and keeping for 40 minutes.
2. Treatment with 0.16% toluene at 37° for an hour.
3. Filtration through a fine porous membrane (750 μl) (filtration through an L3 candle, through Seitz filters, EK filters, etc., is not recommended, because these lead to a loss of 95% of the phage).

Methods of obtaining artificial phagotypes. Anderson and Felix (1953) proposed two methods of lysogenization of typhoid cultures.

1. On the gason of the indicator culture to be subjected to lysogeni-ation, a drop of undiluted mild phage is placed and incubated for 16 hours at 38.5°. The secondary growth is inoculated into 2 ml of broth, and incubated at 38.5° until turbidity appears corresponding to 1-1.5·10^6 microorganisms and tested for interaction with critical test dilutions of all Vi-II typing phages. This method gives good results if the mild phage has a sufficiently high titer (1·10^7 and over) and is capable of developing confluent lysis on an indicator culture.

2. To 1 ml of a lysate containing 10^6-10^7 mild phage particles, a small quantity of the culture to be lysogenized is added (1-10 microbes). The mixture is incubated at 38.5° for 24 hours. The proliferating culture is, as a rule, lysogenic. It is inoculated and the change of the phagotype tested. It is supposed that at such ratio, the probability of an encounter of microbe cells and phage particles during the first hour is extraordinarily low and they do not interact. The culture can proliferate uninhibited as long as the number of microbes is
not sufficient for encounters with phages. The sensitive microbes then die off on account of the proliferating phage and lysogenicity is established in the remaining ones.

This method is used if the phage titer is less than $10^6$ particles in 1 ml. $2 \cdot 10^8$ mild phages are introduced into 20 ml of preheated broth in its maximum concentration. The mixture is left to stand for 8 hours at 38.5°, then overnight in the refrigerator (4°) and then 0.1 ml is transferred into 20 ml of fresh broth. The procedure is similar to that used for producing latent phages with high titers (see there). In contrast to the latter, the passages are continued after the appearance of massive lysis (attesting to a high titer of the phage). During the next transfer rapidly increasing turbidity is observed, due to the proliferation of resistant microbes which had become lysogenic. At the end of the 8 hours of incubation this culture is transferred to Dorset medium and the change in the phagotype checked on several clones.

The typing method is based on the unequal behavior of different cultures to the derivatives of the Vi-phage of serotype II. This phage has the property of adapting with unusual ease to resistant species, thus losing the activity to the original strain. All preparations of typing phages were obtained via adaptation. Unadapted typhoid Vi-phages of different serotypes and O-phages are also included in the scheme. The typing Vi-phages are not differentiated by strains devoid of Vi-antigen, Vi-degradated cultures, and the Vi-I – Vi-IV group. Individual grypes (A, E, C and others) are encountered very frequently within the limits of a single country. Some of these can be subdivided on the basis of their biochemical characteristics and also by means of bacteriophages.

The specificity of the typhoid phagotypes is mainly due to the lysogenicity and represents a particular case of lysogenic conversion.
It is mainly due to the interference of the prophages with the unrelated mild phages.

The type-determining function of individual mild phages was demonstrated in experiments involving artificial lysogenization of phagotypes. These experiments made it possible to establish structural formulae for certain lysogenic phagotypes, which help to predict the lytic properties of the typing phages obtained by adaptation, and to explain the origin of the group lysis reactions, and permit a rational selection of the original phages for producing phage preparations for producing phage preparations for untypable strains. Using the structural formulae, Anderson and Frazer (1955) laid the basis for a classification of phagotypes.

The phagotype characteristic of a microbe is a highly stable property. The application of the method in practice to elucidate epidemiological connections is based on this. Phage typing improves epidemiological research, makes bacteriological diagnosis more accurate and at the same time shortens the time required for the investigation. The method opens up prospects of improved specific phage prophylaxis and phage therapy of typhoid and indicates a new approach to the production of phage preparations.

Manuscript
Page No.

Chapter 3

PHAGE TYPING OF PARATYPHOID A AND B BACTERIA

BACTERIA OF PARATYPHOID A

The method of phage typing of paratyphoid A bacteria was proposed by Felix and Banker in 1948 (Banker, 1955). By means of a phage, isolated from sewerage effluents at Bombay, the strains of paratyphoid A could be subdivided into two groups. By adapting this phage to resistant strains, Banker subsequently obtained another four typing phages which proved to be O-phages and were identical in their serological characteristics. The strains of paratyphoid A, isolated in different countries, were subdivided with the aid of these phages into five phagotypes. Type 1 was lysed by all phages, the other four types only by their own homologous phages.

A certain practical usefulness of the method was demonstrated during investigations of paratyphoid B flareups. The weak point of the method is the frequency of occurrence of unstable phagotypes. This concerns above all the type 4. Following passage through the gall bladder of guinea pigs in the experiments of Rische (1958), some cultures of this phagotype acquired a slight sensitivity to the other phages. The same degraded cultures were isolated together with type 4 from patients and during storage of this phagotype on laboratory media. In the experiments of Buczkowski (1960) in the cultures of 12 out of 147 carriers and patients, obtained in repeated analyses, or different clones of the same culture differed in their reactions with typing phages from each other. For example, in one of the cultures, two clones
proved to be phagotypes 1 and 3. In several patients the phagotype 1 and polylysable types and in others, phagotype 1 together with type 3 or resistant types were isolated simultaneously. Moreover, in to paratyphoid A flareups, caused by type 1, the author noted the appearance of other phagotypes and polylysable cultures. All these data attest to the imperfection of Banker's scheme.

Another deficiency of the method is the prevalence of the phagotype 1 in the tested cultures. Banker (1955) found among 636 strains of paratyphoid A from different parts of the world, the type 1 in 59%, type 2 in 14%, type 3 in 2.2%, type 4 in 7.9%. The degraded strains accounted for 3.1%, the nontypable strains 13.8%. Rische (1958) using 5 typing phages, detected among the strains from 100 patients from Germany, Czechoslovakia, Rumania and Hungary a similar distribution frequency of phagotypes, 67% of them belonging to type 1. In Poland (Buczkowski, 1960) the phagotype 1 was found in 3/4 of patients, reconvalescents and carriers from different foci. Such a preponderance of phagotype 1 naturally reduces the epidemiological value of the method because it gives the impression of a uniformity of the types of microbe population.

A defect of the scheme is also the enormous number of phagotypes which has been detected within this species of bacteria.

It is thought that all the abovelisted deficiencies of the method are a consequence of the erroneous selection of the original phage among the virulent phages of sewerage effluents. As we know, the differentiating action of these phages is slight, they are more suitable for the diagnosis of the species than for intraspecies typing. Mild phages isolated from lysogenic cultures are preferable for phage typing.

BACTERIA OF PARATYPHOID B

Two schemes of typing bacteria of paratyphoid B are known at the
present time: the scheme of Felix and Callow and the natural system of
Scholtens constructed on this basis.

Scheme of Felix and Callow

In 1953 Felix and Callow (Britain) proposed a scheme for the phage
typing of paratyphoid B bacteria, in which adapted, but serologically
heterogeneous phages are used: 1, 2, 3a, 3b, 3al, Jersey, Taunton, Dun-
dee, Beccles and BAOR (Felix and Callow, 1943, 1951). The method of
Craigie and Ian is used for typing.

Typing phages and strains

The phage type 1 was isolated from a lysogenic strain (Felix and
Callow, 1943). The phage selectively lysed cultures isolated during a
flareup of paratyphoid B, which were termed phagotype 1. Later on, via
adaptation of phage 1 to resistant strains, the phages 2, 3a, 3b, 3al,
Jersey, Taunton and Dundee were obtained. The beccles phage was produced
by adaptation of phage 1, isolated from a different lysogenic strain;
the BAOR phage was isolated directly from a lysogenic strain of type 1.

Despite the fact that all typing phages of paratyphoid B are deri-
atives of phage 1, only four (2, 3a, 3al and Jersey) are serologically
identical with it. The others belong to two serological groups. One
group is comprised of the phages 3b and BAOR, the other of the phages
Beccles, Taunton and Dundee.

The serological heterogeneity of paratyphoid B typing phages with
common origin is explained by the fact that the phage of type 1 when
cultured on the original culture may be contaminated with mild phages
of the host strain, which became the starting point for the new typing
phage.

With the above-listed 10 phages one can subdivide the microbes of
paratyphoid B into 10 types, designated in correspondence with the name
of the respective phages. On top of this, about 30 variants of types 1,
2, 3a, 3b, Beccles and Dundee were detected. Part of these (3a vari-
ant of 1, 3a variants of 2 and 3, 3b variants of 2, 3, 4 and others)
are unstable in their reactions with the typing phages while the
others are more constant. It is quite probable that some variants are
new phagotypes.

The method of Felix and Callow was standardized and recommended by
the International Conference of Microbiologists for international use.

The lytic activity of the 10 standard phages with regard to the
phagotypes and their variants is represented in Table 7. The 11th phage
(1010) does not differentiate any phagotype but helps to distinguish
some of them from each other. It can be seen in Table 7, that the
typing phages of the paratyphoid B bacteria differ greatly from the ty-
phoid typing phages in the specificity. Really specific is only phage
1, which lyses only the homologous type 1. All other phages interact
with two or more phagotypes. This is why it is necessary for recogni-
zing the phagotype of a culture to take into account its reaction with
the homologous and with all the heterologous phages.

The phages of the paratyphoid B bacteria, like their typhoid coun-
terparts, are used in critical test dilutions. This is the maximum di-
lution of the phage in which it gives the corresponding reactions with
homologous and some heterologous phagotypes (for the latter it is an
indicator) (see Table 7). For example, the typing phage of Jersey in
critical test dilution should completely lyse the phagotypes 1, 3a var-
iant of 3 and Jersey.

At first the paratyphoid B phages were termed Vi-phages, because
they specifically lysed strains, containing Vi-antigen (detected in
1936 by Felix and Pitt) and did not interact with pure O-variants. It
was soon found that some phages are active on Salmonella cultures which
are devoid of this antigen, in particular on Salm. typhi murium, Salm.
TABLE 7
Scheme of Phage typing of Paratyphoid B Bacteria (Anderson and Williams, 1956)

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<th>Variant b</th>
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| typing phages in critical test dilutions; ml) lysis with secondary growth; (-) designates the absence of lysis.

a) Type; b) variant; c) typing phages in critical test dilutions; d) Jersey; e) Beccles; f) Taunton; g) BAOR; h) Dundee; i) sl; k) ps1; l) ml; m) normal variants.

 abortus equi, Salm. newport and others (Felix, 1956).

Subsequently Kauffmann showed that the Vi-antigen of Salm. paratyphi
3 is the O5-antigen (K-antigen) which is not comparable with the VI-antigen of Salm. typhi. The O5-antigen is not specific for paratyphoid B bacteria and can exist in other Salmonella types of group B. These observations led to the conclusion that the typing phages of paratyphoid B bacteria are typical O-phages.

The stability of the phagotypes of paratyphoid B bacteria was observed in the organism of patients and carriers (during repeated separation from feces, urine, blood, bile, pus, and cerebrospinal fluid) after repeated reseeding and during prolonged storage on nutrient media (Felix and Callow, 1943; Nicolle, Jude, Buttiaux, 1950; R.V. Gordina, 1954; V.A. Kilesso, 1954, and others).

A change in phagotype was observed in 10% of cultures, kept on nutrient media for 1.4 years (R.V. Gordina, 1954). It was nearly always manifested as an extension of the valency to typing phages. It is assumed that the type Beccles is subject to the above described deviations to a higher degree than the others. During prolonged storage sections of the types 1 and 3al (Felix, Callow, 1951), types 3b and 3a (Nicolle, 1957) appeared in it.

causes of the specificity of phagotypes

Prophages (one or several) are extraordinarily widespread in cultures of paratyphoid B (Scholtens, 1951, 1956a, and others). All 10 types of a paratyphoid B bacteria are carriers of mild phages. Some of these are serologically related to and also identical with the typing phages.

In order to confirm the type-determining functions of the prophages, Nicolle, Hamon and Edlinger (1951) carried out several successful transformation of 1, 3a and 3b cultures, by inoculating them with phages isolated from other phagotypes. For example, phagotype 1, when invaded by a phage of type 2, was transformed into phagotype 2; a 3a cul-
ture after treatment with a phage of type 3a1, gave the reactions of
the strain 3a1, the phagotype 3b could be transformed into Taunton and
Dundee. The artificial phagotypes were less sensitive to the test ser-
ies of typing phages than their precursors (compare in Table 7, for ex-
ample, the type 3a and 3a1, 3b and Dundee, and others). In other words
as a result of lysogenization, the valency of the culture to typing
phages was narrowed.

The reverse process, i.e., an extension of the valency of a cul-
ture during action of a mild phage is an exceptional phenomenon. In
particular it was observed by Brandis (1953). Over several years, he
isolated BAOR cultures and atypical variants which interact withpha-
ges 3a and 3a1, to which the normal BAOR types is resistant, from the
feces of a female carrier. The variants contained a mild phage, which
was designated St and which is serologically related to the above 2
phages (Brandis, 1955a). When lysogenized with the phage St, the
strains BAOR and 3b and the nontypable strains were transformed into
atypical variants, sensitive to the phages 3a1 and 3a. Thus, contrary
to the usual behavior, the mild phage imparted to the culture not re-
sistance but sensitivity to serologically related phages. Brandis could
not s- actorily account for this phenomenon. It is possible that in
the culture treated with mild phage, the sensitivity to serologically
related phages appears as a result of replacement of one prophage by
another or freeing from lysogenicity.

On the whole, artificial transformation of the phagotypes was less
successful in paratyphoid B cultures than in typhoid cultures and the
successful experiments were not very numerous. Nonetheless, bearing
in mind that all typing phages of the scheme of Felix and Callow were
produced from latent phages, we may take it that the immunity of these
bacteria to the phages which are serologically related to their pro-
phage forms the basis of the subdivision by phages. In this sense, the mechanism of the specificity of the phagotypes of paratyphoid B, it must be supposed, is different from that in typhoid bacteria, where the type-determining prophages create resistance in the bacteria to the foreign phage.

**Application of the method in epidemiological investigation**

In the course of almost 20 years of application of the method of Felix and Callow the investigators became convinced of its epidemiological value (R.V. Gordina, 1954; Felix, 1944; Corridan, 1951, Nicolle, 1957; Grosso and Saccani, 1958, and others). It has entered the daily practice of numerous laboratories all over the world as a method which makes bacteriological diagnosis more accurate and improves the epidemiological analysis.

International approval of the method of Felix and Callow made it possible to study the distribution of the phagotypes of paratyphoid B in different countries (Felix, 1955). The most prevalent phagotypes in Europe are Taunton, 3a1, 3a, and BAOR. In individual countries, for example, Poland and Norway, the types 1, Dundee and Jersey predominate. The type Taunton is the most widespread. In the GDR it was detected in different years in 39-57.9% (Rische, Schneider, 1960). The types 3a1 (normal) and 1 are found more rarely.

The variants 1, 3a and 3b of the first paratyphoid B are not often found. Thus, in the FRG they constitute only 0.02 to 1.58% of all cultures. Only the 3a1 variant of 1 was detected in a much greater number (5.19%) (Brandis, 1958).

In the years 1953-1957 in the GDR these variants accounted for 6.97% of cultures, of which the 3a1 variant of 1 amounted to 3.12% (Rische, Schneider, 1960).

The methods of additional subdivision of the phagotypes of paraty-
phoid B have not been developed extensively, although they could have been useful, particularly for the type Taunton. The biochemical method of subdivision of the phagotypes of paratyphoid B bacteria were tied in Poland (Lalko, Pietkiewicz, 1962). The biochemical type of the same phagotypes isolated in different foci, is in most cases the same. A connection between the phagotype and the capacity for enzymatic breakdown of rhamnose and inositol has been demonstrated: the phagotypes 1, 3a, 3al, 3b, Beccles, BAOR and Dundee break down both sugars, the types 3al (variants 1, 2) and Taunton only rhamnose. The type Jersey was subdivided into two biochemical types (+-rhamnose and -inositol, and -rhamnose, +-inositol). Biochemical typing was a useful correction for the phagotyping data of cultures from foci.

R.V. Gordina (1945) found a difference in different cultures of type 1, 2, 3a and 3b with respect to inositol, in type 2 to maltose, in type 2 and 3a to sorbitol. The role of the biochemical subgroups in epidemiology has not been studied by this author.

Typing of paratyphoid B microbes in the USSR has been first carried out by R.V. Gordina in 1947 with the five first phages of Felix and Callow. The Soviet researchers became convinced of the great epidemiological value of the method. M.K. Kadyrova (1960b) used phage typing during a flareup in a food manufacturing plant.

Within 2 weeks, 20 persons became ill. During the bacteriological investigation of the contacts, the paratyphoid B bacillus was isolated from the feces of the bread cutter. The duties of the bread cutter included the delivery of beverages from the lemonade factory. Paratyphoid B bacilli were detected in two female workers of the factory, one of whom had contacts with the bread cutter not only in the course of duty. Three months ago the female worker had the disease, diagnosed as influenza. 2 weeks prior to the flareup, the bread cutter also fell ill, but
did not stop working. The symptoms of his illness were reminiscent of the blurred form of paratyphoid (malaise, headaches, fever, etc.). Finally, the following chain was discovered: female operative of the lemonade plant → bread cutter → flareup at the food plant. Phage typing confirmed this sequence: all cultures from the patients, from the bread cutter to the female operator, belonged to phagotype 3b.

The phagotypes 1, 2, 3a, 3al, 3b were detected in the USSR (R.V. Gordina, 1954; V.A. Kilesso, 1954; M.D. Kadyrova, 1960a, and others). The phages Jersey, Beccles, Taunton, BAOR and Dundee were not applied in our country. Comparison with foreign data demonstrates that the relatively rare phagotypes 3b and 2 predominate in the USSR. However, one cannot yet speak of a dominance of certain types and of their geographical distribution because the percentage of cultures which have been typed is too low.

Method

The method of typing of paratyphoid B bacteria with the phages of Felix and Callow (preparation of typing phages, cultures, media, technique of typing) are analogous to that for typhoid bacteria. The phages are used in critical test dilutions. In addition to paratyphoid B phages, typing phages of Salm. Breslau are also used, taking into account that cultures sent with the designation Salm. paratyphi B. often prove to be Salm. typhi murium. The first reading of the reaction is carried out with a magnifier 10x with indirect and direct lighting after growing for 5 hrs at 38.5°C; the second one after 24 hrs incubation.

The anomalous reactions include the following variants (Anderson, Williams, 1956).

Culture of non paratyphoid B — nature: a) The tested strain is not lysed by the paratyphoid B phages, but interacts with a mixture of the phages of Salm. Breslau. b) The strain gives a new, unusual type of re-
reaction with the typing phages and interacts with Salm. Breslau phages.

The antigen structure of the microorganism is carefully checked in either case. Such an investigation often makes it possible to relate the strain to one of the groups of the system of Kaufman-White. It is known, by the way, that Salm. abortus equi (somatic formula 4, 12), Salm. newport (6, 8), Salm. gallinarum pullorum (1, 9, 12), Salm. enteridis (1, 9, 12) are always sensitive to the typing phages of Felix and Callow, although they do not give the lysis pattern which is typical for certain phagotypes.

The culture belongs to a new phagotype. The culture belongs to the paratyphoid B microbes but does not interact with the typing phages or gives anomalous, hitherto unknown reactions with the phages of Felix and Callow. It is not sensitive to Salm. Breslau phages. It is recommended in either case to produce a new phage for the strain to be tested. The original phage can be selected among the typing phages or isolated from lysogenic cultures of Salm. paratyphi B, Salm. typhi and other Salmonella. It is not recommended to use phages sewerage effluents and feces, as phages of such origin are normally not very specific and not suitable for typing.

The Natural Scheme of Scholtens

Although the method of Felix and Callow has gained international recognition, it is far from being perfect. While in most European countries the number of nontypable cultures does not exceed 3%, 30% cannot be typed by means of these phages in the US and in Indochina, even up to 50% of cultures (Nicolle, 1957). Many years of practical verification revealed other defects in the method of Felix and Callow. These were manifested in a discrepancy between the typing data and the epidemiological conclusions. And, although such incidents were extremely
rare, they attracted the attention of researchers. In particular, in the Netherlands, in Lemmer, Scholtens (1955a) isolated two different phagotypes — Beccles and 3a1 variant of 1 from the patients during a large paratyphoid B epidemic, connected by a common infection source. At London, Felix differentiated both strains as the Beccles type, Nicolle at Paris as 3a1. Thus, the weak point of the method of Felix and Callow was revealed — the possibility of a nonobjective identification of cultures. This impelled Scholtens to researches for improving the method.

Mild phages of paratyphoid B bacteria

Scholtens became convinced that the system of Felix and Callow does not by any means utilize all the mild phages of the paratyphoid B bacteria: first-class diagnostic phages isolated from mixed cultures are not included in it.

Only 5 serologically different phages can be isolated from pure cultures. Scholtens (1955b, 1956a) termed them serotypes I, II, IV, VI and VII. These phages differ in the morphology of the sterile spots, their heat stability and the lytic spectrum. The phages of types I, IV and VI develop large sterile spots, and are present in many phagotypes of paratyphoid B. The plaques of the phages II and VII have small and very small dimensions. The phage II has been detected in the phagotypes 1, 3a, 3b, Jersey, and Beccles, the phage VII only in type 2.

Mild phages can be isolated in mixed cultures of two strains, which cannot be discovered in the culture of each separate strain. It is possible that in the mixed culture, as a result of the passage through a sensitive culture, the initially slight virulence of these phages is increased. According to their serological characteristics, most phages from the numerous pairs of Salmonella of paratyphoid B were divided into 2 autonomous groups, identical with the serotypes II and
VI of the phages from pure cultures. The phages from the mixed cultures, however, had a characteristic lytic spectrum. The phages of both groups gave 6 types of reactions with certain test strains (see further on): e, d, c, f, h, b. These reactions were more distinct and stable in the phages of serotype II than in the phages of serotype VI.

Scholtens used the phages from pure and mixed cultures together with the phages of Felix and Callow for differentiating the phagotypes of paratyphoid B. The lysogenicity of the test cultures was studied at the same time. As a result, a new typing system was established, which was termed the natural system (Scholtens, 1959). The term "natural" is connected with the fact that the interaction of paratyphoid B bacteria with the phage preparations in this system is exclusively connected with its lysogenic characteristics. In the natural system, the phagotype is a group of strains in which the lytic spectrum and the lysogenic properties are identical (Scholtens, 1961).

Typing phages and strains

In the Scholtens system, the bacteria are subdivided into groups, each of which includes several phagotypes (Table 3). Correspondingly, some of the phages are group determining phages, others typing or type-determining phages.

The group-determining phages are 5 serologically identical phages of Felix and Callow (1, 2, 3a, 3al, Jersey) and 6 mild phages of serotype II with the lytic spectrum e, d, c, f, h, b, isolated by Scholtens from mixed paratyphoid B cultures. The Scholtens phages are of decisive diagnostic importance.

Depending on the reactions with the group-determining phages, the paratyphoid B cultures are subdivided into 10 groups or series: A, M, S, I, BM and others (see Table 8). All the strains of one group are monotypical and interact with the above-mentioned phages. For example, the
culture belongs to group A, if it is lysed by the phages d and h, to group B, if it is lysed by the phages 3a, e, d, h, b, etc. The strains of a single group, moreover, when mixed with cultures of group A, produce latent phages with the same range of lytic action. Thus, for example, all strains of group M produce phage d. It is active on strains of all the groups except group M. For this reason, the inertia of a culture to phage d confirms that it belongs to group M. With a pair of strains from group A it is possible to produce phage e, which is active on all groups except A, etc. Thus, the group affiliation of the cultures is correlated with their lysogenicity.

The groups are subdivided into phagotypes. For this purpose, the mild phages of the serotypes I, IVb, IVa and VI, extracted from pure cultures are used (type-determining phages). The phage of type I is identical with the phage 3b of Felix and Callow, the phages IVb and IVa, with the phages Beccles and Taunton, respectively. The phagotypes are arranged within the groups in the order of the increase in the number of mild phages contained in them and the corresponding decrease in the sensitivity to these phages. Say, the phagotype 3a (normal) occupies the first place in the group A. In a pure culture it does not give off mild phages and therefore enters into lytic reaction with all 4 type-determining phages (see Table 8). The type Beccles 22 contains the phage of serotype I and interacts only with 3 phages (IVb, IVa and VI). The strains of phagotype 3a, variant 2, contain the phage VI, but are lysed only by the phage I, etc. The other groups were formed on the basis of the same principle. The strains of identical phagotypes contain the same mild phages. In consequence of this, testing of the lysogenicity of cultures may be used for differentiating the phagotype and also for confirming its homogeneity. The lytic reactions, nevertheless play the leading part in type determination.

- 93 -
The bacteria of group I, connected by the reactions with the phages from mixed cultures, are characterized by common biochemical features — they do not carry out enzymatic breakdown of rhamnose, which is decomposed by the phagotypes of all other groups. It must be assumed that in the natural system there exists a possibly profound connection between the biochemical properties and the phagotype of paratyphoid B bacteria.

The groups BT, P and Q and also the phagotype 16 were included later (quoted according to Polanetzki and Reuss, 1961). In these groups the phagotypes are designated by Arabian figures corresponding to the figures designating the mild phages found in these types. The group BT differs from the group B only by the absence of reactions with the phages 31-3al. The types M6 and 18 (Rische and Schneider, 1960) were also added to the natural system in 1960. Thus, the system of Scholtens now distinguishes 37 types of paratyphoid B bacteria.

As can be seen from Table 8, nearly all the 10 phages of Felix and Callow are used in the natural system. Only phage 3al is excluded. Scholtens became convinced of the unreliability and inconstancy of the reactions of cultures with this phage and sometimes with phage 3a, which had been left in the scheme. Rische found that the strains of type 3al (normal) can lose their sensitivity to the phages 3a and 3al and are then typed as Dundee (quoted acc. to Scholtens, 1959). The phages 3a, 1, 2 and Jersey are assigned a secondary part. In recent times Scholtens has been using the new phage Ib which gives more distinct and stronger reactions (Rische, Schneider, 1960) instead of the phage 3b (phage I in Scholtens system).

All the phagotypes of Felix and Callow and also the variants of the types 3a (2, 3, 4), 3al (1, 2) found their place in the system of Scholtens. The third and fourth variants of types 1 and 3b Scholtens
related to Salm. java, as d-tartrate – positive Salmonella. The variants 1 and 2 of phagotype 3al were combined into a single phagotype (3al variant 1 + 2) because the reaction with the BACR phage, on the basis of which they are distinguished, is not constant. Polanetzki and Reuss (1961) did not find this reaction in any of the strains.

Excluded from the scheme are the unstable 3a, variant 1 and 3al, variant 3. As we know, these two variants are distinguished in the system of Felix and Callow on the basis of the feature of inertia towards the Dundee phages. Both variants interacted with concentrated d phage (Dundee) (in 10 fold critical test dilution) in the experiments of Scholtens. Scholtens related the strains 3a, variant 1 to the type 3a (normal) and 3al, variant 3 to the type 3al of Liewarden. The correctness of excluding the variant 3 of type 3al from the scheme was confirmed by Behmer in Belgium. In one epidemic the author isolated strains 3al (normal) and 3al, variant 3. These were differentiated by Scholtens phages as a single type 3al Liewarden (quoted acc. to Scholtens, 1959).

Type 1 in the natural system occupies a special position because it is sensitive to nearly all grouping and typing phages. Its variants (1 and 2) have not yet been differentiated in the system of Scholtens and possibly represent a separate group.

The other variants of the types of Felix and Callow are not referred to in the investigations of Scholtens.

Certain strains in the system of Scholtens which are identical with the phagotypes of Felix and Callow are identified as different phagotypes. The difference concern mainly the phages from mixed cultures. Thus, the strains of type 3a (normal) can be allocated to 3a (normal), 54 and Q1 (see Table 8).
### TABLE 8
### Natural System of Phage Typing of Paratyphoid B Bacteria According to Scholtens

<table>
<thead>
<tr>
<th>Table entries</th>
<th>Symbols: s1) confluent lysis; (s1) inconstant reactions; psl) semi-confident lysis; *) very fine dustlike phage plaques; +1, +2, +3) increasing number of plaques; **) types described by Rische and Schneider (1960).</th>
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<td>Group</td>
<td>Phage types of Felix and Callow; C) phage types of the natural system; D) phages in critical test dilution; E) grouping phages; F) typing phages; G) derivatives of phage I of Felix and Callow; H) phages obtained from mixed cultures; I) typedetermining mild phages obtained from pure cultures; K) additional phages;</td>
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<th>Phage Types</th>
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**Symbols:**
- s1) confluent lysis
- s1) inconstant reactions
- psl) semi-confident lysis
- *) very fine dustlike phage plaques
- +1, +2, +3) increasing number of plaques
- **) types described by Rische and Schneider (1960).
L) type-determining mild phages detected in pure cultures of phagotypes; M) Jersey; N) Beccles-Meppel; J) nomenclature of the preparations in the schem of Felix and Callow; P) Dundee; Q) Beccles; R) Taunton; S) polyvalent O-phage; T) not classifiable types. a) Normal; b) variant; c) Taunton-Hague; d) Taunton-Kampen; e) Schidam; f) Beccles-Midwoud; g) Liewarden; h) Sittard; i) 3a1 (normal) or Dundee; k) sl; l) psi.

The strains of type 3a1 (normal) can include 3a1 Liewarden, 87, 3a1 (Schidam) and Q6.

The Beccles cultures were differentiated into 5 types: Beccles 22, Beccles-Midwoud, Beccles-Meppel, Sittard and P3. These types contain the mild phage 1, and react in the same manner with the type-determining phages I, IV, IVa, VI. They differ in their reactions with the grouping phages e, d, c and f. Moreover, Rische (quoted acc. to Scholtens, 1959) observed Beccles cultures which reacted within the natural system like the type 3a, variant 1 or 2, but did not interact with the phages 3a-3a1. Following reinoculation, these strains were converted into phagotype 3a (normal).

The strains Taunton were divided into 2 groups: Taunton-Hague, which is not sensitive to phage e, and Taunton-Kampen which interacts with phage e.

The strains Dundee were distributed among the strains Dundee (original), Taunton, 87, 3a1 Liewarden, BT6, P4 and the atypical strains. The original phagotype Dundee differs from all the others by its inertia to phage e.

The subdivision of these bacteria according to Scholtens was confirmed by investigators in other countries. Thus, in the FRG in 1958, all Scholtens types (except 18) were found in 1793 cultures of paratyphoid B (Polanetzki and Resuss, 1961). Most frequently encountered were the types Taunton-Kampen (39.2%), 1 (11.7%), BAOR (9.2%), Dundee (7.2%), 3a (5.6%), 3a1, variants 1-2 (5.3%), 3a1 Liewarden (5.1%).
the same time, certain phagotypes (Q1, 87, Q6, Sittard BT1, P1, 16, P3, Taunton-Hague, etc.) are found extremely rarely. They were isolated only in 6% of foci. The types Taunton-Hague, Midwoud, Sittard, Beccles-Meppel, 54, 18, 69 were not found in the GDR (Rische, Schneider, 1960).

Comparison of the system of Felix and Callow with the natural system

As previously remarked, the weak point of the method of Felix and Callow is the possibility of a nonobjective identification of cultures. Putting it more accurately, in flareups with a single source of infection, two, rarely three different phagotypes are detected. True, such cases are relatively rare. For example, in the course of 19 years, only 6 such flareups were observed at the Central Coliform Bacteria Laboratory in England although over 14,000 paratyphoid B cultures were typed (Bernstein, 1960).

In some situations the differentiation of cultures with phages of the natural system helps to give a more probably interpretation of the results. Here are some examples from the literature. The paratyphoid B flareup at Lemmer has been previously mentioned, where cultures of the types Beccles and 3al, variant 1 (Scholtens, 1956b) were isolated. As we know, the phagotype 3al, variant 1 in the system of Felix and Callow differs from Beccles by its sensitivity to the phages 3a-3al. Scholtens found that the reaction with these phages is not constant. Both phagotypes were obviously fundamentally related: they contained the mild phage of serotype 1, they reacted with phage f and were lysed in the same manner by other grouping and typing phages. Scholtens combined them into the new phagotype Sittard.

The phagotype Dundee is most frequently detected together with Taunton and the type 3al (normal) and its variants. Thus, Brandis (1955c) isolated from a carrier simultaneously the types Dundee and
and Taunton. During a flareup in England caused by the phagotype Taunton, the type Dundee variant 1 was repeatedly isolated from one patient pell-mell with Taunton (Anderson, Williams, 1955). From the type Dundee, variant 1 a phage was isolated which transforms Taunton cultures into Dundee, variant 1. A collection of 3 phagotypes (Dundee, Taunton, 3al, variant 4) were isolated by Sloan and others (1960) in a female carrier, who served as a source for a flareup in the environs of Edinburgh. The possibility of the conversion of these 3 phagotypes was revealed in experiments on artificial lysogenization. Such an evolution obviously takes place in the intestine of the carriers or patients. There is evidently also another one: in the system of Felix and Callow the phages 3a, 3al and Taunton show unstable differences between variants and do not show a profound similarity between cultures. This is primarily true for the phagotype Dundee.

In the scheme of Felix and Callow, the strains are counted as Dundee if they interact only with the Dundee phage and are insensitive to the others (see Table 7). Scholtens found that such a pattern of lysis cannot be considered to be diagnostic and this is why: some Taunton strains, having lost their sensitivity to the Taunton phage, and strains 3al (owing to the inconstancy of their reactions with the phages 3a-3al) can react like the type Dundee. For this reason, variants are formed among the cultures of the Taunton type which are erroneously counted as Dundee. As a result, two phagotypes, Taunton and Dundee are detected in an epidemiologically single flareup. The natural system avoids such errors. In the Scholtens system, only the reactions with phages from mixed cultures are recognized as diagnostic. In particular, the authentic phagotype Dundee, in addition to being sensitive to phage d (Dundee) should be resistant to phage 4. Being guided by this characteristic, Scholtens (1955b) ascribed the Dundee cultures, isolated dur-
ing the flareup together with the type Taunton, to the phagotype Taun-
ton. The lysogenicity test confirmed these results: these strains con-
tained a phage of serotype IV, which is not found in the type Dundee
and is normal for the type Taunton.

It is not impossible that the three phagotypes, isolated during
the flareup described by Sloan and co-authors (1960), could be given a
different interpretation in the Scholtens scheme. All that has been
stated above shows that the natural system of typing is more progres-
sive than the system of Felix and Callow. In contrast to all others,
the new system is constructed on the basis of a profound study of the
lysogenicity of phagotypes. A clearly delineated relationship is appar-
ent for nearly all phagotypes between the reactions with the typing
phages and the properties of the prophage. This is the great achieve-
ment of the system. The phages from mixed cultures evidently show up
profound and constant connections and differences between phagotypes.

Hence, the identification of cultures in the natural system is
more objective than in the system of Felix and Callow, where diagnosis
is often based on accidental reactions, say with the phages 3a, 3al
and others.

The representatives of the First Subcommittee for Paratyphoid B
Phage Typing at the International Association of Microbiologists com-
pared in Netherlands, Belgium, FRG and GDR the natural system of phago-
type determination with the system of Felix and Callow and gave a posi-
tive evaluation to the former. The results of typing with Scholtens
phages harmonized with the data of the epidemiological investigations
and eliminated contradictions in some flareups, which had resulted
from the typing with Felix and Callow phages.

Nearly all researchers agree that the natural system is of the
greatest importance for more accurate diagnosis and for correcting the
errors of the system of Felix and Callow. Thus, according to the data of Polanetzki and Reuss (1961), the phagotype according to the Scholtens scheme proved to be different from that determined by means of the Scholtens phages helped in nearly all cases to evaluate the results of the epidemiological investigation more correctly.

The Scholtens phages enabled Rische and Schneider (1960) to refute erroneous epidemiological diagnoses in several foci of paratyphoid B. Thus, a case has been described, when strains of the phagotype 3al (normal) were isolated from a patient. The epidemiological data indicates a connection between this case and a female carrier. Two strains, isolated in this carrier, however, reacted with the phages of Felix and Callow in the same manner as the phagotype Dundee. The application of the Scholtens method removed these contradictions: all cultures belonged to the phagotype 3al Llewarden. Here is yet another example. In the same female carrier the phagotype Beccles was first isolated, and a year later, 3a (normal), another year later Beccles and 3a (normal). All cultures, independent of the phagotype according to Felix and Callow, were lysed only by two phages from mixed cultures (e and h) and were found to belong in the group B of Scholtens.

In order to obtain a more accurate diagnosis of the phagotype, the phage e, which, as previously indicated, makes it possible to distinguish the real type Dundee from strains which react similarly, proved to be particularly valuable. In several of these cases, the isolation of the type-determining mild phages from the cultures proves to be extraordinarily useful. Nestorescu, Popovici and others in Rumania (1961) isolated 11 strains which were differentiated by means of Felix and Callow phages as the phagotype Dundee. When verification with Scholtens phages was carried out, all the strains proved to be sensitive to
phage e and hence the diagnosis "phagotype Dundee" was refuted. 10 of these strains, isolated in a single focus, were sensitive to phage e, d and h and resistant to phages I, IVb, IVa and VI. On agar with dextrose the strains proved to be sensitive to phage 3al. The mild phages I and VI were isolated from the strains. All these data enabled the culture to be grouped among the phagotype 3al Liewarden. The phagotypes Taunton-Kampen and 3al Liewarden were isolated from the strains. All these data enabled the culture to be grouped among the phagotype 3al Liewarden. The phagotypes Taunton-Kampen and 3al Liewarden were isolated in the same focus at the same time from a patient.

In another case, the diagnosis "phagotype Dundee" according to the scheme of Felix and Callow had to be changed to the diagnosis "phagotype Taunton-Kampen" through application of Scholtens phages. Such a case was also described by Rische and Schneider (1960). In strains of phagotype Dundee sensitive to e phage, they detected the phage IVb which, as we know, is present in the phagotype Taunton-Kampen.

A second aspect of the application of the natural system of Scholtens is the additional differentiation of the predominating phagotypes. Subdivision of the type Taunton, which dominates in the microbe population of numerous countries, would be useful. As the investigations showed, of the two subtypes of Taunton, Taunton-Kampen predominates in the FRG and GDR; the subtype Taunton-Hague is found extremely rarely (Polanetzki, Reuss, 1961; Rische, Schneider, 1960). A different ratio may exist in other countries. The phagotype 3al (also fairly widespread) was differentiated in the GDR into 3 different types: 3al Liewarden, 3al Schidam and 87 (Rische and Schneider, 1960), in the FRG in to 2 types: 3al Liewarden (3.3% of foci) and 3al Schidam (1.8% of foci) (Polanetzki and Reuss, 1961). The type Beccles could be subdivided into Beccles 22, Sittard, Midwoud and Beccles-Meppel (Brandis and Storch,
The number of untypable cultures in the two systems is about the same: 2.3-2.5%. At the same time, if a careful investigation by the Scholtens method is carried out, it is possible to differentiate about half of these cultures. Thus, Polanetzki and Reuss (1961) detected among strains which could not be typed with the phages of Felix and Callow, the phagotypes Taunton, M6, 60 and others.

A more extensive verification of the objectiveness of the new improved system of typing is positively necessary. However, even that which is known permits the conclusion: this is a useful, valuable method of differentiating paratyphoid B cultures.

Method

The media and methods of preparing phages and cultures and the methods of typing are the same in the natural system as in the system of Felix and Callow. Let us therefore indicate only the differences.

Scholtens ('955b) preferred meat-peptone agar for type determination: 0.5 kg sausage meat after standing in 1 l water for 24 hrs at 4° is boiled for 20 minutes and filtered. To this volume, 12.5 g commercial peptone is added, 7.5 g sodium chloride and 20 g powdered agar. Following sterilization at 115° for 20 minutes the pH must be brought to 7.3, using a 15% soda solution and sterilization is repeated under the same conditions. Bernstein (1960) dissolved 2% dry Difko broth and 0.85% NaCl in order to prepare liquid media. For solid media, he added 1.3% dry agar.

Preparation of typing phages. The phages 1, 2, 3a-3al, Jersey and Beccles-Meppel are obtained by passing pure lines of the original typing phages of the collection of Felix and Callow through homologous cultures. The phages of the serotypes I, IV and VI are obtained preferably on 24-hr old cultures of Beccles-22, Taunton-Hague and BAOR,
respectively. These 3 serotypes of phages can also be isolated from other cultures (see Table 8). The phage of serotype IV is present in the type Taunton in two variants. One variant (IVb) does not lyse the phagotype Taunton and is analogous to the phage Beccles of Felix and Callow; the other (IVa) is identical with the Taunton phage and reacts with both Taunton phagotypes. The lytic properties with regard to other types are nearly the same in both phages.

The grouping phages of serotypes II and VI with the lytic spectra e, d, c, f, h, and b are obtained from mixed cultures.

The phages e and d are isolated from a mixture of any cultures from the group A and M. It is best to use a mixture of the strains Taunton-Hague and 3b, variant 1. Fino-spot phages of serotype II with a range of lytic action e and d and large-spot phages of serotype VI also with the spectra e and d are isolated from this mixture. The e phages are determined by means of the indicator strain of group M, the d phages with strains of group A.

The c phages are isolated from cultures of the group S, I, EM and B and type 1 when each of these is grown with cultures of group M. The phage is identified with a strain of group M (best of all with Beccles-Midwoud).

The f phages are isolated from mixed cultures of group S and group A. The phage is identified with strains of group S.

The h phages are extracted from a mixture of cultures of group M (type BAOR) and EM (type Beccles-Meppel). They are identified with strains from the group BAOR.

The b phages are obtained in a pair of cultures from the groups A and B. The plaques are identified with strains of group B. The b phages can also be isolated in combinations of the strains of group J or EM with the group A.

- 104 -
Method of obtaining mild phages from mixed cultures. To 60 ml of broth, one drop of each of the cultures is added. The mixture is grown in a thermostat for 5-10 days at 37° and then filtered. The filtrate is checked for the presence of phages on the gasons of both strains. The phages are isolated as indicated above. All the mild phages are purified and passed through a sensitive indicator strain in order to increase the titer.

**Typing technique.** The methods of typing are the same as for typhoid bacteria. The phages are used in critical test dilution. In addition to the typing and grouping phages, indicated in Table 8, it is useful to employ the polyvalentphage of Salm. Breslau (see method of typing with Felix and Callow phages). The first reading of the reactions is carried out with a magnifying glass at 10x magnification after growing for 5 hrs at 38.5°, the second after 24 hrs.

**Anomalous typing results** (see both points of the typing method with Felix and Callow phages). Moreover, Scholtens pointed out the following possible cases.

The cultures react unequally with the phages 3a-3al, but identically in all other reactions. In this case the strains are considered to be a single phagotype because it has been found that the reactions with the phages 3a-3al are not always constant.

The culture gives anomalous reactions, for example, it is typed as the phagotype Dundee but is lysed also by phage 4, which is inactive with respect to the true Dundee type. In such situations it is recommended to study the type-determining phages of the test cultures.

**Demonstration of the type-determining mild phages in cultures of paratyphoid B.** A drop of the broth culture of the strain to be tested mixed with a drop of the indicator strain of paratyphoid B - 3a (B62) is grown in 5 ml of broth. This strain (3a normal type of Felix and
Callow) does not contain any type-determining phages. After 16 hrs of incubation the culture is heated on the water bath at 56° for 15 minutes or filtered. The filtrate is tested for the presence of mild phages on the indicator strain 3a (B62). The phage plaques are transferred into broth and grown for a day. The serological group of the mild phage thus obtained is determined (Scholtens, 1955a). Bernstein (1960) isolated mild phages in the following manner: 10^8 microbes in the logarithmic phase of growth together with the same quantity of indicator culture are introduced into 20 ml of preheated broth; the mixture is incubated at 38.5° for 5 hrs, then centrifuged and the supernatant liquid kept 40 minutes at 56° in order to inactivate the remaining microbes. If heating lowers the activity of the phage, it is filtered through foam glass (pore dimensions 1.2-1.4 μ).

The type-determining phages of the serotypes I, IVa, IVb, VI and in rare cases II (spectrum d) are isolated from paratyphoid cultures by this method. The serotype of the phage is distinguished: 1) on the basis of the characteristic range of the lytic action on 7 cultures of different groups of the systems (Table 9); by neutralization reactions with the corresponding anti-phage sera (Scholtens, 1955b).

### TABLE 9

<table>
<thead>
<tr>
<th>Test-strains</th>
<th>A</th>
<th>B</th>
<th>Lytic reactions of the phages with the test strains of Paratyphoid B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>IVa</td>
</tr>
<tr>
<td>14502</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1153</td>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>821</td>
<td>c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B662</td>
<td>d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>818</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2500</td>
<td>3e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1131</td>
<td>f</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Symbols: s1) confluent lysis.
A) Test strains; B) lytic reactions of the phages with the test strains; C) strain; D) phagotype. a) Spectrum; b) Jersey; c) Beccles-midwoud; d) (normal); e) variant; f) Taunton-Kampen; g) s1.
For the purpose of serological identification of the mild phages the strain to be typed is grown together with the indicator culture 3a (B62) in three broth test tubes, to which the specific antiphage sera I, VI or IV have been added in a dilution of 1:100 (at phage-neutralising titers of the sera: 1:640 – 1:12,800). After 16-18 hrs of incubation the phage is identified as indicated above. The phages of the types I and IV can be present in a culture simultaneously. They can be distinguished by the form of the sterile spots. In doubtful cases, a detailed study of the serological properties of the phages is carried out.

Two systems of phage typing paratyphoid B bacteria exist. In the system of Felix and Callow the bacteria are subdivided with the aid of O-phages into 10 types and about 30 variants. The lytic reactions of the types are practically stable, those of the variants, not always. The specificity of the phagotypes is mainly due to the immunity of the bacteria to phages, serologically related to their prophage. The method has recommended itself as positive in epidemiological investigations. At the same time, cases are known when cultures are not objectively identified, when different phagotypes were found in epidemiologically uniform flareups. The profound study of the causes of this led to the creation of the second system of typing paratyphoid B bacteria.

The natural system of Scholtens was created on the basis of the system of Felix and Callow. It makes use of all the typing phages of the latter (with the exception of 3a1) plus the mild phages from mixed cultures of paratyphoid B. The latter are of decisive diagnostic importance.

The bacteria are subdivided into groups, each of which includes several phagotypes. The grouping phages, 5 serologically identicalpha-
ges of Felix and Callow and 6 mild phages from mixed cultures of serotype II, have different spectra of lytic action. The type-determining phages are 4 phages from pure cultures, of which 3 correspond to the phages 3b, Beccles and Taunton of Felix and Callow. These phages subdivide the groups into phagotypes. Strains of identical phagotypes produce the same mild phages. This can be used for the differentiation of the phagotype and for confirming its homogeneity.

All the phagotypes of Felix and Callow and most of their variants found a place in the system of Scholtens. A number of the phagotypes of Felix and Callow are distributed among different groups on the basis of their reactions with the phages from mixed cultures. Comparison of the two systems of typing demonstrates the superiority of the natural system. In the latter, the phages from mixed cultures reveal more constant differences and connections between strains. This makes it possible to eliminate contradictions between the results of typing and the epidemiological conclusions.
Chapter 4

PHAGE TYPING OF OTHER KINDS OF SALMONELLA

The role of the subdivision of the germs of the same serotype for the profound analysis of flareups of Salmonella food poisoning among humans and paratyphoid cases among animals is quite undisputed. First of all, when these flareups are investigated epidemiologically, a large number of infection sources are encountered. In addition to rodents, which are the basic reservoir of Salmonella, pigs, cattle and domestic birds can be infection sources. The infection is transmitted via meat, milk, duck and hen's eggs and other products of animal origin. The sphere of distribution of Salmonella is also still increasing because of the fact that large quantities of food products (in the raw state or ready for consumption) are being exported nowadays from one country to another. Some of these products, for example, eggs, melange [?], etc. are often used for the preparation of pastries and creams which enter into the diet of the population. All the above indicated facts increase the practical value of the phage typing of Salmonellae.

Food poisoning infection is only one of the forms of manifestation of salmonellosis in humans. In recent years the number of cases in which Salmonella were found in patients with manifold clinical forms of illness, which were previously thought to be dysentery, colitis, enteritis, etc. have become more frequent. The number of such sporadic salmonelloses in our country accounts for 20 to 30% of all intestinal diseases. Many undiscussed problems still remain in their epidemiology and pathogenesis.

- 109 -
To these belong, in particular, the data on the importance of humans (patients and carriers) as infection sources, the epidemiological role of Salmonellae, isolated from environmental objects, etc. The method of phagotype determination which makes possible a differentiat approach to cultures of the same serotype, can be of considerable value in the recognition of the mechanism of the flareups and sporadic cases of salmonellosis.

PHAGOTYPE DETERMINATION OF SAIM. BRESLAU

The Salm. Breslau is the most frequent cause of infectious food poisoning. In Britain, for example, it is the cause of 69% of all food poisoning cases. This microorganism also causes much harm to animal husbandry, by causing toxicoseptic diseases of cattle, particularly in young stock.

Work has been carried out in many countries on the creation of a satisfactory scheme of typing for Salm. Breslau. Some researchers used the principle of Craigie and Ian, subdividing the bacteria on the basis of their sensitivity to mild and virulent phages. Others took the course of searching for differences in the properties of the mild phages and strains, stemming from different sources.

Typing According to the Principle of Craigie and Ian

5 schemes of typing of Salm. Breslau are known, based on this principle. One of these was proposed in Sweden (Lileengen, 1947, 1948) (Kallings and Laurell, 1957), another one in the USSR (L.D. Gutorova, 1959) and three in Britain (Felix, 1956; Cellow, 1959). These schemes with the exception of the last-named (Callow, 1959), were examined by us in detail in a previous review (M.D. Krylova, 1961). These schemes use 0-phages of Salm. Breslau isolated from feces, sewerage effluents and lysogenic cultures.

Felix compared 3 schemes for typing of Salm. Breslau, proposed in
Sweden and Britain. Although the phagotypes, determined with these schemes did not coincide, any of the schemes made it possible to identify strains which were epidemiologically connected and gave results which agree with the data of the epidemiological investigation. Under the conditions prevailing in a single country, a series of O-phages can obviously give epidemiologically useful results. Because Salm. Breslau causes infections of domestic animals and does not have chronic human carriers, Felix thought that there was no need for an international standardization of phage typing for this species of bacteria. One cannot agree with this conception. The intensive development of international trade in products such as meat, eggs, melange [?], egg powder, etc. may favor the transfer of Salmonella infections from one country to another. In this connection, the standardization of the method of phage typing of a number of Salmonellae is of no less importance than for typhoid and paratyphoids. The greatest chances of being adopted for standardization has the scheme proposed recently in Britain by Callow (1959).

Callow used 29 phages designated by Arabic numerals (Table 10). The basis of this scheme are the adapted typing phages and strains of the second scheme of Felix (1956). As we know, Felix adapted the paratyphoid B phage 3b to different strains of Salm. Breslau and obtained thus a series of phages with common origin. By means of these he was able to differentiate cultures of Salm. Breslau into 12 phagotypes (1, 1a, 1a variant 1, 1b, 2, 2a, 2b, 2c, 2, 3, 3a, 4). In the scheme of Callow, 19 out of 29 phages have the same serological characteristics and are identical with phage 3b of paratyphoid B and the typing phase 1 of Felix. Callow isolated an active variant of phage 3b, which completely clears up the spots of lysed Salm. Breslau. At the same time, this variant retained a high adaptive capacity. 13 serologically iden-
tical with phage 3b of paratyphoid B and the typing phage 1 of Felix. Callow isolated an active variant of phage 3b, which completely clears up the spots of lysed Salm. Breslau. At the same time, this variant retained a high adaptive capacity. 13 serologically identical phages were produced from it: 3 phages (1, 4, 14) through adaptation of phage 3b directly to the corresponding phagotypes, 10 phages (5, 7, 15, 16, 19, 20, 25, 26, 27 and 28) via adaptation of its derivatives. Besides, 6 phages (2, 3, 6, 17, 23 and 24) — derivatives of the mild phages from lysogenic cultures of Salm. Breslau — proved to be indistinguishable with respect to their serological characteristics from the paratyphoid phage 3b.

The other phages were also obtained by adaptation of phage 3b and its derivatives. Their serological properties nevertheless proved to be different. In particular, 5 phages (9, 10, 11, 21 and 22) were only partially neutralized by the serum of phages 3b and 1; 2 phages (12 and 13) were indistinguishable from the phage 2 of the first empirical scheme of Felix and, finally, 3 phages (8, 18 and 29) did not combine with any of the above-listed sera. It must be assumed that all these 10 phages are derivatives from the mild phages of the cultures, through which the phage 3b or its derivatives had been passed prior to adaptation.

All typing phages had a high titer: the critical test dilutions were from $10^{-3}$ to $10^{-7}$. Such a high activity was attained by producing the phage in agar layers. With the same aim in mind, the phages were not only passed through homologous cultures but also through heterologous ones, where the phages sometimes developed high activity without modifying the hosts. This last-named phagotype must be nonlysogenic.

The Salm. Breslau were differentiated into 34 phagotypes in the scheme of Callow. Among these are all the 12 phagotypes of the second
scheme of Felix, part of them (type 2, 2c, 2b and 2d) being further subdivided. 26 of the 33 phagotypes of Callow are lysogenic, 23 types carrying simultaneously thermostable and thermolabile phages, 3 types only thermolabile phages. In a number of characteristics the thermostable phages were close to the phages of group A of Boyd (see further on), and the thermolabile phages to the phages of group B. Later on, some of the mild phages were used for the identification of new phagotypes.

The scheme of Callow differs favorably from all above described schemes by the number of phagotypes of the cultures. The list of the Callow phagotypes continues to increase: at the present time there are already about 90. The percentage of untypable cultures is close to zero (Anderson, Wilson, 1961).

Epidemiologically valuable results are obtained in Britain in the typing of cultures from humans and animals with Callow phages. Thus, Anderson and Wilson (1961) reported on the results of typing of strains from 873 sources. The strains had been sent to the laboratory from London and South Anglia during a 3 month period in 1958. It was found that in the same locality the same phagotypes are enormously often found in humans and animals (cattle and domestic birds). Thus, among 6 types, which accounted for 57-77% of all finds, 5 (14, 20a, 12, 3 and 2) were the same in humans and animals. Such a similarity could not have been accidental. It may be assumed that it attests to the epidemiological connection between the human and animal cases during this period. In particular, the phagotype 14 was most widespread among domestic birds. To this belonged 74% of the strains from domestic birds (mainly from chicks and eggs). This type was also the most frequent among humans. This could be an indication that a considerable number of salmonelloses in Britain are due to infection from domestic birds.

A study of the phagotype population of Salm. Breslau in humans and
animals revealed the possibility of predicting the sources of infection. Detection of phagotypes in humans, which were widespread among certain species of domestic animals and birds may be a good indication for epidemiological researchers. For example, the appearance of phagotype 20a, 15a or 18 in humans should cause suspicion that the cause of the cases are cattle, because these types predominate in cattle and are hardly ever found in domestic birds.

Anderson, Galbraith and Taylor (1961) presented an example, how phage typing helped the epidemiological analysis of a prolonged flare-up, which was widespread over a large territory, of cases of coliform infections among humans. 90 persons at 37 points fell ill. All the strains from the cases proved to be phagotype 20a. The same phagotype had been detected early during this flareup in calves at a central point from where cattle are distributed to the abattoirs, at an abattoir and in a butcher's shop. Although it was not possible to investigate the products which were the direct cause of the disease in each concrete case, phage typing enabled the nature of the flareup to be elucidated.

Direct Typing Method

As mentioned earlier (see Chapter 1), the mild phages from different strains of Salm. Breslau differ in their heat stability, the morphology of the sterile spots, the range of action and the antigen structure. Boyd proposed to use these differences for differentiating Salm. Breslau (Boyd, 1950, 1952, 1954, 1956, 1958; Boyd and Bidwell, 1957; Boyd, Parker and Mair, 1951). Boyd termed this the direct method of phagotype determination. The success of the method depends above all on the choice of the indicator strain, which should be sensitive to all the phages isolated from the cultures to be tested. In his first work (1950) Boyd used two "wild" strains of bacteria as indicator strains.
However, even in combination they did not reveal all the mild phages of this species of Salmonella. Only in recent years has a strain been found on which nearly all the known mild phages of Salm. Breslau proliferate (Boyd, 1958).

As pointed out earlier, the essence of the method consists in the isolation and study of the properties of the mild phage. In order to have constant characteristics of these phages, the media used for their isolation and study should be standard. It is best to grow the cultures on concentrated broth (Martin or Hottinger), to isolate the phage on 1% nutrient agar with a pH of 7.2 and a NaCl content of 0.75%.

The test culture is grown in 5 ml of broth together with the indicator strain overnight at 37° during the night. The mild phage of the strain to be typed, proliferates during this period. The mixed culture is freed of bacteria by centrifuging for 10 min at 3000 rpm. A dilution of the supernatant liquid is transferred into a dish, inoculated with the indicator strain. After several hours of incubation, sterile phage spots appear on the surface of the dish. A pure line of mild phage is then obtained and part of the phage is sterilized by centrifuging and heating at 58° for 30 min, another part by filtering through an L2 Chamberlain candle. The first method is used for heatresistant phages, the second for thermolabile phages. The inactivation temperature of the phage is determined in a dilution which gives confluent lysis. The pH of the broth is 7.4. 0.5 ml of the dilution is placed on the water bath and heated for 30 min to different temperatures.

In order to study the range of action and the morphology of the plaques, both phage portions are transferred to agar, inoculated with the indicator strains. Plaques appear a day later. These plaques are fine and very fine in the mild phages of Salm. Breslau; they can be examined only with a binocular magnifier under 20x magnification.
certain experience in the morphology of the plaques it is possible to
distinguish the phage types present in the culture. For the identifica-
tion of the phages B4 and B5 (see further on) their capacity (as an ex-
emption) to develop large plaques on some strains of Salm. paratyphi C
is utilized. The other phages are distinguished on the basis of their
range of action on two indicator strains of Salm. Breslau (1404 and
1411) and in doubtful cases, by neutralization reactions with specific
sera (the reaction method is generally used).

A new test has been added in recent years for the identification
of mild phages, the cross immunity reactions (Boyd, 1958). The nonlyso-
genic indicator strain of Salm. Breslau of which we spoke earlier, and
which interacts with all phages, is treated with mild phages.

A series of standard lysogenic cultures is obtained in this manner.
The lytic activity of all the mild phages is determined on them and
their relatedness or difference is gaged on the basis of this character-
istic.

In a study of about one thousand mild phages of Salm. Breslau,
Boyd, Parker and Mair subdivided them into 2 groups, A and B. The A-
phages (A1, A2, A3, A4, etc.) are heatresistant (they can resist a tem-
perature of 80° for 30 minutes), produce large (up to 1 mm diameter)
sterile spots, often surrounded by a halo and with a thickening of sec-
ondary growth in the center, and show mostly, besides the types A3 and
A4, a similar antigen structure. The A-phages are divided into 12 types
by means of cross immunity reactions on standard lysogenic strains.

The B-phages (B1, B2, B3, B4, B5, etc.) are thermolabile (they are
destroyed at 60°) and are differentiated into 13 types in the cross im-
munity reactions.

Salm. Breslau can contain 2 and even 3 different mild phages. Dif-
ferent types of the same group of phages are extremely rarely isolated
simultaneously. As an example, among 598 strains carrying A-phage, only one contained 2 phages of this group. Conversely, combinations of different A- and B-phages are frequent. The combinations of phages A1 + + B2, A2 + B2, etc. have been described.

To avoid confusion with the known systems of phagotype determination, in which the phagotype of the strain concerned is determined on the basis of its sensitivity to the typing phages, Boyd proposed to designate a strain, differentiated by the direct method, instead of by the word "type," by the term "mark." For example, a strain containing the latent phage Al is termed "Salm. typhi murium mark Al." Boyd (1952) presented observations on flareups of cases of food poisoning and other Salmonella cases in Britain, in which determination of the phage mark of the strain made it possible to carry out a more profound epidemiological analysis.

In a certain hospital ward, Salmonella cases appeared periodically among children for a year. All the 62 strains from the patients contained the same mild phage. Although it was not possible to determine the primary source of the flareup, the common nature of the type of Salm. Breslau in the children indicated the presence of cross infection within the hospital.

Although the method of direct type determination gives constant results, it is laborious and requires much more time than the method of Craigie and Ian. In direct type determination the results can at best be obtained within 3-4 days. The typing cultures which contain 2 difference phages (with double mark) presents certain difficulties. One phage is often detected first and later another phage type, which may lead to epidemiological errors. The authors recognized these deficiencies of the method, which prevent its widespread introduction into practice. It must also be pointed out that the method has not proved to be
as universal by far as its authors initially supposed and is not suitable for type determination of all species of Salmonella (Atkinson, 1957). At the same time, careful study of a large number of mild phages of the same species of bacteria led to interesting theoretical data concerning the phenomenon of lysogenicity. To these belong, for example, investigations on the role of mild phages in the resistance of the cell to the action of phages of a related or foreign serotype, etc.

**PHAGOTYPE DETERMINATION ON OTHER SPECIES OF SALMONELLA**

The phagotypes of Salm. enteridis, Salm. dublin, Salm. gallinarum—pullorum, Salm. thompson (Lileengen, 1950, 1952; Smith 1951b) are differentiated on the basis of their sensitivity to specific O-phages. An attempt to determine the phagotypes of Salm. cholerae suis was unsuccessful. Subsequently, Kral (1955) isolated two bacteriophages from the feces of pigs, which were differentiated as the variant Kundendorf from the normal strains of Salm. cholerae suis.

An attempt to type Salm. virchow (Velaudapillai 1959) is known. 49 out of 52 strains, obtained from 11 different countries, were subdivided by mild phages into 7 phagotypes. All the cultures from Denmark, Britain, Sweden and Nigeria had the same phagotype.

A rational method of typing Salmonella was worked out in 1952-1957 by Atkinson, Heitenbeck, Schwann, Wollaston and Bullas (see Atkinson, 1957). Taking into account the extraordinary frequency of lysogenicity in Salmonella, these authors used a simplified modification of the direct method of phagotype determination according to Boyd. In contrast to the direct method, the mild phages in Atkinson's scheme are differentiated only on the basis of the range of lytic activity on a number of standard indicator strains.

2 indicator strains (these are also phagotypes) were isolated from cultures of Salm. waycross: a lysogenic (1) and a nonlysogenic (Ather-
ton). 6 indicator strains were differentiated in Salm. adelaid e, of
which 5 are lysogenic (1a, 1b, 2a, 2c, 3) and one is nonlysogenic (4).
The strains of Salm. bovis morbificans are all lysogenic and are sub-
divided into 5 groups. Certain strains of this species of bacteria car-
ry several latent phages and evidently form additional groups.

The phage typing data on the above-listed Salmonella species
agreed in all cases with the epidemiological findings. When the results
of typing were compared with the conclusions obtained during the sub-
division of the strains according to Boyd, complete agreement was ob-
served which confirmed the possibility of practical application of the
Atkinson modification of the method. A series of indicator cultures of
the corresponding species of bacteria is necessary for this purpose.
These must be kept in the dry state. The strain to be tested is grown
on mowed agar at 37° for 24 hrs in combination with each of the indica-
tor strains. The mixed culture is washed off with 0.5-1 ml of broth and
centrifuged. The supernatant liquid may contain mild phages of the
test and indicator strains. The phages are determined on gasons of
both strains. The phagotype is identified on the basis of the activity
of the phages on the indicator cultures. If the phages lyse the indica-
tor strain, the test is considered to be positive (+), if not, nega-
tive (-). The group of the test culture (phagotype) is determined on
the basis of the range of activity of the mild phages on all indicator
strains. Nongroupable cultures, whose mild phages give a characteristic
range of activity on the indicator strains are tested crosswise. New
phagotypes are thus determined.

The lysogenicity test also reveals the activity of the mild spe-
phages on the strain to be tested. This is the action of the mild
phages from the indicator culture. In doubtful cases this range of ac-
tivity provides additional information for confirming the diagnosis.

- 119 -
Special attention must also be paid to the absence of lytic activity of the mild phages of the strain to be tested on the indicator strain. This is a diagnostic characteristic.

An attempt to type Salm. Breslau by the method of Atkinson has become known (V.A. Kilesso, unpublished data). Mild phages were detected in the great majority of test cultures on 8 indicator strains. About 200 strains of Salm. Breslau, isolated from patients and healthy persons, from corpses, rodents, domestic birds and animals were subdivided into 15 groups. Identical phagotypes were isolated from epidemiologically related objects.

The Atkinson method has advantages over the direct method: it is simpler and does not require accurate identification of the latent phages. It also has the advantage over the method of Craigie and Felix of not requiring the special production and treatment of typing phages. It is true that it is technically more difficult than the method of Craigie and Ian and that it requires also standard indicator cultures.

The principle of the Atkinson method can undoubtedly be useful for the typing of numerous Salmonellae and other species of lysogenic bacteria. It is not always possible and not even necessary to have a series of standard indicator cultures for each species of Salmonella. In fact, the flareups caused by the same species of Salmonella (not counting, of course, Salm. Breslau, Gärtneri and other widely occurring species) are not so frequent as to warrant the creation of a standard scheme of typing for every species. At the same time the need for additional differentiation of Salmonella of rare species is fairly great, particularly when the epidemiological study does not give a clear pattern. The study of the activity of the mild phages from heterogeneous cultures can become extraordinarily useful in these cases. Stock cultures, cultures from other flareups and from sporadic cases can prove
to be indicators. These strains are tested in lysogenicity cross tests with the cultures to be studied. The identity of the spectrum of the mild phages from the test cultures is the basis for attributing them to a single phagotype. Such a simplified approach to typing has been tried by Thal (1957) in Sweden during a flareup caused by Salm. montevideo.

Salmonellae are very suitable for phagotype determination on the basis of their sensitivity to a series of typing phages or of their lysogenicity.

The salm. Breslau are differentiated by both methods. There are several systems of typing on the basis of the sensitivity of cultures to typing phages. All these have epidemiological importance. The most perfect system is that of Callow, in which Salm. Breslau are subdivided into phagotypes on the basis of their sensitivity to 29 phages, from which 19 are derivatives of the phage 3b of paratyphoid B. Another approach to typing of Salm. Breslau is the study of its mild phages (direct method). The direct typing method has proved to be objective and valuable in epidemiological practice. At the same time, the complexity and laborious nature give it an academic flavor and hinder its wider practical introduction.

More promising are modifications of this method, worked out by Atkinson and others for the typing of Salm. waycross, Salm. adelaide, Salm. typhi murium and others. In contrast to the direct method, the mild phages are not isolated and completely identified. The range of their activity on standard indicator strains is merely checked. In the presence of such cultures the method makes reliable differentiation of strains, isolated in flareups and from sporadic cases, possible. Technically the method is simple and has a number of advantages over the method of Craigie and Jan.
Chapter 5

PHAGOTYPE DETERMINATION OF ENTEROPATHOGENIC COLIFORM BACTERIA

Although the etiological role of a number of serotypes of coliform bacteria (O111B4, O55B5, O26B6 and some others) has now been indisputably settled, the epidemiology and pathogenesis of colienenterites are still in a stage of intensive study. Problematical is also the question of the role of the enteropathogenic serotypes of coliform bacteria in the etiology of food poisoning. In view of the fairly widespread distribution of the pathogenic strains of coliform bacteria in the environment and in healthy carriers the possibility of additional differentiation of strains of the same serotype can lead to a correct understanding of the epidemiological connections in many situations.

In the USSR, phages for enteropathogenic serotypes of coliform bacteria were isolated by Z.D. Gogoladze (1959). These help to differentiate the cultures of different serotypes and can have only diagnostic importance. Phages of the same type were obtained by L.B. Borisov and others (1962). These phages which lyse most cultures of its serotype, can hardly be used for typing them as the authors erroneously believe.

Nicolle, Le Minor and others (1952, 1954, 1957), using 25 phages from lysogenic cultures, the feces of patients, convalescents and healthy persons, animal droppings (mainly chicks), differentiated 7551 cultures of coliform bacteria of serotypes O111B4, O55B5 and O26B6 from different countries and regions of the world into phagotypes. Groups of phages could be isolated which lyse the cultures of one sero-
ype and are inactive on the two others. These phages can be tested for
the differentiation of serotypes. The phagotypes are designated by the
names of the towns and countries, where they had been isolated (Table
1).

TABLE 11

| Scheme of Phagotype Determination on Enteropathogenic Coliform Bacteria of the Serotypes 0111B4, 055B5, 026B6 (according to Nicolle, Le Minor and S. Buttiaux, 1957) |
| **Table 11** |

Symbols: sl) confluent lysis; psl) semiconfluent lysis; † designates
inconstant reactions; - designates the absence of lysis.

1) Phagotypes; B) Alessandro and Comes test; C) H-antigen; D) typing
phages; E) serotype; F) sl; G) psl.

The serotype 0111B4 is divided into 11 phagotypes, the serotype
55B5 into 10, the serotype 026B6 into 7. The strains of the last-named
Sero type are difficult to type, giving unclear reactions in 28.56%.

In enteropathogenic coliform bacteria exists a remarkable connection between the relation to the phage, the structure of the H-antigen and the reaction with β-phenylpropionic acid: the test of d'Alessandro and Comes (1952). The phagotypes of serotype O111B4 are clearly divided into 2 groups on the basis of their reaction with β-phenylpropionic acid (Nicolle, Le Minor and others, 1957). In the first group, which includes 5 phagotypes (Montparnasse, Tourcoing, Vienna, Sevres ubiquitous and Lyonaise) all cultures gave a positive reaction with β-phenylpropionic acid. At the same time (and this is the most important) they always contained the flagellar antigen H2 and belonged to the biochemical type I or II on the basis of their reaction with indole. Thus, phagotype determination enabled the strains of serotype O111B4 to be divided into 5 types, identical in flagellar antigen and biochemical properties. The cultures of the second group, which comprises the remaining phagotypes of the serotype O111B4, always gave a negative Alessandro and Comes reaction. These strains either did not contain H-antigens, or had antigens H21 or H12. Biochemically, the cultures corresponded to the types III, IV, V or VII.

Typical is the constancy of the above-indicated connections. If, for example, a culture of serotype O111B4 gives the reaction of the phagotype Sevres Lyonaise, then one can predict with certainty that it as the flagellar antigen H2, will give a positive reaction with β-phenylpropionic acid and will belong to the biochemical type I or, if the culture belongs to the phagotype Bretonneau, this must be O111B4 H12, ith a negative Alessandro and Comes reaction, of the biochemical type II or IV (Nicolle, 1957).

In the remaining serotypes, these connections are less developed. Thus, in the serotype O55E5, only one phagotype (Saint-Christophore)
e the reaction with β-phenylpropionic acid. Interesting is the fact in this phagotype (like the analogously reacting strains of serotype 1B4) had a flagellar antigen H2, but with regard to the biochemical characteristics it was related to type D. The other phagotypes which gave a negative Alessandro and Comes reaction, were characterized by constant H-antigens and different biochemical properties. In the phage 026B6 the Alessandro and Comes reaction was negative for all phages.

The above-described relationships were confirmed by Hungarian researchers in another scheme worked out by them for phagotype determination (Eorsi, Jablonszky, Milch, 1954), Milch, Deak (1961). The authors used undiluted phages, isolated from the feces of newborns. The serotype O111B4 was subdivided into 7 types (111/1-111/7) on the basis of reactions with 5 phages (a, al, aF42, b and b2) and with β-phenylpropionic acid. As in the scheme of Nicolle, this division agreed with structure of the H-antigen. The immobile variants belonged to the phagotypes 111/1, 111/5 and 111/7 and gave a positive reaction with phenylpropionic acid. The strains with antigen H2 were subdivided on basis of this reaction into 2 subtypes. The strains containing anti-H12 did not give this reaction and were subdivided only on the basis of their relationship with phages.

The serotype O55B5 was differentiated by means of three phages (1 and aF42) into 5 phagotypes (55/1-55/5) among which the Alessandro and Comes test was positive only in the variants with antigen H2 in the immobile variants.

The regularities in the correlations between the flagellar antigen and the phagotype can be useful for practical laboratories. While the study of the flagellar antigens requires several weeks and even months, phagotype determination can provide a result within 24 hrs or in urgent...
The phagotype determination of pathogenic coliform bacteria is led out by the method of Craigie and Ian. It is made more difficult certain instability of the phagotypes on nutritive media owing to association, particularly in the serotype 026B6. In order to avoid, it is recommended to type the culture not later than 1-2 days after isolation (Milch, Deak, 1961). To restore normal sensitivity to phages it is helpful to pass the cultures through the organism or to inoculate them on dishes (Nicolle, le Minor and others).

The Allesandro and Comes test is determined by inoculating of the culture on agar, containing 0.02% α-phenylpropionic acid. Positively ting colonies assume a rose, red and brown color even after 8 hrs. reaction attains its maximum intensity within 24-35 hrs.

Both methods of type determination proved to be valuable in epidemiological research. They helped to separate hospital from non-hospital infections and to elucidate the epidemiological chains.

Thus, Buttiaux, Nicolle and others (1956) observed a hospital flare up of enterocolitis by O111B4 of the phagotype Tourcoing, during which a child was brought into the ward, from which the serotype O111B4 of another phagotype was isolated (Sevres). During the next days phagotype Tourcoing was detected in the new cases, and Sevres in 3 hem. In another flareup, caused by the phagotype Tourcoing, the Brétonneau was isolated from one of the children. It had been transferred into the hospital by a healthy carrier who had arrived in ward 7 days prior to the flareup. This case was the signal for a per check of all newly arriving children. Pintelon (1956) tried the method of phagotype determination according to Nicolle during flareups of enterocolitis of the serotype O111B4, arising simultaneously in
is of a children's hospital in Holland. The isolation of 2 different 
serotypes made it possible to delimit these apparently connected 
epidemics.

Milch and Deak (1961) successfully applied their method of phago-
determination of cultures of the serotypes 0111B4 and 055B5 for 
validating the epidemiological connections in the children's depart-
ment.

One of the deficiencies of the method of Nicolle is the precomin-
ence of certain phagotypes. Thus, among cultures of serotype 0111B4, 
ubiquitous Sevres phagotype is found in 43.41%, and Tourcoing in 
25%. Among the strains 055B5 dominate the phagotypes Bethune (36.5%) 
Lomme (29.46%) (Nicolle, Le Minor and others, 1957). This is the 
reason why the methods of further subdivision of the phagotypes is pro-
posed. Hamon (1961) proposed to subdivide the phagotypes of Nicolle 
the basis of their ability to produce colicines and their sensitiv-
to 14 known colicines.

The capacity of a culture to produce colicine is determined in 
the following manner (method of direct contact): drops of the test cul-
tures are applied to the surface of agar. Following incubation of 37°C 
48 hrs, discs of growing culture develop, around which the coli-
diffuses into the gel. The culture is sterilized with chloroform 
for an hour. After removal of the chloroform by ventilation, 
drops of E. coli K12S are applied to the discs of the killed cultures, 
this is an indicator strain for all colicines of pathogenic E. coli. 
drops are applied in such a manner that they partially overlapped 
discs. The results are evaluated after 24 hrs. of incubation at 
The absence of inhibition of the growth of the indicator strain is 
ble around the discs of the colicinogenic cultures. Growth inhibi-
not observed on the discs of the noncolicinogenic cultures and
In order to identify the type of colicin produced by the test strain, colonies of E. coli K12S are collected in the zone of its action. These mutants are resistant to the colicin of the test strain but retain their sensitivity to the other 17 known types of colicin. The type colicin of the test culture is determined by the range of activity of these 17 colicines on the mutant strain: if, for example, the mutant is sensitive to all types of colicines with the exception of E1 and I, it means that the test strain produces the colicines E1 and I.

A second method of identification of the type of colicin is used when the test strain produces only one colicin. A number of mutants is selected by acting on E. coli K12S with 17 known types of colicin. In this manner an indicator series of mutants is received, each of them being resistant to one colicin type only. Drops of a day-old culture of all the mutants are applied in the colicin zone of the test strain and the type of colicin is determined. For example, the colicin of the test strain is attributed to type B if it inhibits the growth of the mutant, selected by colicin B, and does not act on the other mutants.

For the investigation of the types of colicines, produced by colinogenic cultures of pathogenic coliform bacteria, 8 mutants of strain 28 were selected, which are resistant to the colicines and to combinations of colicines E, V + I, G + H, E + B, G + H + V + I, and V. The activity spectrum of the colicines from different phagotypes of the pathogenic coliform bacteria is studied on these mutants. Colicin I V are differentiated by their sensitivity to the microbe prostheses, colicin G to G + H and H by the capacity of colicin H to inhibit the growth of the culture of Proteus OX19.

12 strains of coliform bacteria, producing the following types of
cines: B, C, D, E1, E2, F, G, H, J, (I + Y), K, S3, S4 and V are for the study of the sensitivity spectrum of the phagotypes of cologenic coliform bacteria to the colicines (determination of the colicine type). The determination of the colicine type is carried out following manner: Each of 14 colicinogenic strains is inoculated the second (semi-liquid) agar layer (method of Gracia) in such a manner that 300-400 colonies develop there. By diffusing to the surface of agar, colicine in this case goes into the medium in sufficient quantity. Drops of the test strains (young culture, diluted 1:400) are placed to the agar surface. Following 24 hrs incubation at 37°, the results are evaluated — absence or presence of growth inhibition of test strain. Doubtful results are checked by using the direct technique.

4 types of colicines, I, E, B and G, were detected in the pathogenic serotypes O111B4, 055B5, 026B6. Within each phagotype it is possible to separate colicinogenic and noncolicinogenic strains. An exception are the phagotypes Lomme, London and Jerusalem, all the strains which were noncolicinogenic. Colicine I (alone or in combination with others) was most frequently detected (in 61.47%) in colicinogenic cultures of different phagotypes. Colicine G was found in the least number (only in cultures of the phagotype Bretonneau). Up to 5-7 different colicinogenic states were observed among the colicinogenic strains of certain phagotypes. Thus, for example, in the type Sevres ubiquitous colicines I and the groups E, E + I, B, E + B were found in type Weiler colicine I, and the groups E, E + I, B, G and G + I. The number of colicines and their combinations in the other phagotypes was less. The form of the colicinogenic state was closely connected with the origin of the phagotypes. Thus, all strains of the phagotypeEnterobacteriaceae ubiquitous which are widespread in the North of France, were...
colicinogenic; the strains from Lyon, Cremona and Berlin colicinogenie, those from the North of France produced colicine 31, those from Lyon, Cremona and Berlin colicine I, those from Wernigerode (GDR) a complex of colicines E + I. Strains of the same phagotype from different foci and geographical areas, also had different sensitivity to the 14 known colicines. This made a subdivision of the colicinogenic and noncolicinogenic strains of the same phagotype into colicine types possible. As a result, some phagotypes were subdivided into 2-6 colicine types. Epidemiological observations showed that all strains of pathogenic coliform bacteria from the same focus produce the same colicines and have the same colicine type.

The possibility of further subdivision of cultures of the same phagotype into colicine types was used in some hospitals in France and Germany. Thus, in June 1956, during a flareup of colienteritis in one of the hospitals at Lyon, the phagotype Sevres Lyonaise was isolated in all childrens' wards. In 3 wards, however, the colicinotypes and the colicinogenicity were different: in one ward, all strains proved to be noncolicinogenic, in another colicin I was isolated, in a third, colicine E + I. The strains from these wards had different colicine types. These data attest to the different sources of infection in the 3 above-mentioned wards, which led to corrections in the data of the epidemiological investigation. Conversely, during an epidemic of colienteritis in one of the hospitals of Kiel (FGR [1]) of 27 strains of Sevres ubiquitous produced the colicines E + I and had the same colicine types. This confirmed the hypothesis of the single source of the hospital infections which spread through the wards.

The method of colicine type determination of enteropathogenic coliform bacteria is still in a stage of investigation. At the same time, it
already proved itself as an interesting and promising method, which is the epidemiological and biological description of cultures more found and accurate.

Another possible method of typing of enteropathogenic coliform bacteria is testing their lysogenicity. With this method it was possible to type the strains of serotype 0111B4 (M.D. Krylova, 1962).

The method of typing is not complex and can be carried out at any laboratory. The cultures isolated from different objects, are crossed for the presence of mild phages. The method yields results only if part of the strains, used in the experiment, has a previously on different epidemiological origin. Stock cultures are also useful indicator strains, isolated from different flareups.

In order to obtain mild phages, the pure cultures of all the bac-
lia to be typed are incubated in concentrated Martin broth for 15-18 at 37° and then centrifuged 20 minutes at 3000 rpm. The supernatant liquids must not be heated to free them from the culture even by normal method (at 56° for half an hour) and must not be filtered. Procedures often lead to a loss of mild phage, particularly if the latter is thermolabile. The supernatant liquid is tested for the presence of free phage on 1.3% agar, inoculated with gason of young cultures of all the other test strains.

The lysogenicity of the cultures is gauged by the appearance of
|ly defined plaques at the point where the supernatant liquid had applied. The phage plaques are best examined in transmitted light 10x magnification. In the majority of cases, however, the lysis is so clear that a magnifying glass need not be used.

In the presence of several cultures which proved to be indicator cultures, the evaluation of the results does not offer any difficulty. Strains which produce mild phages can be clearly distinguished from
the nonlysogenic cultures. The latter are combined in a separate group. Nonlysogenic cultures capable of being indicator cultures, may be set apart in this group. The lysogenic strains may consist of only one group if they give off phages identical in the morphology of the plaques and the spectrum of lytic activity. In other cases the strains are subdivided into several groups depending on the range of activity of their mild phages on different indicator cultures. The results are considered as negative, if not a single one of the tested cultures proves to be an indicator strain.

During colilenteritis flareups at the dysentery ward for children of Noril’sk the cultures of serotype 0111B4, isolated from the feces of the child patients and their mothers who were present in the ward with their children, were typed by the above-described method. Also included in the tests were 3 stock cultures of serotype 0111B4. As a result the cultures were differentiated on the basis of their lysogenicity into 4 phagotypes, of which:

- type A contained 2 mild phages, active on the strains 153 (standard) and 364;
- type B produced phages active only on the strain 153;
- type C - nonlysogenic strains and 3643, sensitive to the mild phages of the strains of type A;
- type D, nonlysogenic strains, which did not interact with the mild phages from cultures of type A and B.

A certain relationship between the origin of the cultures and their phagotype has been established. All the cultures of the lysogenic type A without exception were isolated from children of the infantile dysentery ward of the infection hospital during the period from the 27th June to the 12th July 1957. To the same type belonged the strains, isolated from 2 healthy mothers, who were in the hospital with the
children at this time.

Only in 3 patients were other phagotypes found (types D, B and C). In 2 children the first analysis of the stool, taken on the day of arrival, was negative. Coliform bacteria were isolated from patients in the second analysis (on the 3rd day). The detection of the phagotypes D and C, which had not been previously found in this ward, excluded the hypothesis of a hospital infection of the children. Both children arrived at the hospital with the diagnosis "relapse of acute dysentery." The illness which they had gone through at home, was clinically similar to colinenteritis (diarrhea without blood, with mucus, strong vomiting, etc.). The data of the phagotype determination confirmed the assumption of an extra-hospital infection.

Two schemes of typing enteropathogenic serotypes of coliform bacteria on the basis of their sensitivity to mild and virulent phages are currently known. The method of Nicolle and others has been studied more thoroughly; it allows the differentiation of serotype 0111B4 into 11 phagotypes, of serotype 055B5 into 10 phagotypes, and of 026B6 into 7 phagotypes. The epidemiological expediency of type determination has been demonstrated in a number of flareups. At the same time, the scheme is not yet perfect. The serological characteristics of the phages, the stability of their critical test dilutions and the causes of the specificity of the phagotypes have not yet been studied. In view of the predominance of certain phagotypes of Nicolle, further subdivision via testing for colicinogenicity and sensitivity to the 14 known colicines is useful. The method enables the colicinogenic and noncolicinogenic strains within most phagotypes to be isolated. These and others are subdivided on the basis of their sensitivity to the 14 known types of colicine into colicine types.

The typing of pathogenic coliform bacteria through testing of...
their lysogenic properties is also promising.
Chapter 6

PHAGOTYPE DETERMINATION OF DYSENTERY BACTERIA

The epidemiological significance of the phagotype determination of bacteria of the genus Shigella does not require extensive proofs. To this day, dysentery spreads through all countries of the world, using epidemics, sporadic cases and hospital infections. The epidemiological analysis is complicated by the possibility of chronic carriers to have survived the acute infection, and the manifold pathways of transmission. Particularly complicated is the elucidation of the sources and transmission pathways in sporadic cases and in superinfections, in the infection wards and in the wards for reconvalescents, even one germ is superimposed on another. In all these cases further subdivision of the species and serotypes of the microbes may be enormously useful.

LAGOTYPE DETERMINATION OF SONNE'S DYSENTERY BACTERIA

The Sonne dysentery bacteria can be subdivided by their sensitivity to a series of typing phages. The best-known scheme of this kind has been proposed in Sweden by Hammerstrom (1947, 1949). 11 phages (I-7) are used isolated from lysogenic Sonne's cultures, sewerage effluents and the filtrates of the feces of dysentery patients and animals. The Hammerstrom phages are suitable only for typing the pure R-forms of Sonne's bacteria. The slightest admixture of S-forms prevents the differentiation of the culture because pure R-phages are present among the typing phages. Part of the preparations are S+R phages, which are equally active with respect to S- and R-forms of Sonne's bacteria. In
view of these peculiarities of the typing phages the strain of Sonne's dysentery, which is subjected to phagotype determination, must be present in the pure R-form which is not agglutinated by S-serum. In order to control the purity of the rough cultures it is recommended to use phage XII. This gives confluent lysis with the pure R-form. If small quantities of the smooth form are present, resistant colonies appear within the sterile disc. When the proportion of the smooth forms attains 1%, the disc is clouded by a light growth film, and at 40% the lysis becomes hardly distinguishable.

Hammerstrom successfully differentiated 1834 strains of Sonne's bacteria isolated in Sweden by means of the above-described phages. They were distributed among 68 types and subtypes, which were designated by Arabic numerals. The biochemical type — the capacity of enzymatic breakdown of maltose, rhamnose and xylose on standard nutrient media — was determined in all phagotypes. 5 biochemical types were isolated (a, b, c, d, and g). The phagotypes, differing in their biochemical characteristics, figure as subtypes in the scheme of Hammerstrom. The biochemical types of Hammerstrom are close to the types of Boylen.

The method of phagotype determination is similar in principle to the method of Craigie and Ian. Hammerstrom (1949) recommends a standard medium of the following composition:

- Bactoagar 12 g
- Peptone (from caseine) 10 g
- Na$_2$HPO$_4$ 2 g
- NaCl 3 g
- Broth of beef pH = 7.4 1000 g

The phages are used in test dilutions. When the test dilutions are determined, every typing phage is titrated on the host strain and on several known phagotypes with which it should give negative or weakly positive reactions (in the form of isolated sterile spots) at this
ilution. With the host strain the typing phages in test dilution should give confluent lysis. For example, phage I is checked on the types 44, 6 and 4, phage II on the types 38, 61 and 4, etc.

For typing, drops of a 24-hour old broth culture of the strain are applied in the surface of dishes containing 1.25% agar (after drying at 37°C for 1/2-1 hr). Following half an hour's drying in the thermostat, drops of phage in test dilution are applied to the culture. The dishes are incubated in the thermostat for 4 1/2 - 6 hrs, after which the results are evaluated. A stereoscopic microscope is required for reading the negative reactions. The drying and growing times of the dishes in the thermostat must be strictly adhered to, and, most important, a standard nutrient medium must be used.

Hammerstrom demonstrated the reproducibility of the results of nagotype determination and the practical stability of the phagotypes in a large amount of data.

The Hammerstrom method proved useful in the epidemiological investigation of flareups and epidemics of Sonne's dysentery. Somme's phagotype determination is carried out routinely in Sweden.

The Hammerstrom method has been approved by several countries, although the conclusions of the authors are contradictory.

A positive evaluation of the method is given by Junghans (1958a, 1961). By accurately following the method of Hammerstrom she differentiated 97.4% of 7931 strains of Sonne's dysentery, isolated in Berlin in 1955-1960. The phagotypes 2, 3, 5, 6, 7, 12, 13, 65, 69, 70 are isolated. Part of the cultures (4.7%) gave cross reactions with the typing phages; these cultures fit into the system of Hammerstrom and were identified as 6 new phagotypes. The nontypable cultures accounted for only 2.6%. It was shown that the phages are not absolutely specific for Sonne's bacteria and when undiluted, can lyse cultures of
the representatives of different tribes of the family of coliform bacteria (Shigella, Salmonella, Escherichia, etc.). In critical test dilution, though, the Sonne's phages lyse only a small percentage of cultures. Out of 1106 different cultures of Enterobacteriacea, 359 (32%) were lysed by concentrated phages (one or more) and 197 (17%) by phages in critical test dilution.

The author confirmed the practical stability of the phagotypes during an epidemic by repeated isolation from the same patient. In the course of 2-3 years following three inoculations in vitro, the phagotype remained stable in 94% of 1732 Sonne's strains. A variability of the cultures was observed in 6%, manifested in the appearance of resistance or in a change in the type of reaction. A change in the type of reaction with the typing phages in the same culture can be related to nonstandard method, with changes in the composition of the nutrient medium during the test and with degeneration of the culture. It is essential to verify carefully the purity of the R-form with phage XII, particularly in freshly isolated strains. The selection of such forms should always precede the phagotype determination. When stock cultures are typed, difficulties do not arise, and the phage XII need be used only for the control. A variability of the type of the strains is sometimes simulated by mixed cultures.

The great practical value of the method during the epidemiological investigation of flareups and during observation of changes in the microbe type population under large city conditions is emphasized. Thus, all phagotypes, isolated in 1955-1959, were also detected in 1960. The frequency of isolation of individual phagotypes, however, varied considerably over a period of 6 years. The type 65 predominated in 1955 (77%), the type 12 (54%) in 1956. In 1957-1958 and 1960, the type 13 predominated (44.25 and 58%, respectively), in 1959, the type 3.
Other authors refer to the Hammerstrom method with greater reserve. Thus, V.N. Kuznetsova (1956) reported on an unsuccessful attempt at phagotype determination with Hammerstrom phages on 196 R-forms of Sonne's bacteria. Only half the strains could be typed. Other authors commented on the instability of the phagotypes on laboratory media (Mayr-Harting, 1952, Ludford, 1953, Tee, 1955, and others). Such results may largely be accounted for by a non-standard typing technique.

Of no less importance is the purity of the R-form of the culture, because even a slight admixture of smooth variants causes fluctuations in the sensitivity of the culture to the typing phages (Hammerstrom, 1949).

A more serious deficiency of the method is the preponderance of one or two phagotypes in some countries. Thus, more than half the investigated persons in Sweden had the types 3 and 5 (Hammerstrom, 1949), in England, phagotype 3 was detected in 81% (Mayr-Harting, 1952) and in Australia and Tasmania type 19 was found in 85% (Ludford, 1953).

There is no point in developing a train of thought to the effect that the epidemiological value of phagotype determination is slight if the same phagotype is detected in several isolated flareups. It is believed that the preponderance of a certain phagotype is due to the use of virulent phages from the animal feces and from sewerage effluents.

Being convinced of the imperfection of the Hammerstrom scheme, Tee (1955) worked out a scheme for phagotype determination of R-forms. Like Hammerstrom, he isolated his 10 phags from sewerage effluents, human feces and pig droppings. 829 cultures, forwarded from different regions in Britain, were differentiated into 20 phagotypes. The scheme of Tee had the same deficiency as that of Hammerstrom: in 74% the cultures belonged to the same phagotype (L). This monotonic population became particularly noticeable when this phagotype was isolated in 38
out of 52 flareups. Only in 9 flareups were other phagotypes found.
In 5 flareups, the types were heterogeneous and hence, the phagotype
determination data conflicted with the epidemiological data. In the
opinion of the author, one of the causes of the variety of phagotypes
in a single flareup is the change of the phage sensitivity of the cul-
tures in the intestine of the patients and carriers under the influence
of the phages. Similar changes were observed on nutrient media. All
this led the author to the conclusion that the significance of phago-
type determination on Sonne bacteria is limited.

V.N. Kuznetsova (1956) proposed a scheme of phagotype determina-
tion for the S-forms of Sonne cultures with three phages from the fe-
ces of patients. The phages also lysed rough and transition forms. The
122 test cultures were differentiated into 5 phagotypes. The phagotypes
were apparently stable. The above-described method requires verifica-
tion on a large number of data.

Another approach to the typing of Sonne's bacteria was tried by
Abbott and Shannon (1958). They differentiated these bacteria on the
basis of their colicines on 14 specially selected indicator strains.
They included 12 strains of Sh. sonne and 2 strains of Sh. sonne and 2
strains of Sh. schmitzi. A modification of the method of Frederic,
Tibault and Gracia was used to detect the colicin (quoted acc. to Ab-
bott and Shannon).

The agar test culture was spread in a thick streak on the surface
of 1% meat-peptone agar containing 10% horse blood. The horse blood
serves as catalase source. Following 3 days incubation the culture
which had grown on the band was killed with chloroform. For this pur-
pose, a circular piece of filter paper was moistened with chloroform
and attached to the cover. An hour later, the culture was scraped off
with a sharp glass and removed together with a small quantity of the
iderlying agar. The filter paper was again moistened with chloroform
and the dishes left covered for another hour. The filter was then taken off, the dishes uncovered and aired for 3 hours upside down. On the dishes which were free of chloroform, day-old broth cultures of the indicator strains were applied in parallel streaks. The inoculation was carried out with a loop, by thickly applying the culture across the streaks where the test strain had been inoculated. The results were read after 24 hrs incubation at 35-36°C.

The action of the colicines proved to be specific. On the basis of the pattern of the inhibitory effect of these agents on 14 indicator cultures it was possible to differentiate 367 out of 537 cultures into 7 colicine types: 1a, 1b, 2, 3, 4, 5, 6. Colicine could not be detected in 170 cultures. This was possibly due to the absence of suitable indicator cultures. The cultures, which could be differentiated, were obtained in 102 dysentery flareups and foci. The colicine types were uniform and the results of the epidemiological investigation agreed with the typing data in 97 flareups. In 4 flareups out of 5, in which strains heterogeneous with respect to their colicine type were isolated, these proved also to be heterogeneous with regard to their sensitivity to sulfanilamides. It is possible that the dysentery in these flareups was caused by a mixed infection.

A deficiency of the method is the large number (1/3) of untypable trains and also the possibility of doubtful results. The latter depended mainly on the lack of standard media and on variations in the incubation temperature. At a temperature lower or higher than 35-36°C, the lysis zone was less distinct. The method must be verified under standard typing conditions.

Later on, determination of the colicine types in Sonne's dysentery acteria was carried out in Japan (Naito, Sasaki, and Yano, 1961). The
method of agar layers was used. An 18-hr old broth culture of the test strain was centrifuged for 30 min at 3000 rpm. The supernatant liquid (unheated or heated to 55° and kept at this temperature for 30 min) was checked for the presence of colicine. 5 drops of the liquid were distributed for this purpose on the surface of normal meat-peptone agar and slightly dried. 0.5 ml of broth indicator culture (4 strains of Sonne's bacteria) was added to 2 ml of heated agar. The mixture was poured into previously prepared agar dishes. After 15-18 hrs of incubation at 37° the results were evaluated. The colicine produced a cleared-up zone which was easily distinguished from the action of the mild phages because the latter formed isolated plaques. The investigated strains of Sonne's bacteria were subdivided into 9 types on the basis of their ability of producing colicine and mild phages. 4 colicine types were differentiated only on the basis of the characteristic of colicine production. The authors, moreover, took into account the range of activity of colicine on the indicator strains. The epidemiological value of this subdivision was demonstrated: of 581 strains isolated during a dysentery epidemic at a school, 577 belonged to a single colicine type.

Both methods of colicine typing require further study. It is particularly important to determine the stability of the colicine types and the epidemiological expediency of such a subdivision.

PHAGE TYPING OF FLEXNER DYSENTERY BACTERIA

Numerous attempts to use phages for differentiation of this species of bacteria are known. These were made in most cases for identification of the serological types (S.L. Yagud, 1956, and others). The most successful attempts subdivision of serotypes into phagotypes were undertaken by Metzger, Mulczyk and others (1958), Slopek and Mulczyk (1961), and Mulczyk and Slopek (1962). By means of 12 phages (F1, F2, F3, etc.), isolated from sewerage effluents and from human and animal
ices, 2658 strains of Flexner bacteria were subdivided into 40 phagotypes (1-40) (Table 12). Each serotype was further subdivided by the types into 3-12 phagotypes. Thus, serotype 1a included the phagotypes 3-9; the serotype 1b - the phagotypes 1, 2, 10-14, the serotype 2 - the phagotypes 2, 15-18; serotype 2b - phagotypes 1, 11, 15, 19, 2; serotype 3a - phagotypes 26-30; serotype 3b - phagotypes 11, 24, 25; serotype 3c - types 21-23; serotype 4a - types 1, 2, 11, 14, 18, 28, 1-36; serotype 5 - types 11, 37-40. The phagotypes proved to be stable when stored on laboratory media after three passages through the organism of white mice. The phagotypes within the serotype did not differ in their serological or biochemical characteristics.

Most typing phages were prepared in cultures of Sh. Flexneri: phage F1 on serotype 1a, phage 2 on serotype 4a, phage F4 on serotype b, phage 5 on serotype 5, phage F6 on serotype 1b, phages F7 and F9 on serotype 2a. The phages F3 and F11 were prepared on Sh. dysenteriae. The phages F8 and F10 on Sh. sonnei, phage 12 on Sh. schmitzii. The phages had a critical test dilution within the range of $10^{-3}$-$10^{-5}$. They were used in a working dilution which was one dilution lower than the critical.

The phages were divided into 5 groups on the basis of their serological characteristics. The first group included the phages F1, F5, F9, F11; the second the phages F2, F7; the third, phages F3, F4, F9, F12; the fourth the phages F8, F10; the fifth phage F12. In some groups of phages, a correlation was observed between the antigen structure and the spectrum of lytic activity, in others not. The scheme differs favorably from all those described earlier by the fact that the Flexner bacteria are differentiated into a fairly large number of phagotypes. At the same time, serious verification of its epidemiological value is essential.
### TABLE 12

Scheme of Typing Flexner Dysentery Bacteria (According to Slopek and Mulczyk, 1961)

<table>
<thead>
<tr>
<th>Category</th>
<th>A</th>
<th>B Form of Test Reaction</th>
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Symbols: sl) confluent lysis; psl) semi-confluent lysis; k) plaques, situated at the edge of the spot; + designates isolated plaques; - designates the absence of reaction.

A) Phagotypes; B) phages in test dilution. a) sl; b) k; c) psl.

Typing of dysentery bacteria is still carried out at present on a limited scale. The best-known scheme for the subdivision of Sonne's dysentery bacteria is that of Hammerstrom. 12S and R phages, isolated from lysogenic cultures, detected in sewerage effluents and human and
animal feces, are used in this scheme. The phages differentiate only
the R-form of the Sonne's bacteria. The method has been positively
evaluated in the GDR.

The scheme has not found widespread application in other countries
(USSR, Britain, Australia) because the preponderance of a single or of
two phagotypes has been observed. The method has proved extraordinarily
sensitive to deviations in typing technique (composition of the media,
incubation temperature, etc.). Other typing systems have been little
studied. Attempts have been made recently to type Sonne's bacteria on
the basis of their sensitivity to colicines. These methods are in a
stage of development and require careful verification of their prac-
tical value.

A new typing scheme has been worked out for subdividing the sero-
types of Flexner dysenteria bacteria. By means of 12 phages these bac-
teria are subdivided into 40 phagotypes, each serotype being differenti-
ated into 3-12 phagotypes. The epidemiological value of this scheme
remains to be verified.
Chapter 7
PHAGE TYPING OF STAPHYLOCOCCI

The methods of intraspecies subdivision of the plasmacoagulating staphylococci (which we shall term simply staphylococci in the following, for the sake of brevity) attract the attention of an ever growing number of researchers in different countries. Staphylococci, as we know, are counted among the conditionally pathogenic microorganisms. They are fairly widespread among humans; they are found in the nose and pharynx of 40-50% of healthy test persons (Anderson and Williams, 1956), and on the skin in 37-40% (N.P. Nefed'yeva and others). This is one, but not the sole cause of the frequent staphylococcus infections in humans.

The widespread therapeutic and prophylactica application of antibiotica in recent years led to changes in the nature of the microflora, which produces post-partum and postabortus infections in women, septic diseases of the newborn, postoperation suppuration of wounds, etc. The "classic" germ responsible for these infections, the hemolytic streptococcus, has yielded first place to the staphylococcus which is more resistant to antibiotica. Thus, for example, staphylococcus carriers are much more frequent among hospital personnel than among persons with other professions. In one of the maternity hospitals of Leningrad, 57.8% of the staff proved to be carriers, these being observed more frequently among orderlies than among physicians or workers in the kitchen department (T.V. Golosova and others, 1962). Milch and others (1960) found staphylococci in the nasopharynx of 60% of the hospital personnel.
he incidence of carriers among patients is more frequent and may attain 73.6-78% [Kass, Vogelsang (quoted acc. to Milch and others, 1960)].

All this, in combination with possible deficiencies in disinfection measures and a lowering of the standards of asepsis may lead to the appearance of flareups of staphylococcus hospital infections. The problem of the struggle against such flareups has assumed great importance for maternity and surgical wards.

A staphylococcus can also be the etiological factor in cases of food poisoning, which arise when products are consumed which are contaminated by staphylococcus strains producing enterotoxin. Such flare-ups have been recorded everywhere in recent years. They are most frequently caused by milk products (cottage cheese, cheese, ice cream, etc.).

Finally, staphylococcus diseases are also an urgent problem for the veterinary. Staphylococci can often be the cause of suppuration of wounds and mastitis in cows and other domestic animals.

The epidemiology of the above-listed diseases has not been studied extensively. The role of exogenous infection is often not taken into account. If the widespread occurrence of the germ is taken into account, it becomes clear that only a method which involves a differentiated approach to strains of different origin can shed light on the epidemiology of staphylococcus cases.

The biochemical tests for differentiating strains of pathogenic staphylococcus, as we know, are few and sometimes unreliable. Typing on the basis of serological characteristics and also of the sensitivity to antibiotica (on the basis of the antibioticogram) allows differentiation of the culture into a few types only. The method of phage typing as proved to be the most fruitful for the study of epidemiological and clinical problems of human and animal coccus infections.
PHAGE TYPING OF HUMAN STAPHYLOCOCCI

Phage typing of the plasmecoagulating staphylococcus aureus has first been carried out in 1938 by Williams and Timmins, later by Fisk (1942). Wilson and Atkinson (1945) tested 18 mild phages for the typing of staphylococcus, of which 7 were isolated from cultures by the method of Fisk and 11 were obtained by adaptation of the first phages to resistant cultures. All the phages were used in critical test dilution according to the method of Craigie and Ian. The range of action of these phages was narrow. New phages were subsequently included in the typing scheme. Related groups of phages were found, the expediency of using undiluted phages was established, etc. Particularly important improvements were introduced by Williams and Rippon (1952) and Blair and Williams (1961). They worked out in detail a practical method of preparing phages and a typing technique, and gave a rational interpretation of the typing results. The system was standardized and has already been termed international.

Typing Phages and Strains

The principle of Craigie and Ian forms the basis of staphylococcus typing. All the typing phages are mild phages capable of producing a lysogenic state in cocci under certain conditions. The typing phages do not lyse coagulation-negative cocci. Part of the phages of Wilson and Atkinson (1945) and phages added by other authors in different countries are used in the system. The phages are classified on the basis of their serological characteristics into 4 autonomous groups: A, B, F and L (Table 13). More than half the phages were isolated from lysogenic cultures by the method of Fisk and were designated by the number of the culture (for example, phage 52). The others were obtained via adaptation of the first phages to resistant cultures and designated by the number of the original phage and a letter (for example,
It must be pointed out that passing a mild phage through resistant cultures did not by any means always result in true adaptation. Some phages are descendants of mild phages of the staphylococci, which the original phage was proliferated. This is why, for example, the typing phages 42E, 42D, 42B and 42C, derived from phage 42, proved to belong to three different serological groups: A, F and B. It was under the electron microscope that phages which belong to the same serological group, are morphologically similar but differ in size (Chih-Tung, 1960).

TABLE 13

<table>
<thead>
<tr>
<th>Typing groups of strains</th>
<th>Typing phages</th>
<th>Basic series of typing phages (21 phages)</th>
<th>Supplementary series of typing phages (6 phages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>29, 52, 79, 52A, 80</td>
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</tr>
<tr>
<td>II</td>
<td>3A, 3B</td>
<td>53, 77</td>
<td>42B, 73, 52B</td>
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<tr>
<td>III</td>
<td>6, 7, 42E</td>
<td></td>
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</tr>
<tr>
<td>IV</td>
<td>44, 54, 75</td>
<td>42D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>81</td>
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</table>

A) Typing groups of strains; B) Typing phages; C) basic series of typing phages (21 phages); D) supplementary series of typing phages (6 phages); E) Ungroupable strains.

Two series, a basic and a supplementary series, altogether 27 phages, are used. The phages are used in critical test dilution (KTR). This is one of the tenfold dilutions which lyse with semiconfluent lysis (in the form of "sponges") the homologous strain, i.e., the strain, on which the given typing phage was proliferated. This usually allows the dilution which gives confluent lysis.

The overwhelming majority of staphylococcus phages are not speci-
fic, owing to which strains can be sensitive to several typing phages at the same time. The forms of reaction of different cultures with phages are so numerous that the isolation of phagotypes similar to those of typhoid and paratyphoid bacteria is practically impossible.

Typing consists in determination of the form of reaction of the staphylococcus with a limited group of mild phages. The term "phagotype," as applied to staphylococcus, designates a strain which reacts in a constant manner with one or several typing phages. There are phages which normally lyse cultures in combination with other phages. For example, the phage 52 lysed staphylococci mainly in combination with the phages 29 and 52A. This enabled 4 groups to be isolated among staphylococcus phages and strains (see Table 13). The phages 29, 52 and 52A make up group I, phage 3C the group II, phages 6 and 47 the group III and phage 42D group IV. Under the heading "nongroupable" in Table 13, the cultures which are dissolved mainly by phages 81, 187 and some others are shown separately.

From 1 to 10% of all cultures are lysed simultaneously by the phages of group I and III. The number of such strains increases if concentrated phages are used (see further on). Vogelsang and Haaland (1959) observed such reactions in 11.2% of the staphylococci, isolated from the upper respiratory pathways of hospital personnel. Pöhn (1957) proposed that the hidden cause of the appearance of these strains is the insufficient group specificity of individual phages of group III. In particular, more than half the staphylococci of group I interacted in the investigations of this author with one phage of group III (73) and more than 1/4 with three (7, 42A, 75). Pöhn proposed that the hidden cause of the appearance of these strains is the insufficient group specificity of individual phages of group III. In particular, more than half the staphylococci of group I interacted in the investigations of
his author with one phage of group III (73) and more than 1/4 with nree (7, 42A, 75). Pöhn proposed that lysis of the culture by these hages of group III should not be taken into account and that it should e ascribed to group I. A culture which is sensitive only to these hages should be related to group III with reservation. Vogelsang and aaland (1959) confirmed only the data with respect to phage 73. This as later excluded from the basic series.

The list of typing phages continues to be augmented to this day Wallmark and Finland, 1961a).

G.N. Chistovich in the USSR (1960) proposed his own scheme for yping staphylococcus. Of 30 lysogenic strains of pathogenic staphylo- occus he isolated 46 races of phages. The phages were divided into he 4 groups A, B, V and G on the basis of their activity on 20 indica- or test strains. The test strains were correspondingly divided into he same number of groups, each of which included several phagotypes. 6 phages proved to be suitable for typing. This range was successful- y used for differentiation of pathogenic staphylococci (G.N. Chisto- ich, M.I. Rivlin and D.N. Bocharova, 1961, 1962). The group G produced neumonia in most cases, the group B intestinal injury and suppurating rocesses (V.A. Khrushcheva and F.M. Teytelbaum, 1961). A number of he phages of G.N. Chistovich are identical with the international hages, others are not identical with them. The phages of G.N. Chisto- ich help to describe the results of typing with standard phages in reater detail (G.N. Chistovich, and others, 1962).

ethod

For typing staphylococci in accordance with the international meth- d, the laboratory should dispose of the basic and additional series of yping phages and standard phagotypes. Phage typing of staphylococci is arried out on the media used for coliform bacteria. It is useful to
add to the broth and agar (1.2%) 0.004 M calcium chloride prior to sterilization which promotes the development of a higher phage titer by activating the adsorption on the cocci (particularly in phages of the serological group B (Rountree, 1955, White and others, 1959). The calcium chloride is prepared in the form of 1 M solution in distilled water. The solution is sterilized in the autoclave. Some authors recommend the addition of 0.1-0.2% glucose or dextrose to the broth and agar.

Production of phages on dry medium. Nearly all phages proliferate on phagotypes with identical number; the phages 52A and 79 are prepared on the phagotype 52A/79, the phages 42B and 47C on the type 42B/47C, the phage 75 on the type 75/76. The reaction of the breeding strains with the typing phages in KTR and 1000 KTR should correspond to those indicated in Table 15. If a single strong (++) reaction with the phage in 1000 KTR appears or disappears, the breeding strain is replaced. The dishes are filled with 1.3% nutrient agar in a layer with a height of 5 mm. 6-7 drops of a 4-5 hours old broth culture of staphylococcus are then carefully spread over the agar. The homologous phage is applied on top of the culture layer in a dilution which has been calculated in advance to give confluent lysis with the given quantity of culture after overnight incubation in the thermostat at 30°. This dilution should be more concentrated than the critical test dilution. A dilution is normally used corresponding to 10-100-fold concentration of the critical test dilution. A good growth of staphylococcus should be present in the control field after 24 hrs. If spontaneous lysis of the culture takes place, the dishes are discarded because the "spontaneous" phage can contaminate the typing phage. The phages normally attain their highest titer, when the field of confluent lysis is covered with a fine bloom of secondary growth. If the surface of the field is shiny, the
Page title is lower. It is possible that in the latter case an excessive quantity of the phage dissolves nearly all the cocci immediately after the beginning of incubation. Owing to lack of substrate the number of subsequent phage generations is limited.

For the purpose of extracting the phage, the dishes are frozen at 30° for 24 hrs and at -60° or in solid carbon dioxide for 1-2 hrs. During the subsequent thawing the agar is broken and the phage enters into the liquid which is sucked out. The agar can also be emulsified in broth (5 ml per dish). After being kept for 24 hrs at 4°, and centrifuging at 300 rpm for 20-30 min the phage-containing broth is separated from the agar.

Method of semiliquid agar. The phage and culture are mixed into 7 ml of 0.5% agar in such quantity that 2.5 x 10⁷ cocci and one KTR of the phage are present in 1 ml of agar. The mixture is poured on top of a layer of 1.5% agar. The dishes are placed into the thermostat overnight at a temperature of 30° (or 37°). The semiliquid agar is then washed off with 6-10 ml of broth, and centrifuged 10-20 min at 3000 rpm. The supernatant liquid is sucked out.

Preparation of phages in liquid medium. (Anderson, Williams, 1956; Arster, Knight, 1959). The phages are immediately prepared in the necessary reserve volume. The optimum ratio of phage and culture is determined beforehand empirically. For staphylococcus phages and strains it is normally such that from ten to one tenth of the critical test dilution of the typing phage is consumed by a day-old broth culture of staphylococci, diluted 100 fold in the volume of the medium. The mixture is incubated for 6 hrs at 30°. The phage thus produced is filtered.

Decontamination of phages. The phage is freed of the staphylococci by centrifuging and subsequent filtering through foam glass (5/3), porcelain or membrane filters. Filtering does not lower the concentra-
tion of phages with high titer but weakens phages with a titer under
1:100 and renders them unsuitable for typing. It is undesirable to heat
the phages to 58° because they are thermolabile and are killed before
all the staphylococci have died off. Sterilization of phages with thym-
mol is also not applicable because the latter inhibits the growth of
some staphylococcus strains even in low residual concentrations.

**Determination of the critical test dilutions of phages.** The sta-
phylococcus phages should have a critical test dilution of $10^{-6}$ but in any case not less than $10^{-3}$. On each newly produced phage fil-
trate, the titer is determined on the homologous strain (breeder strain)
and on 16 standard test strains. The phages are first diluted tenfold
and titrated against the homologous strain on 1.2% nutrient agar, in-
oculated with the gason from 4-5 hr old broth culture. The dishes are
incubated overnight at 30°. The KTR is determined the next day. As pre-
viously mentioned, this is the dilution in which the phage lyses the
homologous strain with semi-confluent lysis (in the form of "sponge").
The activity of the filtrates is then checked on 16 test strains. Pha-
ges with a KTR of $10^{-4}$ and less are tested undiluted, phages with
large KTR are diluted 10-100 times in order to exclude inhibition re-
actions. The filtrates are then titrated on all sensitive strains. The
results are designated as indicated in the standard Table 16. The dilu-
tion which gives the least strong reaction (+++) on the test strain is
compared with the dilution which gives approximately the same reaction
on a homologous strain. If these two dilutions coincide, the reaction
is designated by the number 5. If a $10^{-2}$ times higher phage concen-
tration is required for the appearance of such a reaction on the test
strain than on the homologous strain, the reaction is designated by
the figure 4; if the required phage concentration is $10^3$-$10^4$ times
stronger, by the figure 2. The figure 1 designates very weak reactions,
the symbol 0/2 inconstant and the symbol 0 inhibition reactions.

**TABLE 14**

Characteristics of Typing Phages (Blair, Williams, 1961)

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*Data of the Central Staphylococcus Laboratory.
**the phage 73 is resistant to heating to 50° for 60 min and is sterilized by this method.

A) Phage; B) breeder strain; C) serological group of phage; D) Ca consumption; E) conditions of preparation; F) designation of phage; G) number of phage; H) phagotype; I) method of production; J) optimum concentration of phage per mi; L) temperature (°C); M) incubation time (hr). 1) Absolute; 2) partial; 3) semiliquid agar; 4) the same; 5) broth; 6) broth; 7) broth or semiliquid agar.
TABLE 15
Reactions of the Standard Phagotypes and Test Strains (Blair and Williams, 1961)

<table>
<thead>
<tr>
<th>Phagotype</th>
<th>Number of strain</th>
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<td>10017</td>
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<td>3B'71+++</td>
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± = less than 20 plaques  
+ = 20-50 plaques  
+++ = over 50 plaques  
° = inhibition reaction (only with concentrated phages)  
A) Phagotype; B) number of strain; C) KTR. a) Non-typable.
**TABLE 16**

Lytic Spectrum of Typing Phages (Blair and Williams, 1961)

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<tr>
<td></td>
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<td>1 1 1 1 1</td>
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</table>

Note: The breeder strains were not included in the Table because all reactions on them can be designated by the figure 5.

A) Test strains; B) phages.

Phages with a lytic spectrum similar to the standard are suitable for typing.

**Storage of phages.** Undiluted staphylococcus lysates are best kept dried in the vacuum refrigerator without addition of sera. The liquid phages are stored at 4° up to 3 years. A tendency to a decrease in the titer is observed in some preparations after 6 months storage. At 4° the critical test dilution of the phages retains its activity for several weeks. In order to obtain reliable typing results, the effectiveness of the phages retains its activity for several weeks. In order to obtain reliable typing results, the effectiveness of the critical test dilution must be determined once a week on standard homologous strains. A critical test dilution which does not develop lysis in the form of a "sponge" is replaced by a new one. If the titer is reduced by more than one dilution, new phage must be prepared.
Typing technique. The basic methods of typing staphylococci are similar to those used for the phage typing of typhoid bacteria. Let us merely point out the differences. The strain to be tested is grown in broth at 37° for 4-5 hrs. The dishes with agar are slightly dried at 37° for 2-6 hrs. Oswald and Reedy (1954) recommend covering the Petri dishes with porous porcelain lids and to let them stand overnight for drying at room temperature.

The strain is first typed with the basic series of phages (21 phages) in critical test dilution or in 10 fold greater concentration than that of the critical test dilution. The inoculation of the dishes (0.2 ml of culture) and the technique of inoculating with phage, see in the section "Phage typing of typhoid bacteria." The cultures are grown 18 hrs at 30°. The typing results are then summed up. The results can also be observed after 48 hrs. Slight variations in the typing technique do not distort the results to any great extent (Williams and Rippon, 1952). If necessary, an 18-hr old culture can be typed (instead of a 4-5 hr culture); the dishes must be inoculated with 4-5 drops of culture with subsequent rubbing with a spatula instead of pouring on and removing the excess culture; the dishes with the culture are dried open for 1-2 hrs and incubated 5-6 hrs at 37°, then overnight at room temperature. Growing at 37° all night leads to copious secondary growth which distorts the typing results.

The following symbols are conventionally used for recording the results:

1) ± weak lysis (less than 20 plaques);
2) + medium lysis (from 20 to 50 plaques);
3) ++ strong lysis (over 50 plaques and also semiconfluent lysis having the form of a "sponge," confluent with secondary growth and confluent lysis without secondary growth).
According to the international nomenclature the phagotype of the staphylococcus is conventionally designated by the names of the phages which form over 50 plaques on it in critical test dilution — strong lysis. Thus, the staphylococcus which is lysed by the phages 3B, 3C and 55, has the designation 3B/3C/55. The list of phages is often concluded with a plus sign (for example 6/7/47/53/54/75+). This indicates that the culture can be lysed by ± or + phages which are not included in its designation (Fig. 3).

However, a neglecting of average and weak reactions can sometimes lead to a seeming difference in the phage characteristics of strains, obtained in the same focus. The intensity of lysis can fluctuate as a result of variations in typing technique or in the biology of the phages and cultures. As a result, the strains are given difference characteristics, i.e., they are described as different phagotypes. In order to avoid this, Williams and Rippon (1952) recommend as far as possible to type all epidemiologically related strains on the same day and that the entries in the recording journal should correspond to the designation of cultures in a Table, where not only strong, but also medium and weak reactions are given. If typing is carried out on different days, it is useful to include 1-2 strains from the first series as standards. Differences by one strong reaction are normally not taken into account and such strains are classified as being identical. Two cultures are considered to be different phagotypes if they differ by at least two strong reactions, the phages, which give these strong reactions on one of the cultures, not giving weak or medium reactions on the other strain. In the opposite case, the cultures are considered to be identical phagotypes (see Table 17). Taking into account the wide distribution of plasmacoagulating staphylococci, it is useful to type several strains from the same object.
TABLE 17
Recording and Evaluation of the Forms of Reaction (Blair, Williams, 1961)

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<th>C</th>
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</tr>
<tr>
<td>4</td>
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</table>

A) Number of strains; B) reaction with phages; C) recording of the form of reaction; D) evaluation; E) single phagotype; F) differ from the strains 1-4 and from each other.

By means of the basic phages in critical test dilution it is normally possible to classify 50-60% of staphylococci from patients with various forms of mastitis, suppurating diseases, from wounds, from vomit and food in cases of food poisoning, and from the pharynx and nose of healthy persons.

If a culture is not lysed by any of the phages in the basis series, typing is repeated with more concentrated preparations. Phages are normally used in concentrations 100 and even 1000 times greater than their critical test dilution. Only the strong reactions are taken into account in the recording of results. Ortel (1958), by using the above-indicated method, differentiated up to 75% of the test strains of staphylococcus aureus. Pöhn (1957) up to 82.3%, MacLean (1956) up to 90%.

A deficiency of the above-indicated method is the nonspecific lysis of staphylococci, which takes place as a result of the adsorption of certain concentrated phages. Alternatively this is termed the inhibition phenomenon. It is also often manifested in the form of confluent lysis (Fig. 4). Such an inhibitory effect of the phage is differentia-
Fig. 3. Forms of reaction of different phagotypes of staphylococci with typing phages in critical test dilution. a) Lysis with phage 52A(++)
    ) lysis with the phages 3A, 3B and 3C(++); c) lysis with phages 6, 7, 7, 53, 70(+++) and phage 42E(+) (center of the third row) (according to Anderson and Williams, 1956).

Fig. 4. Inhibitory effect of concentrated staphylococcus phages (acc. to Williams and Rippon, 1952).

...ed from specific lysis with reproduction by testing the activities of the following phage dilutions. The formation of isolated sterile spots in the culture confirms the specific nature of lysis because plaques are not formed in presence of an inhibitory effect. The inhibitory effect is not taken into account in the analysis of the results and is designated by the symbol "0".

The percentage of differentiated cultures can also be increased by parallel typing of several colonies of the same strain. Pühn (1957), for example, was able to determine the type by this method in 17.2% of initially nontypable strains and in 2.2% of "atypical" cultures, which have lysis with the typing phages.

- 161 -
Anderson and Williams (1956) proposed a second series of phages for the differentiation of cultures which cannot be determined with the first phage series. The series consisted of 21 phages which were first used in a mixture of several phages of the same serotype. The number of phages of the supplementary series has now been reduced to 6. These are the phages 42B, 73, 47C, 78, 52B, 69 (Blair, Williams, 1961). Finally, it is recommended to produce new phages for the cultures which cannot be classified with the international phages. Two methods can be employed.

Adaptation of one of the typing phages. Petri dishes are seeded with the gason of the test strain and slightly dried. Drops of undiluted typing phages are applied to the gason and after their adsorption placed into the thermostat (30°C) overnight. The sterile spots detected on the following day are lifted from the underlying culture and agar, added to 1 ml of broth and subjected to further passages. The range of lytic action, the serological properties, etc. are studied on the new phage.

As previously indicated, the adaptation of staphylococcus phages includes not only a true change of the phage particles under the influence of a new host strain, but in a number of cases also an element of selection of the typing phage from the mixture. In any phage reproducible on a lysogenic strain, mild phages of the host strain may get in which are sometimes capable of developing on the new strain. The phage 42C (Rauntree, 1949a), for example, was obtained by this method.

Isolation of new mild phages from lysogenic cultures: a) a 2-4 hour old culture of several previously known lysogenic strains is centrifuged at 3000 rpm for 10-20 minutes. The supernatant is sucked off and applied dropwise to the test strain, seeded with gason in Petri dishes (2-4 hr old broth culture). Following overnight incubation at
30°, a sterile spot with the surrounding culture is transferred into 1 ml of broth and repeatedly passed through the test culture until a phage with sufficiently high titer is obtained.

b) 0.5 ml of 3-6 hr old broth culture is poured into a Petri dish and irradiated with ultraviolet in a dose of 4-5 erg for 30-90 seconds with slight shaking (Corill, Gray, 1956). The irradiated culture is centrifuged 20-40 min at 3000 rpm. The supernatant is tested on the gason of the indicator strain for the presence of phage, as indicated in point "a".

Phagotype 80/81 and Rapid Methods of Its Detection

Staphylococci are normally lysed immediately by several typing phages. An exception from this rule is the phagotype 80 (or 80/81). Strains of this phagotype were first isolated in Australia during a large epidemic in one of the hospitals (Rauntree, Freeman, 1955). These were resistant to all the typing phages of Williams and Rippon. Only phage 52 and 52A produced on them a small number of sterile spots. By adaptation of phage 52A to the resistant cultures a new phage — 80 — was obtained. On the basis of its serological properties it was included in group B. It is possible that this phage is a virulent mutant of phage 52A because it is not capable of lysogenizing a culture of 80 (Comtois, 1960). The strains of staphylococcus, which are lysed by the B-phages 52 and 52A, always interact with phage 83. Bynoe and others (1956) isolated a similar strain in Canada and adapted the A phage (42B) to it. The new phage was given the designation 81. It was also virulent. The Australian phagotype 80 was lysed very strongly by phage 81 and, conversely, the Canadian phagotype 81 by phage 80. This confirmed the identity of the types 80 and 81 and Rauntree proposed to term them 80/81. Some researchers continue to term the phagotype 80/81 type 80, if the typing phage 81 is absent in their diagnostic series.
Strains have been detected in Australia in recent years which interact either with phage 80 or 81, but these are rarely encountered (Rauntree, 1959). Hence, the phagotypes 80, 81 and 80/81, unless specially stipulated, will be considered in the following to be identical.

The type 80/81 causes enormously frequent staphylococcus infections in hospitals and maternity homes, and was thus termed "epidemic" or "hospital" type (see further on).

The wide distribution of the phagotype 80/81 makes rapid methods of its detection useful.

White (1961) proposed to use for this purpose the specific sensitivity of the type 80/81 to the phage 81. The method enables the presence of the phagotype in a smear from the nasopharynx or a wound of the patient to be determined within 24 hrs from the beginning of the investigation. The smear is taken with a cotton wool swab, which has been dipped into broth containing additional calcium (0.004 M), placed into 3 ml of broth and shaken for 5 minutes. The smear is then diluted tenfold in the same broth and in the lysate of phage 81 (critical test dilution 10⁻⁶). 0.1 ml of each dilution is seeded on the surface of nutrient agar and grown overnight at 37°. If staphylococci are observed only at the points where the broth dilution of the smear had been applied, the test is considered to be positive and the strain is considered to belong to phagotype 80/81; if the staphylococci are also found in the lysate of phage 81 and in the broth, the test is considered to be negative. In this case the colonies are numbered and tested for their capacity to produce coagulase and for their sensitivity to the typing phages.

By the above-described method the author has typed cultures from the noses of 100 patients. Staphylococci were found in 30 patients, the phagotype 80/81 being present in 17 patients. In this last case the
Staphylococci were absent in the phagolysate 81, but in the broth there were 600 to 4,320,000 colonies of the type 80/81. Other types (I, II, and III group) were isolated from 13 patients, the staphylococci being present in both samples. The value of the method has also been demonstrated in the typing of staphylococci from wounds.

Another rapid method ("test tube assay") was worked out by Asheshow (1961). The method is based on the reaction of increasing phage titer, proposed by V.D. Timakov and D.M. Gol'dfarb (1955, 1956) for the tracing of typhoid, dysentery, etc. bacteria. In principle the method is based on the fact that the highly specific indicator phage proliferates in presence of the homologous bacteria. The increase in the phage titer attests to the presence of such bacteria in the substrate under investigation.

Asheshow uses phage 80 as indicator phage. 3 test tubes containing 2 ml of nutrient broth each, with 0.08 ml of sterile 1% CaCl₂ solution added, are taken for the test. The smear from the test object is suspended in the first test tube (test) and 0.1 ml of phage 80 is added from a solution, containing 10⁵ particles per ml. The standard strain type 80/81 and 0.1 ml of phage 80 are introduced into the second test tube (positive control). Into the last test tube (negative control) only 0.1 ml of phage is placed. All 3 test tubes are incubated for 5-7 hrs at 37°. 0.02 ml from each is then transferred to meat-peptone agar, inoculated with the indicator mixture: a 4-6 hr broth culture of terramycin-resistant strain 80/81, to which terramycin (100 units per ml of culture) had been added immediately before the test. The antibiotic inhibits the growth of the incidental microflora and makes the lysis pattern clearer. The dishes are incubated 4-5 hrs at 37°, after which the results are determined. If the result is negative, the procedure is repeated after the test tubes have been kept in the thermostat over-
night.

The results are considered to be negative if the number of phage particles in the experimental test tube is not greater than in the negative control, and positive if the sample from the experimental test tube gives confluent lysis. If the lysis is not confluent, but the number of particles in the experimental test tube is slightly higher than the initial, the results are considered to be doubtful. In all cases it is necessary, after having obtained a tentative result, to carry out the typing of pure staphylococcus cultures in order to make a final diagnosis.

286 smears from the noses of personnel and from septic wounds were investigated by the above-described method. Staphylococci were found in more than half the objects. In 75% of the positive tests, the staphylococci were resistant to phage 80. In 17 tests, the phagotype 80 was detected, being accompanied in all these cases by a positive reaction of increase in phage titer.

Both these rapid methods have the same disadvantage — their application is limited to a single phagotype. Besides, strains exist (which are very frequently encountered) which are lysed by phage 81 (or 80) in combination with other strains. Such cultures will first be erroneously ascribed to the phagotype 80/81. As this will be clear from the following, it is true that some of them (52/52A/80/81) have a great affinity with the type 80/81.

Stability and Variability of Phagotypes

The forms of reaction of staphylococci with phage preparations are fairly stable: different clones of the same strain and numerous subcultures passed in vitro and in vivo retain their primary nature of phage sensitivity unchanged (Blair, Carr, 1953; Williams, 1957, and others). In 6-11% of cultures from patients and the environment the authors iso-
ted staphylococci of a different phagotype than 1-2 days previously. 
is may account, but not in all cases, for the reinfections with such 
unusually widespread microorganism, as the plasmacoagulating staphy 
ococcus aureus. Sometimes the researchers encounter a true variabilit 
the phage reactions of staphylococcus. Different clones of the same 
agotype may lose or acquire new reactions with phages. Williams and 
pon (1952) also observed such changes in vivio. The authors could 
determine whether these depended on the composition of the medium 
ed for typing or whether a true variability of the culture existed 
this case. They are inclined to think that variations of this kind 
ot yet imply an evolution of the phagotype.

The difficulty in the study of the variability of staphylococcus 
agotypes in the organism is due to the necessity of constant differe 
ation of these phagotypes from reinfection. Nonetheless, fairly con 
cing indications have now been presented concerning the variability 
phagotypes within the limits of their groups. This concerns primari 
the phagotypes 80, 81, 80/81, 52/52A/80 and 52/52A/80/81.

The phagotypes 80/81 and 52/52A/80/81 are often isolated together 
us, Asheshow and Rippon (1959) during a long-lasting epidemic of sta 
ylococcus infections, caused by the phagotype 80/81, very often, par 
cularly towards the end of the epidemic, detected the phagotype 52/ 
2A/80/81. Milch and others, Rauntree (1959), Comtois (1960) and 
hers reported similar findings.

As previously mentioned, the "classic" phagotype 80/81 interacts 
ly with the phages 80 and 81 and it is thus comprehensible that cul 
ires 52/52A/80/81 are in practice interpreted as a different phago 
type. However, the frequent coexistence of these two phagotypes compels 
to assume a close relationship between them and the possibility of 
transformation of one into the other. This hypothesis has been con-
firmed in experiments of Rauntree (1959), Asheshow and Rippon (1959) and others. Because these facts are closely connected with the phenomenon of phagotype specificity, they will be discussed in the following section.

**Phagotype Specificity**

Staphylococci may contain mild phages of all the serotypes (A, B, F, L) which make up the series of typing preparations. This is comprehensible because all typing phages were isolated from lysogenic cultures of staphylococci. The form of the reaction of the staphylococcus with the typing phages depends on the peculiarities of the prophages carried by it. Thus, the typing A-phage 47 can impart to the standard phagotype 47 resistance to A-phages 47, 47B and 47C during lysogenisation (Lowbury, Hood, 1953). The phage A from a culture of 52/52A/80/81 imparted to the staphylococci 80/81 resistance to the A-Phage 81, transforming them into the phagotype 52/52A/80 (Asheshow and Rippon, 1952). The phage B from the phagotype 81 can impart to the staphylococci 52/52A/80 and 80/81 a resistance to the B-phages 52, 52A and 80, transforming both cultures into the phagotype 81 (sensitive only to A-phage 81) (Rauntree, 1959).

In all the above-presented experiments there are typical phenomena of immunity of a culture to phages which are serologically related to the prophage: lysogenisation with phage A gives immunity to A-phages, the B-phages impart resistance to B-phages, etc.

It was found in the experiments of the same authors that this phenomenon is not universal. Following lysogenisation with the typing A-phage 7, the standard typing strain 7 acquired not only resistance to A-phages (7, 47, 47C, 47B, 72, 75A), but also to the B-phage 76 (Lowbury and Hood, 1953). The phage F from the phagotype 52/52A/80 imparted resistance to the A-phage 81 to the phagotype 80/81.
such properties were isolated from carriers: in 6 cultures of phagotype 80, resistant to the A-phage 81, the phage F was detected (Ashe-show and Rippon, 1959). Finally, the phage B from phagotype 81 imparted resistance not only to B-phages (52, 52A and 80) but also to the A-phage 47C to a culture of 47C/52/52A/80/81 and transformed it into the type 81 (Rauntree, 1959). Hence, resistance to A-phages can be created in staphylococci by F- and B-phages and, conversely, A-phages can impart resistance to F-phages. This is the phenomenon of interference of the prophage with a serologically foreign virus. Such a form of resistance has been described for the 3 mild phages λ, Pl and P2 (Lederberg, 1957, Bertani, 1958), on which the system of typing typhoid bacteria is based (Anderson and Felix, 1953).

Thus the reactions of plasmacoagulating staphylococci with the typing phages are determined in some cases by the immunity to virus, serologically related to the prophage, in other cases by prophage interference. Only experiments can show which mechanism is specifically involved in each concrete case. It is thought that the large number of staphylococcus phagotypes and the great variety of their reactions with typing phages are explained just by this diversity of forms of specificity.

Lysogenisation also plays an important part in the appearance of nontypable cultures. The phagotype 80/81 and untypable strains are often isolated simultaneously from patients. Untypable strains have been successfully artificially produced at the laboratory by lysogenisation of the type 80/81 (Comtois, 1960).

The study of the causes of specificity and experiments involving artificial transformation of one phagotype into another help to delimit reinfections from the true variability of phagotypes. The phagotypes 7/81 and 52/52A/80/81, which, as previously mentioned, are often found...
together, may serve as an example, Rauntree (1959) assumed originally, that the staphylococci of type 80/81 were derived from staphylococci of type 52/52A/80/81 via lysogenisation of the latter by the phages 52 and 52A. A preliminary investigation did not support this hypothesis because nearly all staphylococci of phagotype 80/81 proved to be nonlysogenic (Asheshow and Rippon, 1959; Rauntree, 1959). On the contrary, the staphylococci of phagotype 52/52A/80/81 contained the mild phages of the serotypes A and F. Two phages, later termed $a$ and $n$, transformed the staphylococci of phagotype 80/81 into the phagotype 52/52A/80/81. Rauntree termed these "converting" phages. The phages 52 and 52A are B-phages. In these experiments, the cultures acquired sensitivity to phages which were serologically foreign to the infection strains.

Remarkable is the fact that in Rauntree's experiments on cultures, transformed into the type 52/52A/80/81, the undiluted typing A-phage 47C no longer produced the growth inhibition reaction which it had given earlier. The staphylococci became completely immune to this phage. This phenomenon was extraordinarily specific. The author proposed that the presence or absence of inhibition reactions with the A-phage 47C may indicate a corresponding presence or absence in the strains 52/52A/80/81 of the prophage which had converted them from strain 80/81. "Converting" phages were indeed isolated from all cultures of the natural phagotype 52/52A/80/81 which did not give "inhibition" reactions with phage 47C. The cultures which interacted with phage 47C were nonlysogenic. It has been shown in later researches that during the transformation of the type 80/81 into 52/52A/80/81, not only a purely lysogenic conversion, but replacement of one prophage by another takes place (Rauntree and Asheshow, 1961). Following numerous unsuccessful attempts at elucidating the lysogenicity of phagotype 80/
/81, a completely defective prophage 80' was found in it. It could not be detected by the usual methods. Its existence was revealed on account of its capacity to give a genetic recombinant with both converting phages (b phage). The recombinant plaques appear on a gason of type 52/52A/80/81 after inoculation of the latter with individual cocci 80/81. A similar case has been described by Cohen (1959) for E. coli B. These bacteria contained a completely defective prophage, the presence of which was established only on the basis of its capacity to give recombinants with phage P2.

The prophage 80' imparts to cocci 80/81 immunity to the phages 52, 52A and b. It is possible that the prophage "blocks" their loci in the chromosome. Following lysogenisation by converting phages (a or n), this immunity disappears: the staphylococci become sensitive to the three above-mentioned phages. The converting phages evidently replace the prophage 80/ in the cocci, thus "curing" them from it. An indication of the loss of prophage 80' consists in the fact that if the phage a is passed through the artificial phagotype 52/52A/80/81, phage recombinants are not found in the lysate. Moreover, traces of prophage 80' are not found in mutants of 52/52A/80/81, which arise spontaneously in a culture of type 80/81: they are sensitive to b-phage and do not give recombinants under the corresponding conditions.

It is remarkable that the phage recombinant in turn is capable of lysogenising cocci of the converted type 80/81 (i.e., of the artificial phagotype 52/52A/80/81), in which the prophage 80' is replaced by prophages a or n. Clones with double (a + b or n + b) lysogenicity thus appear. From certain phagotypes only one phage b is isolated. In such clones there is no "blocking" of the proliferation of the phages 52 and 52A. In other words, interference with phages 52 and 52A is innate only in the prophage 80' but not in its recombinants. The loci of the pro-
phages b, a and n in the chromosomes of staphylococci are evidently different from the locus in the prophage 80'.

The possibility of an evolution of other phagotypes under conditions prevailing in the living organism has been demonstrated in the investigations of Sompolinsky, Korn and others (1961). During mastitis flareups in a herd, the authors isolated from the milk of sheep and cows staphylococci of three species: resistant to all phages, 0(42E/44A) and 44A(29/52A/79/80/42E/44). During further study, all three types proved to be enormously closely related. In particular, all strains 0(42E/44A) contained mild phages which interacted in vitro with the staphylococci 44A(29/52A/79/80/42E/44A) and transformed them into 0(42E/44A) and resistant types. These experiments explained the great variety of types of reaction of staphylococci and showed that cultures which differ in their type of reaction can sometimes be of common origin. Staphylococcus phagotypes are evidently not as stable in their reactions with typing phages as is the case with typhoid bacteria.

Practical Utilization of the Method

A serious impediment in the interpretation of the typing results is the enormous variety of reactions of staphylococci strains with typing phages. In fact, a large number of phagotypes can be distinguished within each of the 3 groups. At the same time, some reaction types are encountered more often than others.

Williams and Jevons (1961) presented the typing results obtained on over 8000 strains and demonstrated that 26 phagotypes of staphylococci are very frequently encountered. These were the phagotypes 29, 52, 52A, 79, 52A/79, 80, 52/52A/80, 52/80, 29/52/80 in group I; 71, 3C/55/71, 3B, 3C, 55, 55/71 in group II; 6/7/47/53/54/75+, 6/47/53/54/75+, 7/47/53/54/75+, 42E, 47/53/75/77, 53/75/77, 53/75/77, 75/77, 77, 53, 73, 83A in group III and 42D (group IV). To these belonged 65.4% of
the strains from healthy carriers, 61.2% of the strains from patients with food poisoning and 78.3% of the strains isolated from hospital infections. Remarkable is the fact that within each group of patients their own characteristic phagotypes predominated and that their number was less (for example, in the cases of food poisoning only 17 phagotypes predominated).

Numerous observations in different countries showed that in all forms of staphylococcus infection phagotype determination of the strains can be of real help in the elucidation of the pathways of transmission and the sources of infection and the epidemiological analysis of flareups. In particular it has been shown that staphylococci, dwelling in the nasopharynx and on the skin are by no means harmless to man.

Preponderant in the nasopharynx of healthy carriers can be staphylococci of group I (Milch and others, 1960; Ortel, 1958, and others) of group III (N.P. Nefed'yeva and others, Fusillo and others, 1954), Williams, Rippon, Dowsett (1953), Vogelsang and Haaland, 1959. Identical phagotypes are more often found in the pharynx, the nasal mucosa and the skin of one and the same carrier than different phagotypes. Carriers may have staphylococci of the same phagotype in the nose or on the skin for very long periods. Thus, Roodyn (1960b) detected the phagotype 3B/3C in the nasal cavity of the same person for 3 years (in 1952, 1953 and 1956), in another for 6 years (in 1951, 1953 and 1958); the phagotype 52A on the skin (with constant absence of staphylococci in the nasal cavity) for 4 years.

Phage typing indicated that recurrent furunculosis, styes, and suppuration of accidentally inflicted wounds is most frequently caused by the phagotype which inhabits the human nasopharynx (Tullach, 1954, and others). Roodyn (1960a) showed that in individual families the
staphylococci from the nasopharynx can serve as etiological factor for suppurating diseases for years. Thus, in 11 of 17 families, the method of phagotype determination helped to demonstrate the existence of cross infections from one member of the family to another for the duration of several years. For example, in one family during the period 1952-1958 the staphylococcus of phagotype 52A/79 caused in the mother a suppuration of a burn on the chin and leg for several months in 1952; suppuration of a wound on the finger in 1958; in the daughter, a suppuration of a burn on the leg in 1953; in the father, an abscess on the legs in 1953, and a suppuration of a burn on the hand in 1958 and also small abscesses in other children during different years. The same type was isolated from all members of the family from the nasal mucosa. In other families suppuration of wounds in the skin and styes were caused for ages by staphylococci of the phagotypes 3B/3C, 52/79/80, 3C/55.

The hypothesis of a possible cross infection could be excluded in 6 families by phage typing: no less than 3 phagotypes were responsible for the suppuration of wounds and burns of the family members.

The phenomenon of autoinfection with "one's own" phagotypes of staphylococcus has also been demonstrated for septic wounds (Waage, 1952), for injuries on the hands of miners (Atkins and Marks, 1952). In the latter case the authors had detected the infectious phagotype of the staphylococcus in a miner several months before the onset of the inflammation. Valentine and Hall-Smith (1952) cured furunculosis patients by measures which entailed removal of the staphylococci from the nose.

Schmidt (1962), using the method of phage typing, demonstrated the possibility of a double staphylococcus infection in cases of mastitis, furunculosis and abscesses.

The use of the method of phage typing has proved to be extraordin-
arily useful in the epidemiological analysis of flareups of staphylococcus diseases in the maternity and surgical wards. The staphylococci of group I and III are most frequently isolated in hospital infections. According to the data of Ortel (1958), 61% of 1555 types strains belonged to group I, 24.3% to group III and 7% to group II. Pöhn (1955), Blair and Carr (1953) isolated mainly staphylococci of group I from patients and personnel, which interacted with phage 52 and 52A. In other flareups, a preponderance of the cocci of group III was noted (Fustillo and others, 1954; MacLean, 1956, and others). At the same time, the number of epidemic phagotypes is relatively small. According to the data of Williams and Evans (1961), 70.6% of 5616 strains from epidemic foci in different hospitals in Britain belonged to 14 phagotypes. Among these predominated the phagotypes 80 (31%), 52/52A/80 (13.4%) and 83A (6.6%) (the phage 83A was added to the series in 1959). 6 strains [the three above-listed plus 47/53/75/77 (3.4%), 52A/79 (3.3%) and 6/7/47/53/54/75 (2%)] caused 50% of all flareups. The phagotypes 79, 52A, 77, 53/77, 75/77, 6/47/53/54 and 73 were also included among the epidemic types.

Epidemic phagotypes were rarely found outside hospitals. To these types belonged only 17% of 710 strains, isolated from healthy carriers and 28% of the strains, isolated from nonhospitalized patients (contact with the hospital could not always be excluded in the last group). It was found that some phagotypes can be widespread among the personnel without, however, causing flareups of infections.

Particularly frequently isolated at the hospital (31%) was the type 80. And this was not only so in Britain. In 1954, this phagotype caused 74 out of 92 flareups of sepsis of the newborn in Australia (Rauntree and Freeman, 1955) and caused extremely grave skin injuries in individual cases. During the ensuing years it retained the domi-
ting position in hospital flareups, the majority of pneumonia cases with fatal issue during the grippe epidemic of 1957 being caused by this type of staphylococcus.

In Norway, the phagotype 80 also predominated among the cultures isolated from the personnel of a large hospital at Bergen (Vogelsang and Haaland, 1959). In one of the hospitals of Boston during the period 1955-1958, 1/3 of all isolated staphylococci belonged to the phagotype 80/81 (Wollmark and Finland, 1961). This is not a new phagotype. Among the stock staphylococci, isolated in 1927-1947 (prior to the application of the method of phage typing) it was detected in 22.1% (Blair and Carr, 1960). The wide distribution of type 80 has impelled a search for methods of its further subdivision (Wollmark, 1961).

The phagotype 71 has been described as the basic etiological factor of contagious impetigo, pemphigus and some other suppurating diseases (Barrow, 1955, Spittlehouse, 1955, Anderson and Williams, 1956, and others).

By using the method of phage typing it is possible to trace the propagation of hospital strains among personnel and newly arrived patients. Phage typing of staphylococci in lying-in hospitals can determine with maximum accuracy the causes of infections of mothers and newborn babies, thus extremely facilitating the practical counterepidemic measures. Matejovská and Raška (1961) ascertained that diseases of mothers (mastitis) and of the newborn (conjunctivits, pemphigus, pyoderma) in maternity hospitals are caused only by epidemic hospital strains. In the investigations of these authors, these were the phagotypes 52/5-A/30 and 80, sometimes 7/75 or 3A/71. The staphylococci brought into the maternity home by the mothers (in the pharynx, nose, vagina) also spread among the newborn and mothers, but did not cause infections.
Phage typing helps to determine the rate of spreading among patients and personnel in a hospital. The nurses acquire a new staphylococcus strain soon after the onset of labor in the ward. It is remarkable that the staphylococci spread more rapidly among non-carriers.

The patients receive the hospital type of staphylococcus equally rapidly. On the day of arrival, staphylococci were present in the nasopharynx of 10.2% of patients, the percentage of carriers increased to 35.5 within 3 days, and within 12 days to 71.1% (Milch and others, 1960). Dowling and others (1953) ascertained that staphylococcus carriers upon their return home spread the hospital type within the family circle. It is obvious that the hospital conditions (close contact in the wards, etc.), and the weakening of the macroorganism promote the proliferation and dissemination of staphylococcus.

Three kinds of staphylococcus epidemic can be distinguished in surgical and maternity departments (Anderson and Williams, 1936). In the first two kinds, all or practically all staphylococcus strains from wounds or skin injuries belong to the same phagotype. One or two carriers of this epidemic type can be found in flareups of the first kind and it is easily shown that these are the ones spreading the infection. The epidemic can be liquidated by removing the carrier. Such flareups are rare.

In a flareup of the second kind, the epidemic type of staphylococcus is present in the nasopharynx of numerous patients, in most service personnel and on the objects in daily use at the hospital.

Such a flareup has been described by Blair and Carr (1953). In a ward of a New York hospital, 62% of staphylococci from wounds, abscesses, the blood, from children's beds, soap and the floor interacted only with phage 52A. 63% of the staphylococci from the nasopharynx of children and the personnel belonged to this phagotype. In other wards of the
hospital, the type 52A accounted only for 9% of all strains. This provided a basis for concluding that the personnel is the most important cause of the prevalence of wound infections at the department.

Epidemics like the above-described, naturally cannot be stopped by the removal of certain carriers. Only careful observation of asepsis and of chemotherapy of the nasopharynx of the carriers can help.

In flareups of the third kind, several different phagotypes are isolated from wounds, some of which are also detected among the personnel (A.F. Morox, 1961). In such a situation it is difficult to determine the true epidemic type of staphylococcus and the source of infection. The epidemic may be a consequence of a lowering of the standards of asepsis and sometimes of widespread presence of carriers of pathogenic staphylococci among personnel and patients. Complex measures of cleaning and disinfecting rooms and measures to improve the standards of asepsis in combination with assanation of the bacillus carriers with antibiotica help to reduce the percentage of carriers. This markedly reduces the incidence of staphylococcus infections.

It is obvious that the main function of phage typing investigations into hospital infections consists in determining what form of epidemic is present. This is only possible if staphylococci from a maximum number of wounds are typed. If the epidemic was caused by a single phagotype, this justifies a search for carriers among the personnel.

The interpretation of most flareups of staphylococcus infections does not present any special difficulty. At the same time, changes in the reactions with typing phages in cultures, isolated repeatedly from the same patient, must be extremely carefully treated. An intra- and inter-group variability of the phagotype must be distinguished.

Changes in the phage sensitivity within a single group is a frequent phenomenon. Milch and others (1960) observed such variations in
An intra-group evolution of the phagotypes is very often found in the phage group I. For example, in the same flareup, part of the strains interacts with several phages of group I in critical test dilution, another only with concentrated phages. A loss of the sensitivity of a culture to one or two phages is also possible. For example, the phagotypes 52/52A/80/79; 52/52A/79 and 80 are isolated together. The variations within a phage group may be a direct consequence of a modification in the typing technique (Williams and Rippon, 1952; Anderson and Williams, 1956, and others) and also of a lysogenization of the cultures in the organism (Rauntree, 1959; Asheshow and Rippon, 1959). Great care must be exercised in describing such variations as a result of reinfection.

It is a different matter when we are dealing with a change in the group of the staphylococcus. Experimental indications of such far-reaching variability of staphylococci are lacking; it is more probable to consider them as superinfections.

T.V. Golosova, V.A. Shenderovich, et al. (1962) used phage typing in the control of the efficiency of assanation of staphylococcus carriers in a maternity home. The investigators compared the phagotypes of the staphylococci, isolated prior to assanation and a month later. The change in the phagotype proved that the carriers detected a month after assanation, were the result of the reinfection with new strains of pathogenic staphylococci in some individuals.

Staphylococci of the phage group III and sometimes untypable cultures are isolated, as a rule, in cases of food poisoning. Thus, according to the data of Anderson and Williams (1956), of 18 strains, isolated in 18 flareups of food poisoning in different countries, 15 belonged to group III, and only 3 strains to group II. The authors assumed that contamination of the object cannot be excluded in the last.
case. Williams and Evans (1961) found a phagotype of group III in 7% of flareups of food poisoning (210 strains were studied), groups I and II accounting for 3.5 and 2.4%, respectively.

The types of reaction of the staphylococci, isolated in flareups of food poisoning, are normally not as manifold as in hospitals and in the investigation of healthy carriers. Thus, N.P. Nefed' yeva and others (1961) isolated cocci in 28 flareups, which were lysed by different combinations of the phages 6, 7, 47 and 53. Typical is the fact that, as a rule, the same phagotypes of the staphylococci were isolated from the excretions of the victims, as in the incriminated (food) product. One flareup was caused by phagotype 42D (group IV), one by type 3A (group II), and untypable cultures were isolated in 2 flareups. During staphylococcus poisoning in Czechoslovakia, staphylococci with the reactions 6/54 and 47/54 were most frequently found (Matejovska, 1957), in Britain, the phagotypes 6/7/47/53/54/75 (12.5% of all foci), 6/47/53/54/75 (12.9%), 53 (10.5%) (Williams and Evans, 1961), in Italy, the type 6/7/42E/47/53/54/75 and related types (Gallotti and Spano, 1961), in the USA (from meat), the types 83 and 53 in different combinations with others (Jay, 1962).

Examples of successful application of phage typing during investigation of staphylococcus food poisoning are widely known. The literature is rich in descriptions of flareups, in which phage typing helped to establish positively the infection source.

Wilson and Atkinson (1945) described a flareup in which strains of the same phagotype of staphylococcus aureus were isolated from vomit from patients and from leftovers of ham consumed by them. The same phagotype was isolated from the nasal cavity of worker in the kitchen department, who was engaged in the cutting of sandwiches on the day prior to the poisoning. As another example we may quote the flareup
described by S.L. Petrovich (1961). 21 men came to the health station of a work on a hot summer day. All the victims had partaken 1-4 hours previously in the dining room of a cold soup of kvass, vegetables and boiled meat. From the wash water of the patients, the soup, and the smears from the utensils, 8 strains of coagulase-positive staphylococci were isolated, 7 of which belonged to the phagotype 6/53+. The same phagotype was detected in the nasopharyns of the 2 cooks, who had prepared the soup and also in the smears from the board used for preparing the boiled meat.

As we know, the laboratory tests for staphylococcus enterotoxin are complex and cannot always be carried out. It follows from the observations of British microbiologists (Anderson, Williams, 1956) that cultures of the phage groups I, II and IV produce enterotoxin enormously rarely, at the same time as those of group III produce it very frequently. There is no basis, however, for assuming that any strain of group III can secrete enterotoxin: group III includes good as well as poor producers of toxin; phage typing cannot distinguish between them. In short, phage typing is not suitable as an indirect method of determining the capacity of staphylococci to produce enterotoxin, although it often helps to predict this property.

The fact that nearly all strains isolated during food poisoning in different countries, regularly belong to group III, differing in the combinations of the 9 phages of this group which lyse them, leads to the thought that the sensitivity of staphylococci to certain typing phages and their capacity for producing enterotoxin are mutually dependent properties. Such a relationship has also been observed between the phagotype and the hemolytic activity of staphylococci. Thus, in strains which do not produce hemolysin and which are resistant to phage 42F, the appearance of this capacity was observed simultaneously with
the acquisition of sensitivity to phage 42F (Davidson, 1961). Such variants were found during inoculation of the culture. The clones of the variant and the parent strain were dissociated, giving hemolysin-producing mutants. However, the type of reaction with phages did not always change simultaneously with the variations in hemolytic activity; variants sensitive to phage 42F appeared in the hemolytically inactive clones.

As we know, the pathogenicity and other biological properties of staphylococci are closely connected with lysogenicity. Staphylococci, which are pathogenic on the basis of in-vitro tests (coagulase reaction, fibrinolysis reaction, formation of pigment, hemolysin and enterotoxin) as a rule, are lysogenic. Staphylococci which are not pathogenic on the basis of these tests are nonlysogenic and are resistant to the typing phages of the basic and auxiliary series (Ciucu, Popovici, 1961). At the same time it has been shown that the sensitivity to a certain typing phage is closely connected with the peculiarities of the prophages.

It has been found in recent years that in some cases lysogenization can be accomplished by considerable modifications in the metabolism of bacteria. In particular, nonlyogenic, avirulent strains of diptheria bacteria can be transformed into toxic ones as a result of lysogenization. It has been shown that this process is not connected with a selection of mutants. Its mechanism is not yet clear. One fact, however, is beyond doubt: by penetrating into the bacterial chromosome, the prophage modifies the course of protein synthesis in some way. It is not impossible that similar phenomena also take place in the strains of toxic staphylococci. It may be assumed that among the mild phages of staphylococci there are some whose penetration into the cell exerts an influence on the metabolism of the latter, thus imparting the capacity
for enterotoxin synthesis. Enterotoxin production may be connected with certain varieties of mild phages or possibly with certain of their combinations. The above hypotheses are mainly based on a single fact: the capacity of the toxigenic strains to be lysed by a constant group of phages. Light can only be shed on these processes by careful experiments on the study of the properties of the mild phages of the strains of group III and their capacity for exerting an influence on the enterotoxicity of the staphylococci.

Comparison of the Method of Phage Typing with Serological Methods and the Antibioticogram

In addition to phage typing, the determination of the serotype and the study of the sensitivity to antibiotics are used for further subdivision of the coagulase-positive staphylococci.

The serological classification of staphylococci according to Oeding (1952), who distinguished 10 antigens in living cultures of pyogenic staphylococci: a, b, c, d, e, f, g, h, i, and k, has now won recognition. The antigen d is detected in all cultures, the antigen a in nearly all, the antigens f, h, i, k are found extremely rarely. Most staphylococci belong to the 4 serotypes: ae, abe, ab and abc.

In practice, serological typing is carried out by agglutination of 5-hour old cultures of staphylococci on a glass slide with 8 sera. Agreement between the results of phage and serologic typing is rarely observed. Strains of one phage group can belong to different serotypes and vice versa (Oeding and Williams, 1958, Pulverer, 1961). True, if the strains were isolated from the same epidemic focus, the results of their serological and phage typing coincide. A deficiency of the method of serological typing as compared with phage typing is the small number of types, into which the cultures can be subdivided and also a certain academic aspect. A merit of the method is the small percentage of un-
Testing for the resistance to antibiotics is used as the simplest method of typing staphylococci. Its importance is limited because the diversity of types of sensitivity to antibiotics among the cultures is not great and they change during the process of investigation even within the same phagotype. Phage subdivision has proved a more successful and reliable method.

Many researchers have tried to discover a connection between the resistance of staphylococci to antibiotics and their phage group. The most antibiotic-resistant staphylococci are those of group I and III, which cause human diseases most frequently (Vogelsang, 1953; Vogelsang and Haaland, 1959; Rauntree, 1952, 1953; Milch and others, 1960). The number of penicillin-resistant cultures can attain 86.1-92% in these groups (Cetin, 1962). Antibiotic-resistant strains are more rarely found in the phage group II (0-40.7%) and particularly in group IV, which are rarely isolated from humans.

As regards the connection between the phagotype of the staphylococcus and its antibioticogram, epidemiological factors must be assumed to play a decisive part. It is to be expected that if some single phagotype has spread in a hospital ward, its antibioticogram will predominate among the cultures.

Phage typing of staphylococci with simultaneous study of the antibioticogram has proved fruitful in the study of the regularities of distribution of staphylococcus diseases in hospitals. Most of these investigations demonstrate convincingly that staphylococcus hospital cross infection is not only a widespread phenomenon, but is also extremely often responsible for the preponderance of antibiotic-resistant cocci in the hospital. This is true with respect to postoperative complications of wounds in maternity homes and suppurating and septic diseases.
of the newborn.

The study of the antibioticogram and phagotype of staphylococci helps to solve the problem of reinfection. If a wound staphylococcus, having acquired penicillin resistance, changes its phagotype, it must be assumed that the development of resistance in this case is not the result of penicillin therapy carried out on a given patient, but a consequence of reinfection. Conversely, the appearance of resistance in staphylococci without a change in phagotype attests to the absence of cross infection. Study of the antibioticogram of staphylococci confirms the hypothesis that only a change in the phage group of the staphylococci can be unconditionally considered as reinfection. In such cases the antibioticogram varied by 47%, in variations within a group, by 11% (Milch and others, 1960).

Oeding and Sompolinsky (1958) compared all three methods of typing of staphylococci in postoperative complications in hospitals, during flareups of food poisoning in military camps and during investigation of carriers. "Outside" staphylococci, as a rule, were resistant to phages, sensitive to many antibiotica and were best differentiated on the basis of their serological characteristics. The hospital staphylococci could be easily typed with phages and on the basis of the antibioticogram. The clearest and most reliable results were obtained by combination of several methods (Kretzschmar, 1961).

PHAGE TYPING OF STAPHYLOCOCCI OF ANIMALS

The phages of the international series proved to be unsuitable for differentiating the coagulase-positive staphylococci of animal origin. Only about half of them and rarely more than half the cultures of staphylococci isolated from the udders of cows, from milk, butter, cheese and other products reached with them (Coles and Eisenstark, 1959, Nakagawa, 1960a; Seto and Wilson, 1958). A particularly low percentage of typable
cultures obtained the authors who used phages in critical test dilutions. The use of concentrated phages (in concentrations 10 and 100 times higher than the critical test dilution) increases the percentage of typable cultures. For example, in the experiments of Nakagaw (1960a), the phage 3A was inactive in critical and even in 10-fold critical test dilution. In a concentration 100 times higher than the critical test dilution it lysed some cultures from cow's milk. Only with some phages (79, 3C, 7 and 42D) the concentration did not have such a strong effect on the typing results.

The phages of the international series do not give a clear demarcation of animal staphylococci into 4 phage groups. Intergroup reactions are extremely frequent (for example, between group III and I, III and IV and I).

In animals and in products of animal origin, staphylococci of different phagotypes and phage groups are detected, as a rule, than in humans. Cultures from cattle rarely show any sensitivity to phages of group II. These phages are obviously of little value for typing staphylococci of such origin (Davidson, 1961). The type 42D (group IV) is extremely often found in animals. It predominated among strains, isolated from the udders of cows in the USA (Coles and Eisenstark, 1959), from cow's milk in the USA (Smith, 1948a and b) and in Japan (Nakagawa, 1960a). Nakagawa detected 42D strains, sensitive to phage 81 and also a considerable number of cultures of group III with weak sensitivity to the phages 42E, 6, 47 and 75. In the USA, the phagotype 44A, which had been eliminated from the international scheme, has often been detected in certain droves of cows with mastitis (Seto and Wilson, 1958) and also in synovitis of turkeys (Smith, James, and others, 1961).

At the same time, the uniformity of the staphylococcus phagotype population in animals is deceptive. The strains of the phagotypes 42D
are not identical. It is possible to subdivide animal staphylococci of type 42D by means of mild phages (Nakagawa, 1960b; Smith, 1948a and b). Within the phagotype 44A 9 types were differentiated by means of phages isolated from staphylococci from turkeys (Smith, James and others, 1961).

In connection with the above-described deficiencies, the international series of phages has been recognized as unsuitable for typing of staphylococci of animal origin. Special typing schemes have been worked out for these microorganisms. Depending on the origin of the typing phages, three kinds of scheme can be distinguished.

Mild phages from lysogenic staphylococci of animal origin are used in the schemes of the first kind. This condition is particularly important because there exists a certain known relationship between the origin of cultures and their sensitivity to phages (Smith, 1948b; MacLean, 1951). This relationship is so marked that, for example, phages from bovine staphylococci must be isolated for the typing of staphylococci from cows, for staphylococci from turkeys, phages from turkey staphylococci, etc.

By means of 10 mild phages, Barnum and Fuller (1956) differentiated 81.2% out of 591 strains of hemolytic staphylococcus isolated from the milk and udder of healthy cows and cows in different stages of mastitis, into 5 phagotypes: A, Al, B, Bl, Ba. The staphylococci of each phagotype were lysed by 3-5 phages. The authors demonstrated the epidemiological value of their method of typing in 7 herds, where cases of bovine mastitis were frequent. They isolated the same epidemic phagotype in 5 droves from the milk and udder of healthy cows and cows with different forms of mastitis and also from smears from different utensils, used in milking. The staphylococcus phagotype did not change following the treatment of the cows with antibiotica, disinfection of the udder.
Nakagawa (1960b) subdivided by means of 16 mild phages 72.2% of 442 staphylococcus cultures from milk into two clearly delimited groups: A (with subgroups Al and A2) and B. The use of concentrated phages (100 times more concentrated than the critical test dilution) confirmed the marked difference between the groups A and B; not one of the cultures of group B was transformed into group A or vice versa. Strains which were differentiated as group III-IV by the phages of the international series made up only one group in the system of Nakagawa. Cultures of group II and partially of group I could not be typed. The advantages of the scheme of Nagagawa were limited to staphylococci from cow's milk. Only 6.8; 10; 23.1 and 8.1% respectively of the staphylococci isolated from humans, sheep, pigs and horses, could be typed.

In the schemes of the second kind, phages of the international series, adapted to the staphylococci of animals, are used. The phage which lyses the staphylococci of the given species of animal most actively is selected as the starting phage. Adaptation also enlarges the activity of the phages with regard to resistant cultures.

Coles and Eisenstark (1959) adapted 8 standard phages (79, 52A, 47C, 42D, 42E, 7, 42B, 81) to bovine staphylococci. By means of these new phages (A5, A6, A7, A8, A9, A10, A11 and A13), the authors subdivided 168 cultures from cows into 8 main groups, including 25 subgroups and 13 mixed categories. 39 forms of reaction were established in all. Only 18.8% of cultures were resistant to the action of the adapted phages. Typical is the fact that among the staphylococci predominated strains, lysed by derivatives of phages 42D and 81.

A series of typing phages can be obtained by combining the mild phages from lysogenic cultures with adapted phages. Coles and Eisenstark (1959) used 6 phages. Three of these were obtained by adaptation.
of phages 42D, 81 and 7 to strains of animal origin and three were isolated from lysogenic cultures. By means of these phages it was possible to differentiate most of the cultures isolated from herds of cows with mastitis. Phage typing helped to establish the existence of a large number of staphylococcus infections: several phagotypes of staphylococcus aureus were isolated from all herds. Moreover, in the same cow 2-3 phagotypes were taken from different ventricles of the udder. The same diversity of phagotypes in a single herd was observed by other authors as well (Seto and Wilson, 1958; Price and others, 1956).

The utilization of special typing systems for staphylococci of animals has the consequence that the analysis results obtained in veterinary and medical bacteriological laboratories cannot be compared. This creates difficulties in the analysis of flareups of staphylococcus poisoning, when products of animal origin are suspected as the cause (cottage cheese, milk, cheese, etc.). At the same time, if only the Williams and Rippon phages are used in such cases, a large proportion of cultures may remain undifferentiated. Besides, a deceptive uniformity of the type population (for example, isolation everywhere of type 42D) may in individual cases misrepresent the true importance of certain products.

This contradiction is to some extent eliminated in the scheme of Davidson (1961). For typing the staphylococci of cattle the author selected 10 phages from the standard series of Williams and Rippon and 9 phages obtained from lysogenic bovine staphylococci. Among 13,966 cultures, isolated over a period of 6 years from the milk and udders of cows in herds at Weighbridge (Britain), only 1.47% were resistant to these phages in critical test dilution. The lysogenic test confirmed this subdivision. It is remarkable that for periods of 3 months to 3-1/2 years the mastitis phagotypes were stable in the stronge types of
reaction. This confirmed the epidemiological value of this typing scheme.

The new series of phages was tested on 222 cultures, taken from 1000 cultures of herds in different parts of Britain. The phages with low specificity were discarded. The 15 phages established in correspondence with the international scheme were subdivided into 4 groups: I-III (29, 52, 42E, 101 and 110), III (31B, 53, 77), IV (42D, 102, 107, 108, 111) and 78 (78, 115). The phages from lysogenic bovine staphylococci belonged to the same serological groups as the phages of the international series: to group A belong the phages 101, 109 and 115, to group B the phages 102, 105, and 111, to group C the phage 107 and to group F the phages 106 and 108.

By means of series of phages, 106 phagotypes were determined in 222 cultures. The phagotypes of group I-III (43.7%) and IV (38.3%) predominated. Owing to the coincidence of the groups in both series and the presence of one or several phages of the standard series in each Davidson group, it is possible in the scheme of Davidson to compare the results with those obtained by using the international series.

As in the scheme of Nakagawa, the Davidson phages proved to be applicable only for typing of staphylococci of cattle and not very suitable for typing of staphylococci from other animal species. The scheme can be used for the study of epidemiological connections in mastitis herds and also as an aid in the investigation of flareups of staphylococcus food poisoning, when milk products are suspected as the pathways of propagation.

The standard international phages are used for typing the plasma-coagulating staphylococci of humans (basic and additional series, including 27 phages). Part of these phages has been isolated from lysogenic cultures, another was obtained by adaptation of the former to
resistant cultures. The phagotypes are differentiated on the basis of their type of reaction with the typing phages. The phagotypes are not standard because the forms of reaction of staphylococci with phages are extremely numerous. There are also phages which normally lyse culture in combination. This allowed 4 groups to be isolated among staphylococcus phages and strains. The forms of reaction of staphylococci with the standard phages are fairly stable. A variability has been observed only within the phage group. This could be a consequence of a selective of mutants, of lysogenic conversion, or a replacement of one prophage by another. The specificity of the phagotypes of staphylococci like that of other groups of microbes, is controlled by the prophages. In some cases, this is a manifestation of the immunity of the cell to virus serologically related to the prophage and in others, of prophage interference.

The staphylococci isolated during flareups of hospital infections are characterized by a great diversity of reaction with the typing phages which naturally makes epidemiological analysis more difficult. The detected phagotypes are correlated with one of the four phage groups, taking into account that the phage reactions may vary within the group. The variation within a group can be treated as a variability of the phagotypes, and variations outside the limits of the group as reinfection.

A smaller number of phagotypes is isolated during flareups of food poisoning than in hospital infections. These are most frequently staphylococci of group III. The ability of elaborating enterotoxin is mainly innate in the above-named group of staphylococci. At the same time, no all strains of group III produce enterotoxin and phage typing cannot, therefore, give an indication with respect to this ability. The staphylococci are also typed on the basis of their serological characteris-
and on the basis of the antibioticograms, other methods can be auxiliary importance by making the results of phage typing more significant and precise. The most reliable results are obtained by a combination of all three methods.

The phages of the international series are not very suitable for typing of staphylococci isolated from animals. Several typing schemes have been proposed for such staphylococci. The phages were obtained lysogenic staphylococci of animals and also by selection and adaptation of the phages of the international series to such staphylococci. New schemes cannot be used for typing human staphylococci. When investigating flareups of food poisoning, caused by milk products, it is useful to combine the international phages with phages which differentiate staphylococci of animal origin.
Chapter 8

PHAGE TYPING OF PSEUDOMONAS AERUGINOSA

definite increase in the number of cases caused by Pseudomonas
noth has been observed in recent years. This microorganism is
ionally pathogenic. The most frequent habitat of Pseudomonas
ossa is the intestine of humans and warmblooded animals, some-
the mucosa of the nasopharynx and the skin. Under certain condi-
Pseudomonas aeruginosa can cause general sepsis as well as in-
tion injury to various organs; inflammation of surgical wounds,
et types of otitis, conjunctivitis, meningoc-encephalitis, mas-
flammation of the urinary pathways, gastrointestinal disorders,
mation of the trachei, bronchi, lungs and other organs, ulcers
cubitus. The increase in the number of diseases of this type in
Pseudomonas aeruginosa is found, is largely connected with the
oral, prolonged use of many antibiotica, which depress the normal
lora of the pharynx and intestine and possibly stimulate the pro-
tion of Pseudomonas aeruginosa. Pseudomonas aeruginosa infection
ally manifested by debility or sickness of the organism. Small
en and old people are particularly prone to it. Flareups of dis-
cased by Pseudomonas aeruginosa have also appeared frequently
ent years in maternity homes and surgical wards: contagious enter-
of the newborn; mastitis in mothers, mass complication of wound;
nder these conditions, a subdivision of the strains of Pseudomonas
ossa of different origin into phagotypes can be of assistance in
idemiological analysis in establishing the chief sources and path-
of its propagation in the wards, and in carrying out the necessary

Several schemes for phage typing of Pseudomonas aeruginosa have

proposed to date. All these are far from perfect but they indicate

theless a certain possibility of typing this microorganism on the

of its sensitivity to a range of mild and virulent phages, and

basis of its lysogenicity and pyocinogenicity. A certain epidem-

ical value has been demonstrated for some of these schemes. The

attempt at phage typing of Pseudomonas aeruginosa was undertaken

rner (1950). By means of 19 phages isolated from lysogenic cultures,

author determined several phagotypes in this microorganism.

Pavlatou and Hassikou-Kaklamani (1961) proposed their method of
g, also based on the principle of Craigie and Ian. With 12 phages,

which (1, 2, 3, 4, 9 and 10) were isolated from lysogenic cultures

(5, 6, 7, 8, 11, 12) from sewerage effluents, 174 strains of

omonas aeruginosa (from pus, urine, feces, sputum and the blood
tients and healthy children) were differentiated into 12 phago-

(I-XII). Only 2 phages were specific: I(for type I) and 2 (for

II). The other phages lysed only some phagotypes which differed

e pattern of sensitivity to the 10 typing phages. Some reactions

extremely inconstant. In individual cases this was a serious im-

ent for the identification of cultures because they simulated a

ility of the phagotypes. The authors assume that a variability

e reactions with phages is possible if cultures are stored for

periods on laboratory media and also in the organism of patients.

thus best to type fresh cultures, preferably taken prior to the

istration of antibiotics.

In spite of the above-presented deficiencies, the method has proved

ically useful. The differentiation of cultures isolated from pa-
Ind theiIr everyday utensils at a children's hospital made it pos-
trace the pathways of propagation of Pseudomonas aeruginosa
establish the fact of spreading of the infection within wards.

one ward, strains of phagotype I were isolated from 3 sick new-
ies, which confirmed the connection between the infantile cases.
ner ward, 6 strains were isolated for several days from the
f newborn children. All these belonged to phagotype III. The
of Pseudomonas aeruginosa isolated from a Dettol solution which
od uncovered in the same ward for several days, belonged to the
agotype. At the same time, cases are described in which differ-
gotypes were isolated from the same patient. Thus, type I was
in one child from a furuncle of the upper lip and type III
e blood. In another patient, phagotype V was found in abscesses
left and right buttocks, type III and a strain resistant to all
from abscesses on the thighs. The authors proposed that this di-
of types may be a consequence of reinfection or of a variabil-
the phagotypes in the organism. This problem requires further

A other scheme for typing Pseudomonas aeruginosa was proposed by
McLeod (1960). The authors used double (serological and phage)
Most typing phages were isolated from lysogenic cultures, some
werage effluents. Using 16 phages, the authors differentiated
ins of Pseudomonas aeruginosa isolated in 3 uro-
le urine of operated and unoperated patients and from utensils of
se into 5 phagotypes (I, N, QS, W and Z). Several subtypes could
mined within the types. The subtypes were not rigidly different-
their reactions depending on the biological peculiarities of
ures and on variations in typing technique. On the basis of
rological properties in correspondence with the presence of
- 195 -
ological properties and the phagotype were interrelated in most cul-
es. Thus, the strains of group \( \alpha \) belonged to phagotype \( N \), those of
up \( \beta \) to type I, those of group \( \gamma \) to the type QS. In some of the
ains, however, these characteristics did not coincide. This was par-
ularly noticeable during the typing of cultures from other towns
regions. Thus, for example, from 9 strains of serotype \( \alpha \) (obtained
a Birmingham) 5 belonged to phagotype \( B \) and 4 to phagotype \( N \). The
od has proved useful in the epidemiological analysis of the causes
postoperative suppuration. It was found that Pseudomonas aeruginosa
exist in the human organism for fairly long periods: the phagotypes
cultures, found on the skin, in the urine and feces of carriers,
e the same in repeated investigations. The same phagotypes caused
postoperative infection. Only in two patients could a new phago-
e be observed after the operation, being combined in one of the pa-
ts with the type found prior to the operation. Phage typing showed
 cultures from feces are rarely detected in inflammations of the
ther; its main cause are normally strains which inhabit the urine.

The method of phage typing in the two above-described schemes is
same as that used for typhoid bacteria, etc. At the same time, cer-
n peculiarities must be taken into account when working with Pseudo-
as aeruginosa (Gould and McLeod, 1961; Pavlatou and Hassikou-Kakla-
i, 1961). A thin layer (2 mm) of solid (2-2-5%) agar (pH = 7.2) is
red into a Petri dish. In the thick layer of softer agar, used for
ing other species of bacteria, iridescence and intense secondary
with of resistant forms develops at the points where the phage drops
been applied, which makes evaluation of the results more difficult.
test strain is grown for 6 hrs on 1-3% peptone water and transfer-
into the slightly dried agar surface. After drying a little, drops
typing phages are applied to the culture. The phages are used in indicator cultures in 1% peptone water. They are used undiluted at critical test dilution. The phages were stored at 4°. Under these conditions they were available for several months.

exist for the typing of Pseudomonas aeruginosa on the basis of the properties of the mild phages and the pyocine. As we know, strains of Pseudomonas aeruginosa secrete bacteriocines into the host cell media, which are active against many microorganisms. Among these is thiocine. This is a proteinlike substance. It is liberated in the medium on lysis of the bacterial cells and can also dissolve several strains of Pseudomonas aeruginosa. The pyocines are specifically adsorbed by sensitive (indicator) strains of Pseudomonas aeruginosa and cause their death. In contrast to the phages they are not reproduced in an injured cell.

In the experiments of Halloway and others (1960) 75% of the studied cultures were pyocinogenic or lysogenic.

Some strains carried 2-4 different mild phages. According to their serological properties, most phages belonged to 6 groups (A, B, C, D, E, F). Within each serological group the phages were divided into subgroups on the basis of the range of their hosts. Some of the phages are still being studied and possibly belong to additional groups.

Halloway (1960) made an attempt to type strains of Pseudomonas aeruginosa on the basis of the lytic properties of the mild phages and pyocines. The author used the 3 strains of Pseudomonas aeruginosa (L) and 29 as indicators.

The method of Gracia was used for the study of the mild phages and pyocines. 0.001 M of calcium chloride was added to the basic media. Culture of each of the 3 indicator strains was prepared in the following manner. 2 drops of a day-old broth culture of the indicator
The mixture was poured on the surface of normal meat-peptone and left to solidify. The surface layer was slightly dried at 37° for 30 minutes. Drops of day-old broth cultures of the strains to be tested were then applied with a loop. Up to 20 drops were placed into a dish. The results were evaluated after 24 hrs incubation at 37°. Differentiation of the cultures was carried on the basis of the nature of lysis around the spot. The lysis zone varied from hardly visible to 2 mm. The edge zone could be sharply delineated or diffuse, uneven or serrated. If necessary, the phages could be differentiated on the basis of the plaques and the antigen characteristics as proposed by Halloway and coauthors (1960). The pyocine was differentiated from the phage firstly on the basis of certain peculiarities of the lysis zone and, secondly, on the basis of the inability to give is sterile spots (similar to those formed by phages) when the indicator strains were inoculated with series dilutions. The pyocine gave rise to a lysis zone or growth inhibition, whose intensity decreased with degree of dilution.

214 cultures isolated in several Melbourne hospitals were typed in the above-described method. The hospital strains were subdivided into 18 phagotypes on the basis of their capacity of producing mild phages and pyocine on 3 indicator strains. The epidemiological value of this method was demonstrated in individual hospitals.

Pseudomonas aeruginosa can be differentiated on the basis of its sensitivity to a series of typing phages, derived from lysogenic cultures and sewerage effluents and also on the basis of the properties of mild phages and pyocines. A certain epidemiological value has been demonstrated for all typing methods. At the same time these are still far from perfect and have certain defects. Thus, for example, it was...
that the phagotypes are not stable and that the results depend on variations in the times and conditions of typing. Further action and practical verification of these methods are essential.
Chapter 9

PHAGE TYPING OF CHOLERA VIBRIOS, DIPHTHERIAL BACILLI
AND SOME OTHER BACTERIA

The feasibility of phage typing has been demonstrated to date for species of pathogenic microorganisms. A scheme of phage typing of the vibrios has been worked out recently in India (Mukerjee, 1959, Mukerjee and others, 1957, 1959). The cholera phages proved to be fairly uniform. Comparing 623 phages from the feces of patients, river water and effluents and from lysogenic cultures, the authors identified 4 main serological groups among 606 phages. The phages of group IV have not been detected in lysogenic cultures, the phages of groups I, II and III are ubiquitous. 17 phages did not belong to the listed 4 groups, they were heterogeneous and unsuitable for the typing of cholera vibrios because they had a broad lytic spectrum. By classification of the phages of the 4 groups, vibrios could be differentiated into 7 phagotypes, and the phagotypes and serotypes did not coincide. Vibrios on which typing is based, should be present in the smooth-rough (S+R) form. This is due to the fact that phage II does not type with the rough forms of vibrios. Even the phagotype I which is typed by all typing phages loses its sensitivity to phage II upon transition to the rough form.

The smooth and mixed forms are typed in a high percentage. Thus, 630 vibrios, isolated during a cholera epidemic at Calcutta, the culture strains accounted for only 1%. These were rough forms, sensitive to phages I and III and smooth forms with ambiguous phage reac-
A very rigid correlation was found to exist between lysogenicity agotype of the vibrio. Thus, strains of phagotype I were alwaysogenic. The other phagotypes mostly (73% out of 165 studied cul-
carried mild phages, about 90% of the cultures of the 2nd and 
be containing phages of group I, 70% of cultures of the 3rd pha-
phages of group II, and 50% cultures of the 5th type phages of 
III. The above-described lysogenic characteristics are closely 
ted with the sensitivity spectrum of the phagotypes. For example,
es of type 5, containing mild phages of group III, do not inter-
th phage III. Evidently, as in many other species of bacteria,
ence of mild phages in vibrios imparts to the cell an immunity 
ges of the related serotype.
ol proof of the value of this method in epidemiological investi-
s has been provided. In 5 localized epidemics the method helped 
be the epidemiological chains.
schemes for typing of diphtheria have been known. Keogh and 
(1938) distinguished the type, davis, mitis and intermedius by 
of 2 phages. Later on, Fahmy (1943) using 5 phases, divided 
thia bacteria into 5 phagotypes. These phagotypes did not coincide.
mitzer and Fredericq (1956) worked out a scheme for typing dip-
mild phages. The phages A, B, D and O were isolated di-
from lysogenic cultures. The phages C and I, are variants of 
A, phage N is a variant of phage C, phage N₂ a variant of phage 
variants were obtained by adaptation of the original phage to 
ant cultures.
50 cultures of diphtheria of the type gravis were differentiated 
he aid of the above-mentioned phages into 9 phagotypes, which 
to be stable on laboratory media and in the organism. A correla-
ion was observed to exist between the phagotype and certain biochemical properties of the strains (enzymatic breakdown of glycogen). The strains which interacted with phage A or its derivatives (C, I, N or N₂) broke down glycogen. The strains which were sensitive to the phages D or O did not have this property. The proposed scheme has been applied with success in epidemiological research.

Prevot and Thouvenot (1961) differentiated 212 strains of anaerobic corynebacteria into 11 phagotypes (I-XI) by means of 6 mild phages. 5% of the cultures belonged to the type I. The typing phages were related serologically but differed in their heat stability and were inactive on diphtheria corynebacteria.

The possibility of typing strains of Proteus hauseri with bacteriophage has been reported. Vieu (1958) divided the strains of this species into 10 phagotypes, using 12 undiluted phages, isolated from sewerage effluents. Evaluation of the results was carried out after 6-8 hrs of incubation at 37°C. This method is currently used at the Pasteur Institute at Paris.

Attempts at phage typing of hemolytic streptococcus have become known. Evans (1934) was the first to subject streptococcus phages to a careful study. These were isolated from sewerage effluents and divided into 5 races on the basis of their serological characteristics: A, B, C, D and E. The phages differed also in their range of action on hemolytic streptococci of different origin. Streptococci of different origin could be differentiated by means of 4 phages into 8 groups (I-VIII). A definite relationship between the origin of the cultures, their serological characteristics according to Lansfield and their sensitivity to phages was demonstrated.

The phages A, C and E acted on a limited number of strains of group A, phage E lysing almost exclusively strains of the serotype XVII
Griffiths (Evans, 1942; Evans and Sokrider, 1942). Phage C interacted with scarlatinal cultures (Evans, 1935). Phage B acted only on reptococci of the serological group C and F, isolated from animals (Evans and Verder, 1938; Evans, 1940). Phage D lysed only streptococci of group O (Evans, 1934, 1942). The author could not discover a rigid correlation between the serological type of the streptococcus according to Griffiths and its sensitivity to phages (Evans, 1940).

Attempts have been made in recent years to type streptococci by means of mild phages. It was found that lysogenicity is as widespread among streptococci as in other groups of microbes (A.A. Totolyan, 1961). Nearly half the cultures may be lysogenic. The best method of detecting mild phages is the action of ultraviolet light. A coincidence of the serological characteristics of the test and indicator strains is essential for determining the lysogenic properties of the culture. Id phages can be determined in a pair of strains of hemolytic streptococcus which are either different or identical with regard to their antigen structure. By means of 6 phages, most of which were mild (with the exception of phage CA1). A.A. Totolyan subdivided 165 stock and fresh cultures of hemolytic streptococcus of group A into 5 phagotypes.

Noteworthy is the fact that the same phagotype (with the exception II) included cultures of different serotypes. For example, group I contained the strains I, IV, X, XXIV, XXVI, XXVII and XXIX of the Griffiths serotypes. At the same time the cultures of some identical serotypes belonged to different phage groups. In other words, some kind of correlation between the serological properties and the phage sensitivity of a culture could not be found.

The phage resistance and sensitivity of cultures was accounted for the lysogenic properties: the cultures were resistant to "their" ages and sensitive to phages isolated from cultures of other groups.
Furthermore, phage resistance was also connected in some instances with the presence of a protective layer of hyaluronic acid in the streptococci.

Some streptococcus phages had marked adaptive properties. Thus, the phage CAl could be adapted to a strain of serotype IV, as a result of which it acquired activity with regard to strains IV and some others, losing the capacity of lysing streptococci of the types XXIV and XXIX.

A curious fact is that strains of identical phage types had a similarity in some culture characteristics, such as: the nature of growth in serum broth, and peculiarities of the hemolysis zone. In the view of the author this could be connected with structural differences in the surface layers of the cells (thickness of the protective layer of hyaluronic acid, etc.).

The markedly widespread distribution of lysogenicity among streptococci, which has been demonstrated in the researches of A.A. Totolyan, provides a realistic basis for the creation of a more perfect scheme of phage typing for this species of microorganism. The need for such a scheme would also follow from the fact that the role of hemolytic streptococcus in the etiology and pathogenesis of scarlet fever, rheumatism and other diseases forms the subject of scientific disputes to this day. And it is clear that a method of differentiating strains of different origin, but of identical serotypes, can help to bring some clarity into the problems of the etiology and epidemiology of streptococcus diseases.

The possibility of phage typing of streptococci has been demonstrated (Evans and Chinn, 1947). Some enterococcus phages are capable of differentiating enterococci of different origin (for example, strains isolated from human intestine, from the enterococci of coldblooded animals, cows, horses, and wasps) (N. Akhmedov, 1959).

Following the isolation of a number of bacteriophages of mycobac-
ia by Gardner and Weiser (1947a, b), Hauduroy and Rosset (1948),
so and others (1949) proposed a scheme for the phage typing of para-
erculosis bacteria, in which the phages were used in test dilutions.
tko (1953) subdivided M. phlei and M. smegmatis into 2 phagotypes.
dra (1962) typed 377 strains of mycobacteria (pathogenic, saprophy-
typical) by means of 10 phages, isolated from soil and designated ording to the species of mycobacteria, through which the phage had n passed (Phagus phlei, Phagus pellegrini, etc.). 106 strains proved be sensitive to certain phages, only Phagus phlei being specific for tures of M. phlei, which in turn, were not lysed by other phages.
pathogenic mycobacteria were lysed by Phagus smegmatis and minetti.
All these researches permit us to assume that most pathogenic bac-
ia can apparently be typed by some phage method. The ease with which ferent bacteria can be subdivided is not uniform. It depends greatly the possibility of obtaining bacteriophages and on the peculiarities lysogenicity of individual species of bacteria.

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- 243 -


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