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BACTERIOPHAGE TYPING OF STRAINS OF SALMONELLA TYPHI OCCURRING IN MICHIGAN WITH SPECIAL REFERENCE TO ISOLATIONS FROM TYPHOID CARRIERS

Thesis for the Degree of M. S.

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Norman D. Henderson

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THESIS

This is to certify that the

thesis entitled

Bacteriophage Typing of Strains of Salmonella Typhi Occurring in Michigan with Special Reference to Isolation from Typhoid Carriers.

presented by

Norman D. Henderson

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BACTERIOPHAGE TYPING OF STRAINS OF *SALMONELLA TYPHI* OCCURRING IN MICHIGAN WITH SPECIAL REFERENCE TO ISOLATIONS FROM TYPHOID CARRIERS

by

Norman D. Henderson

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INTRODUCTION

In 1900 there were 5,122 cases of typhoid fever reported to the Michigan State Board of Health with 824 deaths (1901). During 1947, a total of 77 cases and five deaths were recorded by the Bureau of Disease Control of the Michigan Department of Health (1948). Figure I depicts the reported death rate due to typhoid fever from 1900 to 1947 (Michigan Department of Health 1948). In spite of modern sanitation, public health education and the development of better laboratory techniques, typhoid fever persists as a small residuum due for the most part to the existence of carriers of Salmonella typhi*.

The majority of cases occurring in 1947 was of a sporadic nature. One outbreak, which was caused by a previously undetected carrier, accounted for 17 cases and one death. Bradley (1943) refers to these sporadic cases as "chinks in the complex armor of modern sanitation" and states that "it is only by counting these sporadic cases which fail to initiate outbreaks that we have a true measure of the efficiency of our system." Presumably the chronic carrier is always somewhere in the background, but his relationship to a series of cases may be remote. Bradley followed 23 sporadic cases in adjoining counties in England. Using epidemiological methods and the bacteriophage typing technique, he was able to locate and identify the responsible carrier on a dairy farm

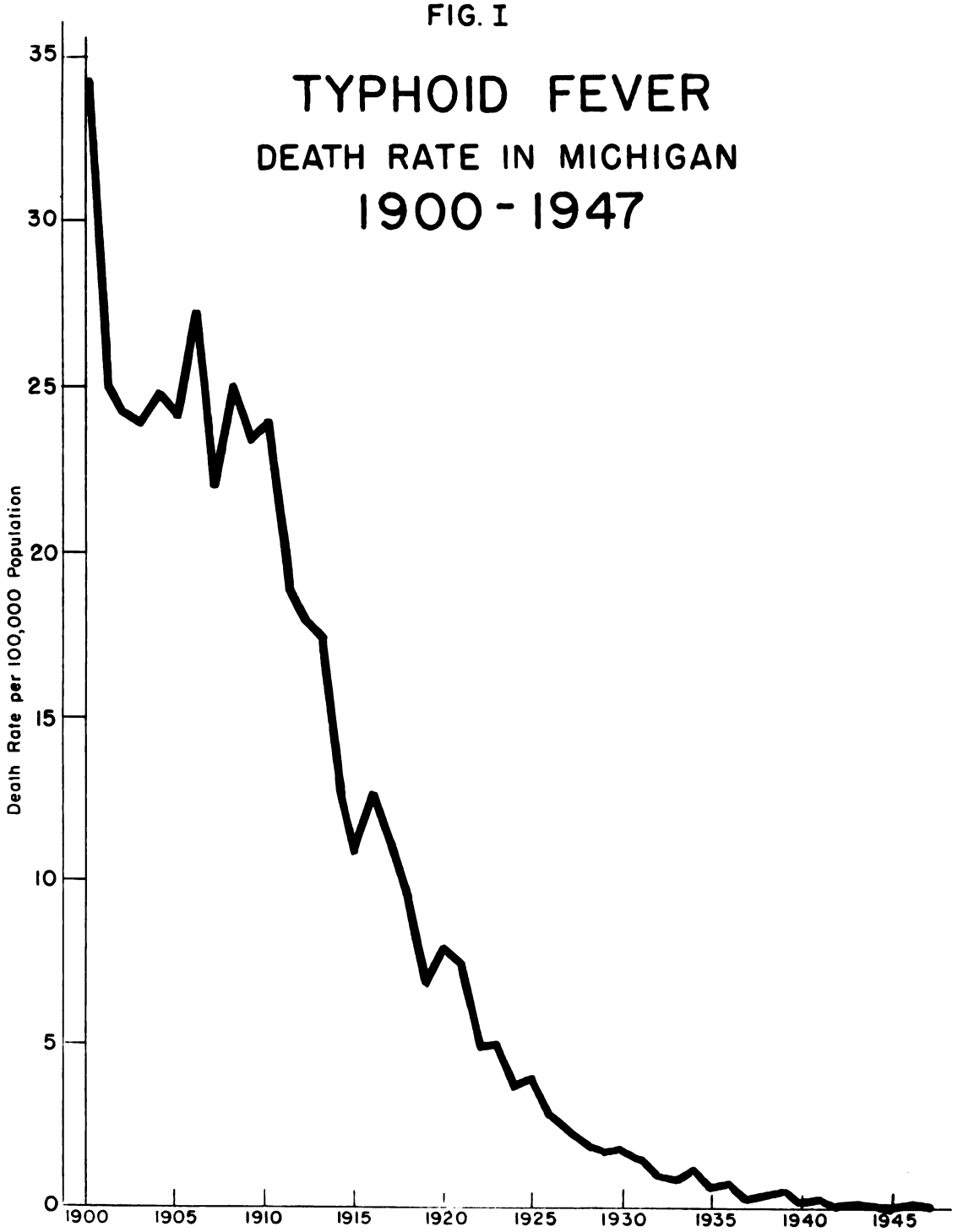
*The nomenclature used in this paper is that of the Kauffmann-White Schema.

100 miles from the locality of the cases.

Bacteriophage typing supplies the epidemiologist with a tool which enables him to trace an outbreak or a series of isolated cases to the source.

Once it has been ascertained that a person is a typhoid carrier or case, it is now possible by a relatively simple laboratory procedure to identify not only the typhoid organism itself but the actual type being excreted by the individual. This laboratory procedure is the bacteriophage method of typing the typhoid bacilli.

FIG. I


TYPHOID FEVER
DEATH RATE IN MICHIGAN
1900 - 1947
Death Rate per 100,000 Population
35
30
25
20
15
10
5
0
1900
1905
1910
1915
1920
1925
1930
1935
1940
1945

HISTORICAL

The question of phage specificity has been investigated since the earliest days of bacteriophage study. d'Herelle (1922) stated "It (the bacteriophage) attacks a certain number of species at the time of isolation, and possesses for each of these a variable virulence." Thus, a phage active against Shigella dysenteriae may exhibit some lytic action on Salmonella typhi. The other condition also exists in which a phage, showing activity against one species, will demonstrate none when applied to a species of a different genus. Furthermore, a particular phage may be active against one strain of a bacterium, and not against a second strain of the same species.

Burnet (1927) demonstrated that there was a close correlation between the action of a particular phage on a certain bacterial species and the surface or somatic antigens of that species. For example, a phage will show activity against S. typhi, Salmonella enteritidis, and Salmonella pullorum, each of which has in common the somatic antigen IX,XII as designated in The Kauffmann-White Schema. The application of bacteriophage typing to the classification of the genus Salmonella has not met with success, because organisms possessing the same antigen were lysed by one bacteriophage.

Felix and Pitt (1934a) announced that virulent living strains of S. typhi were resistant to agglutination by a pure "O" antiserum. This announcement was followed by a second report from the same authors (1934b) stating that virulence and resistance to

agglutination by "O" antiserum were correlated with the presence of an antigen which they called the Vi (virulence) antigen. Kauffmann (1934) elaborated on the terminology of the Vi antigen by introducing the terms V form and W form. A strain of S. typhi that possesses the Vi antigen is said to be in the V form, while a strain not demonstrating this antigen is said to be in the W form.

By applying the terminology of Kauffmann to the work of Felix and Pitt, it is apparent that a strain which contains the Vi antigen (V form of Kauffmann) will be agglutinated by a pure anti-Vi serum and will not be agglutinated by a pure anti-O serum. Accordingly, an organism devoid of the Vi antigen (W form of Kauffmann) will be agglutinated by a pure anti-O serum and will not be agglutinated by a pure anti-Vi serum. Kauffmann made an extensive study of this phenomenon and found that an organism may exist in the V, W, or V-W form; the latter being that condition in which a strain will exhibit partial agglutination in both a pure anti-Vi and pure anti-O serum.

The discovery of Felix and Pitt served as the starting point for a new line of research, and several authors, Sertic and Boulgakov (1936), Scholtens (1936, 1937), Craigie and Brandon (1936) and Craigie and Yen (1938), working independently, established the existence of bacteriophages specific for the Vi antigen of S. typhi. The action of these phages was highly specific and lysis did not occur with typhoid bacilli devoid of the Vi antigen.

Craigie and Yen (1937) examined different strains of bacteriophage which were active against the Vi antigen and found that the phages could be grouped into four types (see Table I).

TABLE I

CHARACTERISTICS OF THE VI BACTERIOPHAGES

Vi Phage	Relative Particle Size	Thermal Death Point	Neutralization by antiphage serum				Lytic activity for V forms of S. typhi
			I	II	III	IV	
Type I	Large phage	67-70° C	+	-	-	-	Lyses all V forms
Type II	Medium phage	69-72° C	-	+	-	-	Develops a high selective lytic activity for the type of S. typhi on which it is propagated
Type III	Small phage	61-64° C	-	-	+	-	Lyses majority of V forms
Type IV	Medium Phage	59-62° C	-	-	-	+	Lyses majority of V forms with exceptions of type F and D1

From the information contained in Table I, several conclusions may be drawn:

1. The four phages are specific for the V form of S. typhi.
2. Serologically, the four types of phage are distinct.
3. A variation occurs in the lytic action on V form strains of S. typhi, particularly in the case of Type II phage. Bacteriophage propagated on a strain of S. typhi develops a high selective lytic activity or specificity for the substrate strain.

The variation exhibited by phage II attracted the attention

of Craigie and Yen (1938). They found that the affinities of this phage could be conditioned to a particular strain of S. typhi on which it had been propagated. Using classical strains of S. typhi, they determined that phage II, when grown on either the Rawlings or Watson strain, developed an equally high lytic activity for both strains, but lysed strain Ty2 only when applied in strong concentrations. On the other hand, when grown on strain Ty2, phage II lost its ability to lyse the Watson and Rawlings strains and became highly specific for strain Ty2. By changing the propagating strain, several preparations of phage II were obtained that showed a selective affinity for a particular strain of S. typhi. The epidemiological aspects of this discovery were realized by Craigie and Yen, and the factors responsible for this phenomenon were investigated.

The hypothesis, developed by Craigie and Yen to explain the variation of phage II, was that the phage exists as a series of mutants which differ from each other in their affinity for different strains of S. typhi. This hypothesis, in turn, is predicated on the supposition that varieties of S. typhi occur which differ from each other only in their Vi antigen. The variations or differences in Vi antigen are demonstrated only by the action of Phage II. Thus, the increase in specificity of a phage preparation may be explained on the basis of selective propagation of the mutants specific for the substrate strain of S. typhi, with a corresponding decrease in the other mutant particles.

By selective propagation of the mutants of phage II, a

series of lytic preparations was built up which demonstrated a higher relative affinity for the homologous strain than for the heterologous strain of S. typhi. Such preparations, diluted so as to contain only an effective number of phage particles of the dominant homologous mutant, were employed to identify strains of S. typhi similar in type to that on which they were grown.

The hypothesis of Craigie and Yen explains: (1), the ability of the phage to adapt itself to bacteria of heterologous type; (2), the high titer for strains of homologous type; (3), the lower titers for the various heterologous bacteria, and (4), the constant ratio between these titers.

To standardize phage reactions so that the preparations could be used for typing strains of S. typhi, Craigie and Yen decided on a "critical test concentration"*. This was defined as that dilution of the phage which, under the conditions of the test, just produced a sufficient number of plaques to give an area of confluent lysis on the homologous strain of S. typhi. The actual dilution employed was determined by two factors:

1. The time and condition of incubation of the tests.
2. The diameter of plaques produced by the phage on the homologous strain of S. typhi.

Upon extending their procedure to a number of isolations of S. typhi, Craigie and Yen found that these strains were differentiated into well defined groups or types. New phages were built

*Abbreviated as C.T.C.

against representative strains of each type and then checked against the original phage preparations. In this manner, it was discovered that similar type strains yielded similar phages. This technique was applied to strains isolated from a known carrier and from persons known to have been infected by the carrier. The results obtained indicated that all the strains related to a single carrier were identical in type. Further testing produced new types, until at the time of their first comprehensive report, Craigie and Yen (1938) had studied 625 cultures representing 11 different phage types.

These were designated A, B1, B2, C, D1, D2, E. F, G, H and L. The letter "I" was intentionally omitted in the designation of types to avoid possible confusion with phage I.

Other authors have added to the number of types originally reported by Craigie and Yen until there are now 25 known types and subtypes. The reactions of these phages at their "critical test concentration" when tested against the corresponding series of Vi type strains of S. typhi are given in Table II.

Experience has proved the stability of Vi phage II. It is now generally accepted that the phage preparations should be taken as the standard and not the culture of type strains of S. typhi. From the information available, it is apparent that preparations of Vi phage II, once propagated, tested and diluted to their critical test concentration, are, for all practical purposes, fixed in their specificity. The particular form of the Vi antigen of a given strain of S. typhi is thought to be a reasonably permanent characteristic. The epidemiological value of phage typing is based on this observation.

TABLE II

STANDARD PHAGE PREPARATIONS AT C.T.C.

Type of S. typhi	A	B1	B2	B3	C	D1	D2	D4F	D4	D5	D6	E1	E2	F1	F2	G	H	J	K	L1	L2	M	N
A	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	SCP	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
B1	±	CL	-	+++	-	-	-	-	-	-	-	++	-	++m	+++m	++m	++m	-	-	±	±	±	±
B2	-	-	SCP	±	-	-	-	-	-	+m	-	-	-	-	-	-	-	-	-	-	+m	-	-
B3	±	-	±	CL	+++	±	SCP	-	-	±	-	+++	±	SCP	-	+++	+++	++	±m	±	++	SCP	SCP
C	-	-	-	-	CL	+m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	-	-	-	-	-	CL	CL	-	SCP	±	-	-	-	-	-	-	-	-	-	-	-	-	-
D2	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D4F	-	-	-	-	-	-	-	SCP	SCP	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D4HP56	-	-	-	-	-	-	-	+++m	SCP	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D5	-	-	-	-	-	-	-	-	-	SCP	++	-	-	-	-	-	-	-	-	-	-	-	-
D6	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-	-	-
E1	-	-	-	-	-	-	-	-	-	-	-	CL	SCP	-	-	-	-	-	-	-	-	-	-
E2	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-	-	-	-
F1	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	CL	-	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++m	-	-	-	-	-	-	-	-
G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCP	-	-	-	-	-	-
J	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-	-
K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CL	-	-	-	-
L1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	SCP	+++	-	-
M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
N	-	-	-	-	-	-	-	-	-	-	±	±m	-	±	-	±m	-	±m	-	-	-	-	SCP

CL - Confluent lysis ± - Few plaques

SCP - Semi-confluent plaques +++ - Numerous plaques

m - Micro plaques

A few exceptions have been noted, but these are apparently rare in occurrence. Type A is sensitive to all Vi phage preparations. A recent report by Craigie and Felix (1947) indicates that this type may result from dissociation. For example, a Type A culture was isolated from a Type T and a Type O carrier. The indication is that this change may take place in carriers of long standing. Type A has also been isolated from cultures of Types B1, C, D5, F1 and N. This point is of great importance to the epidemiologist because the isolation of two types in an outbreak raises the question of a double source of infection.

Helmer et al. (1940) have reported on the isolation of Types F1 and F2 from a single case of typhoid fever. A careful epidemiological study failed to disclose a double source of infection.

Type O and Type C are peculiar in that they yield phages which are variable as to their plaque size. This condition seems to be a characteristic of the particular type of S. typhi involved because it has been found to exist in freshly isolated cultures.

In addition to these variations within the types, there are other conditions which must be considered in the typing of strains of S. typhi. The variation between the V form (Vi antigen) and the W form (O antigen) has already been discussed. It will be recalled that the V forms are lysed by phages I, II, III and IV, and show considerable variability in the reactions with the type II phage. Generally speaking, the organisms sensitive to the type II phages can be further subdivided into three large groups. The alpha group is defined as being composed of those strains which are fully

sensitive to Type II phage. In addition, when used as the substrate strain for propagating phage II, they produce a phage with a clear-cut lysis of that type. Thus, if phage II is transferred from one alpha type to another, it develops a distinct affinity for the new type, while retaining no residual lytic action for the preceding type.

The beta group is made up of those types of S. typhi which when used as a substrate strain produce a phage that retains its affinity for the original strain even after several passages on the new type. The gamma group is made up of those strains which are resistant to the action of phage II. The three groups are arranged as follows:

Alpha Group	Beta Group	Gamma Group
Type A	Type B2	Resistant to
B1	B3	phage II, but
C	D1	sensitive to
D2	D3	phage I, III
D4		and IV.
E1		
E2		
F1		
F2		
G		
H		
J		
L1		
L2		
M		

The practicability of the method of Craigie and Yen has been well substantiated. Lazarus (1941) in the United States, Crossley (1942) in Canada, Buckle (1946) in Australia, Crocker (1947) in South Africa, and Cruickshank (1947) in England have reported the results

of phage typing in their countries. Two salient facts stand out in these various reports:

1. The world-wide distribution of types appears to be fairly uniform and the phage types of S. typhi are, for all practical purposes, stable.
2. Typhoid fever persists due to a small number of cases which occur in spite of our existing methods of sanitary control.

Both of these facts are of great importance to the epidemiologist. The stability of types provides him with a guide whereby he may trace a particular outbreak back to its source. The persistence of cases presents a constant challenge to his efforts to control and eradicate this disease. Craigie (1941) has suggested that on occasions the task of the epidemiologist might be facilitated if records were available regarding the type excreted by every known carrier in the area. Therefore, typings from carrier isolations have been included as a separate section of this study.

MATERIALS

The twenty-three standard Vi-phage II preparations used in this study were supplied, in concentrated form, by The Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada. The corresponding Vi-type strains of S. typhi were also supplied. Instruction included with the phage kit directed that the phage preparations should be diluted to the C.T.C. and then tested against each of the Vi-type strains.

To facilitate the typing of cultures, the 23 phages were combined by us into seven pools. Dilutions were computed so that each phage was present at its C.T.C. (Table III)

Late in the study, Vi-phages I, III and IV were supplied by Mr. Arnold Juenker of the Salmonella Typing Station, Michigan Department of Health Laboratories. These preparations had been furnished originally by J. M. Desranleau of the Quebec Provincial Health Laboratories.

The media necessary for typing were prepared originally according to the instructions supplied by The Connaught Laboratories. These were as follows:

Agar Medium

'Bacto' nutrient broth (dehydrated)	20.09 gms.
Sodium chloride	7.59 gms.
'Bacto' agar	20.09 gms.
Distilled water to	1000.00 ml.

TABLE III

COMPOSITION AND PREPARATION OF POOLED PHAGES

Pool	Individual Phages	Amount of each phage	Total Phage	Broth	Total Vol.	Final Dilution
I	A,C,H	0.05 ml.	.15 ml.	4.85 ml.	5 ml.	1-100
II	E2,G,J	0.05 ml.	.15 ml.	4.85 ml.	5 ml.	1-100
III	F2,B2,B3	0.05 ml.	.15 ml.	4.85 ml.	5 ml.	1-100
IV	K,D2,D4,F,D5	0.05 ml.	.20 ml.	4.80 ml.	5 ml.	1-100
V	L2,M	0.05 ml.	.10 ml.	4.90 ml.	5 ml.	1-100
VI	B1,D1,E1,F1	0.05 ml.	.20 ml.	4.80 ml.	5 ml.	1-100
VII	D4H,D6,L1,N	0.05 ml.	.20 ml.	4.80 ml.	5 ml.	1-100

Phage dilution broth

'Bacto' nutrient broth (dehydrated)	8.09 gms.
Distilled water to	1000.00 ml.

Phage culture broth

'Bacto' nutrient broth (dehydrated)	15.09 gms.
Sodium chloride	7.09 gms.
Distilled water to	1000.00 ml.

Original tests were made using these media. However, Craigie and Felix (1947) have stated that any good meat digest agar may be used as an alternative providing that the standard reactions are reproduceable. Therefore, in the interest of economy and for convenience, we have substituted the following media in place of the 'Bacto' nutrient broth and 'Bacto' nutrient broth agar:

#2 Broth

Veal infusion	1000.0 ml.
'Bacto' peptone	10.0 g.
Sodium chloride	5.09 gms.

Adjust to p H 7.6 and filter

#2 Broth Agar

'Bacto' agar	15.0 gms.
#2 Broth to	1000.0 ml.

Craigie had recommended that the cultures of the Vi-type strains should be maintained on Dorset Egg medium (3 parts whole egg, 1 part saline, slanted and inspissated). Medium of this formula was made up and dispensed in small screw-cap glass vials. In this form,

the Dorset Egg Medium was used for maintaining the stock strains.

All 23 stock strains were transferred to fresh slants at six month intervals following passage on nutrient agar and tests for specificity. Slants of the egg medium were also supplied to all laboratories in the State that would submit strains for typing.

Cultures that proved refractory to Vi phage II were tested serologically for O and Vi antigens in addition to being tested with phages I, III and IV. For this purpose, two antisera were used:

1. A pure "O" antiserum containing antibodies against the IX, XII antigen. Salmonella gallinarum was used for immunization.
2. An anti-Vi serum prepared by us. Salmonella bollerup was used for immunization.

METHODS

Our procedure for typing strains of S. typhi by the bacteriophage method has been essentially that of Craigie and Yen (1938) and Craigie (1941). Because we have followed Craigie's procedure with close attention to detail, the following description is quoted directly from Craigie (1941).

> "Two standard platinum loops, 2.75 mm. in internal diameter, No. 20 B & S gauge wire, are used alternately to apply the culture and phage to the plate. While one loop is actually in use, the other is cooling after sterilization. In transferring a loopful of broth culture to the plate, the loop is used merely to direct the natural spreading of the broth and should not be rubbed over the agar. The shank of the loop is angled so that the loop will lie parallel with the agar surface during inoculation, the loopholder being grasped between the thumb and first two fingers like a pen. A full loopful of broth culture is obtained by sharply moving the culture tube so that the loop quickly breaks the surface of the fluid. Then with the holder held in the way described and with the forearm and side of the hand resting on the bench, the loop is lowered until the drop held in it touches the surface of the agar. At this point, the loop is given a slight horizontal circular movement by moving the fingers, guiding the spreading of the drop over an area approximately 15 mm. in

diameter. A number of areas, corresponding to the number of phage preparations used in the test, are inoculated with each strain. When the agar has absorbed the fluid which has been applied (optimum 5 to 10 minutes), the phage is applied from the standard loop to the center of the inoculated area. In this case, the loopholder is best held horizontally between the thumb and fingers, these pointing downward with the side of the arm resting on the edge of the bench. The loopful of phage is lowered until it almost touches the surface of the agar, when slight rotation of the loopholder will move the loop into a vertical plane, thus allowing its contents to flow onto the center of the inoculated area. It is to be noted that the drop of phage must be allowed to spread naturally on the plate, and the actual contact of the loop and inoculated area is to be avoided as far as possible.

"When the fluid which has been applied had dried sufficiently to permit of the plates being safely moved, they are incubated for 2 hours at 37° C. and transferred to the cold room overnight. They are returned to the incubator and are ready for reading in 4 to 6 hours. It should be noted that the plates must not be stacked, since at temperatures significantly below 37° C., B. typhosus will grow without exhibiting lysis with Vi phage. The method of interrupted incubation has two advantages. It permits the phage reactions to be observed at their optimum and before they are obscured by late growth in the case of some strains. Further, the intermediate

period in the cold room permits diffusion of the phage into the surrounding normal culture, thus enhancing the clarity of the reactions. The reactions are best observed by oblique artificial illumination and against a dark background."

The results obtained when the diluted phages and stock strains were tested on #2 broth agar are reproduced in Table II.*

To aid in the preliminary screening of test strains, pooled phages were used. Stock strains were tested against the pooled phages and yielded the results reproduced in Table IV.

*The form used in Table II is that of Craigie and Felix (1947).

TABLE IV

POOLED PHAGE PREPARATIONS

	Phage Pool						
	I	II	III	IV	V	VI	VII
Strain	A,D,H	E2,G,J	F2,B2,B3	K,D2,D4F,D5	L2,M	B1,D1,E1,F1	D4HP56,D6,L1,N
A	CL	CL	CL	CL	CL	CL	CL
B1	-	±m	±m	-	±m	CL	-
B2	+++	+++	CL	+++	+++	+++m	+++
B3	+++	+++	CL	±m	+++	+++	SCP
C	CL	±L	±L	±m	±L	±L	±L
D1	-	-	-	SCP	-	CL	CL
D2	-	-	-	CL	-	-	-
D4F	-	-	-	SCP	-	-	CL
D4HP56	-	-	-	CL	-	-	SCP
D5	-	-	-	CL	-	SCP	SCP
D6	-	-	-	-	-	-	CL
E1	-	+++	-	-	-	CL	-
E2	-	SCP	-	-	-	-	-
F1	-	-	CL	-	-	CL	-
F2	-	-	CL	-	-	-	-
G	±	CL	±	±m	-	-	±
H	SCP	±	±	-	±	±	±
J	-	CL	-	-	-	-	-
K	-	±m	±m	CL	++	±	±
L1	-	-	-	-	CL	-	CL
M	-	-	-	-	SCP	-	-
N	-	-	-	SCP	±	SCP	CL

CL - Confluent lysis
L - Large plaques
m - Micro plaques
± - Few plaques
SCP - Semi-confluent plaques
+++ - Numerous plaques

ROUTINE TYPING PROCEDURE

Strains of S. typhi submitted for typing were usually received on Dorset egg slants. Those submitted on different media were streaked out on nutrient agar plates and six V form colonies picked and transferred to an egg slant. A small amount of culture from the egg slant was inoculated into 2 ml. of nutrient broth and the tube incubated at 37.5 C for 3-4 hours. At the end of the incubation period, eight inoculation sites were made on a nutrient agar plate as described above. After the broth had dried, one standard loopful of each of the seven pooled phages was applied to a separate inoculation site, the eighth spot being left for control purposes. After the phages had dried, the plate was inverted and incubated for two hours and placed in the icebox overnight. In the morning the plates were returned to the incubator for four hours of additional incubation. The phage action resulting from the various pools was recorded and compared with the standard reactions as given in Table IV.

If confluent lysis was demonstrated in each spot, the culture was recorded as type A and no further tests were performed. If phage action was apparent in one or more spots, the culture was retested against the specific phages making up each pool involved. Incubation was repeated as before and the results read the following morning.

As an example, let us examine a culture which, when tested with the pooled phages, produced confluent lysis in Pool I

FIGURE II

Reaction with pooled phages

Reaction with specific phages

with only a few isolated plaques apparent in the other inoculated areas (Figure II). This culture was tested with the individual phages making up Pool I, i.e., phages A,C, and H. The results, read the next morning, showed confluent lysis with phage C and only a few isolated plaques on the other two inoculation sites. By comparing these results with Table II, it can be seen that these reactions were comparable only with those of type C and that the culture was therefore type C.

If no phage action was observed with any of the pooled phages, two additional tests were performed. The strain was inoculated into 2 ml. of broth and tested with phages I, III and IV using the procedure outlined above. If the culture demonstrated lysis with the three phages, it possessed the Vi antigen and was resistant to phage II for one of two reasons:

1. The culture was in the gamma form and was therefore untypeable.
2. The culture was of a new type not included in the present schema and a phage would have to be propagated on it, tested against the stock strains and standardized.

A culture refractory to the action of phage II was also tested serologically as a check on the action of phages I, III and IV. This test was performed on a glass slide using a IX, XII antiserum, a Vi antiserum and a saline control. The strain reacting strongly in the IX,XII serum and weakly or not at all in the Vi serum was in the W or degraded V form. If the culture reacted

strongly in the Vi antiserum and weakly or not at all in the IX,XII serum, it was in the V form. If this culture yielded lysis with phages I, III and IV then it was apparently a gamma culture. (See Table V).

TABLE V

REACTIONS OF THE VARIOUS FORMS OF S. TYPHI

Form	Sensitivity to Vi Phages				Agglutination	
	I	II	III	IV	Vi Serum	"O" Serum
V	+	+	+	+	++++	-
W	-	-	-	-	-	++++
Gamma	+	-	+	+	++++	-

\+ - lysis
++++ - complete rapid agglutination

The differences between the V, W and Gamma forms are apparent from the reactions obtained with the Vi phages. These same differences are confirmed by the use of Vi and "O" antisera. However, no combination of these reagents will distinguish between a true gamma form culture and one that is of a new Vi type and therefore not included in the type II phages. To separate these cultures, we have resorted to what we will call the homologous phage technique.

A loopful of a concentrated phage corresponding to one of the alpha types was applied to an inoculation site of the test strain. If no lytic action was observed, the culture was in the gamma form. However, if a few isolated plaques were observed, several were cut out so that the plaques, a small amount of culture and the underlying

agar were removed. This section was placed in a tube containing 2 ml. of nutrient broth. The tube was incubated until lysis was observed, or for four hours. The tube was then placed in a 56 C water bath for 45 minutes. This destroyed the bacteria but did not affect the phage. Serial tenfold dilutions of the phages were made and tested against the culture in the usual manner. If the phage action appeared strong, the new phage was tested against the stock strains and the results compared with the standard reactions. If the results were comparable with any of those in the standard chart, then the culture was merely a variant of the existing type. However, if the reactions differed from those presented in the standard chart, we were dealing with a new phage type, and the culture would have to be tested exhaustively with the stock strain to determine its own standard reactions.

After all tests with the phages were completed, the original broth suspension was used to inoculate media containing certain biochemicals to confirm the identity as S. typhi. The following media were inoculated: Liquid single sugars, dextrose, lactose, sucrose, maltose, mannite, sorbitol, xylose and dulcitol; other media, Urea medium, Peptone water for Indol and H_2S production, Voges-Proskauer medium, Simmon's citrate agar slants and 0.3% agar for motility.

Three cultures submitted for typing proved not to be S. typhi. All others produced typical biochemical reactions with some variation in the fermentation of xylose and dulcitol, the production of H_2S and the degree of motility. There was no apparent correlation between these variations and the phage types identified.

TABLE VI

VI PHAGE TYPES OF SALMONELLA TYPHI ISOLATED IN MICHIGAN SEPTEMBER 1947 TO MARCH 1948

Phage Type	ISOLATIONS FROM CLINICAL CASES			ISOLATIONS FROM CHRONIC CARRIERS			TOTAL Isolations
	Original	Repeat	Total Case Isolations	Original	Repeat	Total Carrier Isolations	
A	6	0	(6)	17	12	(29)	35
B1	0	0	(0)	1	2	(3)	3
B2	0	0	(0)	4	3	(7)	7
B3	0	0	(0)	3	3	(6)	6
C	3	5	(8)	15	11	(26)	34
D1	5	1	(6)	4	2	(6)	12
D4	2	0	(2)	5	1	(6)	8
D6	0	0	(0)	2	0	(2)	2
E1	33	51	(84)	18	19	(37)	121
F1	7	8	(15)	3	2	(5)	20
M	0	0	(0)	1	0	(1)	1
	56	65	(121)	73	55	(128)	249
W Form	2	0	(2)	18	8	(26)	28
Gamma Form	9	11	(20)	14	16	(30)	50
FINAL TOTAL	67	76	(143)	105	79	(184)	327

DISCUSSION OF RESULTS

Table VI contains a summary of all typings attempted between July, 1947, and May, 1948. The diagnostic and carrier cultures submitted for typing were about evenly divided. The division between original and repeat specimens is also about equal. The submission of duplicate specimens provided us with the opportunity to make a thorough check on the stability of types. In over 300 typings, we have found only one case exhibiting a possible discrepancy.

During the period covered by this study, we have had one opportunity to study the stability of types in a limited carrier-borne epidemic. An outbreak of typhoid fever, apparently water-borne, involving 17 persons occurred in one of Michigan's cities. Family S., living on the outskirts of the city, supplied about a dozen families, as well as themselves, with water from a shallow well. During the latter part of July, the family discontinued the use of the well and installed city water. The installation of a flush toilet required a septic tank which was installed near the well. Meanwhile, the neighbors continued to use water from the well.

During late July and August, a total of 15 cases of typhoid fever developed in the vicinity of family S. Upon investigation, it was discovered that the well water was dangerously polluted. The indicated source of pollution was the new septic tank and the well was condemned as soon as its menace was discovered.

A total of 30 strains of S. typhi from 17 individuals

involved were submitted for typing. All were phage type E1. Among these strains were several from two members of Family S. who were proved to be carriers. The original source in the family was the mother who had had typhoid fever 15 to 20 years before. A grandchild, presumably a contact carrier, was also found to be discharging S. typhi, type E1. Attempts made to culture S. typhi from the well water proved unsuccessful. Up to the time of this report, 60 cultures isolated from this outbreak have been received for typing. All were identified as type E1 and none proved to be untypeable.

The cultures identified as D4 were all of the sub-type HP56. These cultures represent the newest of the phage types. Craigie (1946) identified this type among cultures submitted for typing from an outbreak of typhoid fever in one of Michigan's larger cities in 1946. The disease was contracted by 22 people at a wedding breakfast. Epidemiological investigation indicated contaminated food. A previously unknown carrier was discovered among the people who served and prepared the food. Craigie made an extensive study of the 17 cultures submitted and reported Type D4HP56 as being isolated from the carrier and eight cases. Nine isolations proved to be type D1. Arrangements are being made to study the organisms being excreted by the carrier. All cultures of type D4HP56 identified during this study were isolated from persons known to have been infected as a result of the outbreak described above.

The two cultures identified as Type D6 were isolated from two chronic carriers. Reactions originally obtained with the pooled phages were indefinite, partial lysis being observed only in Pool VII. The homologous phage technique was employed and the phage produced

was identified as being similar to the standard phage D6.

Untypeable cultures in this series represent 23.85% of all isolations as compared with 21.93% reported by Buckle in his survey of the literature. As indicated in Table VI, the untypeable cultures were divided into the W and gamma form. The W form cultures made up 8.56% of the total strains, while 15.29% were in the gamma form. Among the carrier strains, 14.14% were in the W form and 16.30% were in the gamma form.

Included among the 23 W form cultures are seven cultures from a carrier and a single contact which were proved to be small colony varieties of S. typhi. The cultural characteristics were similar to those described by Morris (1941, 1943). However, we were not able to reproduce the satellite phenomenon; neither were we able to secure a successful typing on any one of the strains submitted.

SUMMARY

A study of the Vi phage types of S. typhi is presented. A detailed review of the literature was made to develop the lines of research leading up to the discovery of the bacteriophage typing method by Craigie and Yen. The recent literature has been examined briefly to assess the value of the method as a laboratory technique and as an aid to the epidemiologist.

We have examined 327 cultures of S. typhi by the phage typing method. Strains included in this study were submitted from the laboratories of the Michigan Department of Health and other laboratories in the state. The cultures received were divided roughly into two groups: (1), isolations from clinical cases and (2), isolations from chronic carriers. There were 143 strains (43.73%) submitted from individuals clinically diagnosed as having typhoid fever. This total was made up of 67 original and 76 repeat strains. We were able to secure positive typing on 121 cultures (84.61%) while 22 cultures were untypeable. The typeable strains represented seven types, i.e., A, C, D1, D4, D6, E1 and F1. E1 was the most prevalent type, making up 69.42% of the positive typing secured with clinical isolations.

There were 184 strains (56.26%) submitted from carriers (chronic or contact) for typing. Type M, not represented in the identifications from clinical cases, was identified in a culture from a chronic carrier. Successful typings were secured on 14 strains which proved to be sub-types of type B. This type, as well as D6, was not represented in the cultures obtained from clinical cases. This fact

might be of more than academic interest because it could be conjectured that there is a variation in the pathogenicity of the various phage types. If true, this might account for the preponderance of certain phage types. The V form could not be recovered from 56 carrier strains (33.93%).

Positive typings were completed on 249 (76.14%) of the 327 strains of S. typhi received for typing. Further applications of the homologous phage technique should make it possible for us to reduce the number of untypeable strains. We also have received three new type strains not represented by phages in our typing kit. Phages propagated against these and tested against the so-called untypeable strains should further reduce the percentage of unsuccessful typings.

CONCLUSIONS

1. The types of S. typhi which have been identified during the course of this study are limited in number and are readily distinguishable.
2. Typing results indicate that the phage type is a relatively stable characteristic of S. typhi. The epidemiological value of phage typing is based on the fact that the form of the Vi antigen, and therefore the phage type, is stable following passage from one person to another.
3. The information obtained, as a result of phage typing, is of epidemiological significance, especially as concerns the typing of strains isolated from known typhoid carriers.

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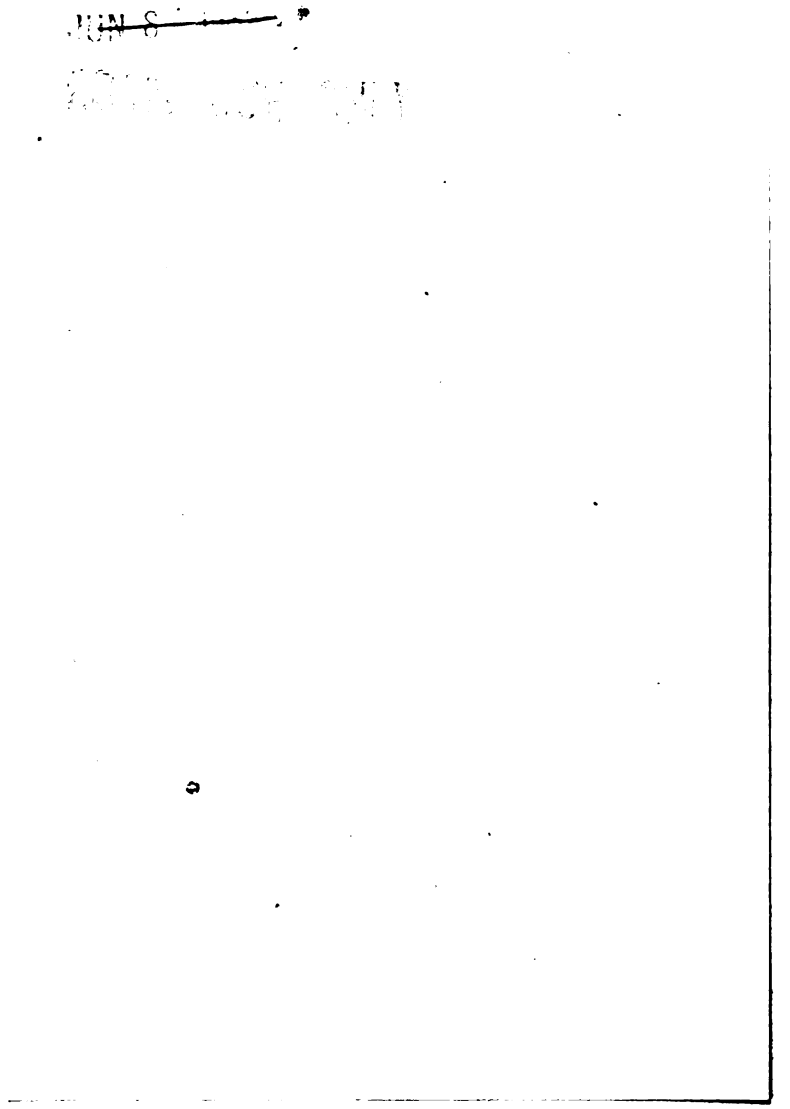

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