BACTERIOPHAGE, VIRULENCE AND AGGLUTINATION TESTS WITH A STRAIN OF *SALMONELLA TYPHI* OF LOW VIRULENCE

By A. FELIX and E. S. ANDERSON

From the Central Enteric Reference Laboratory and Bureau, Public Health Laboratory Service (Medical Research Council), London

CONTENTS

INTRODUCTION

The typing of strains of *Salmonella typhi* by the Vi-bacteriophage method (Craigie & Yen, 1938) is now generally considered to be an indispensable aid to successful epidemiological field work. Two factors of great practical importance contributed to speedy recognition and almost general acceptance of the method, namely, the remarkable constancy of the types and the circumstance that about 90% of the strains can be typed (Felix, 1943, 1948).

The routine typing of strains of *Salm. typhi* is now carried out in most countries according to the revised typing scheme and standardized technique suggested by Craigie & Felix (1947). The recommendations of these workers were approved by a committee of experts during the Fourth International Congress for Microbiology, held in Copenhagen in 1947, and were adopted as the provisional international standard method. The Central Enteric Reference Laboratory of the Public Health Laboratory Service in London acts as the International Reference Laboratory for enteric phage typing. Standard Vi-phage preparations and the corresponding Vi-type strains are distributed to the National Reference Laboratories in various parts of the world, and the latter send to the International Reference Laboratory any supposed new Vi-type strains for confirmation and eventual inclusion in the typing scheme (International Committee for Enteric Phage Typing, 1949).

From experience gained during the past three years it can be stated that the outcome of this co-operative inquiry has proved highly satisfactory. The phage typing by means of the standardized method gives on the whole uniform results in the hands of workers in different parts of the world. The results were reviewed at the Fifth International Congress for Microbiology in Rio de Janeiro (International Committee for Enteric Phage Typing, 1951) and five new Vi-phage types have been added to the twenty-four recognized types and subtypes contained in the typing scheme of Craigie & Felix (1947). The complete typing scheme of *Salm. typhi* now in use is shown in Table 1.

Some of the difficulties encountered in the phage typing of typhoid strains have been briefly discussed in the paper dealing with the standardization of the method (Craigie & Felix, 1947). The chief complications met with are those due to 'degradation' of the cultures and to fluctuations in the composition of the agar medium used for typing. The object of the present paper is to describe the difficulties that arose during the investigation of the Oswestry typhoid outbreak in 1948 and how they were overcome. Full accounts of the special features of the outbreak and of the laboratory and field work in connexion with it have been published by Bradley, Evans & Taylor (1951) and Jones (1951), and the reader is referred to these papers.

DIFFICULTIES ENCOUNTERED IN THE Vi-PHAGE TYPING OF THE OSWESTRY STRAIN

(*a*) *Results of routine typing at the beginning of the outbreak*

The cultures isolated from the first four patients in this outbreak were received for phage typing on 11 and 12 September 1948. They were examined in routine manner with the twenty-four standard Vi-phage preparations, but could not be assigned to a specific type because lysis of varying degree was produced by most of the phages. We now designate an untypable strain exhibiting partial sensitivity to several or all of the typing phages as a 'degraded Vi strain', whereas the term 'untypable Vi strain', introduced by Craigie & Felix (1947), is reserved for strains that are resistant to all the existing typing preparations of adapted Vi phage. A helpful criterion in distinguishing between a 'degraded' and an 'untypable' Vi strain is the reaction of the culture to the standard phage A applied in a concentration twenty times that used as the routine test dilution. A 'degraded Vi strain' is fully susceptible to concentrated phage A, whereas an 'untypable Vi strain' is found to be completely resistant or to yield only a few plaques.

Cultures giving the reactions of a 'degraded Vi strain' are often found at the Central Enteric Reference Laboratory among those sent for typing after prolonged subculture at the laboratory where the strain was first isolated. Most of these cultures give clear-cut typing results when a number of single-colony subcultures are examined. Fortunately the number of typhoid strains encountered in Britain that cannot be typed by means of the first twenty-four adapted Vi phages is very small. During the 8-year-period 1942–9 'untypable Vi strains' represented 9·4% and 'degraded Vi strains' only 3·0% of strains of *Salm. typhi* indigenous to this country (Felix, 1951).

Table 1. *Reactions of Vi-type strains of* Salmonella typhi *to routine test concentrations of typing phage preparations*

Vi-type strains	Vi-phage preparations																										
	A	B 1	B 2	B 3	C	D 1	D 2	D 4	D 5	D 6	E 1	E 2	F 1	F 2	G	H	J	K	L 1	L 2	M	N	O	T	25	26	27	28	29
A	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
B 1	+ +s	CL	+ +s	+s	+s	–	–	–	+s	+m	+s	+s	+s	+s	+s	+s	–	+s	+s	+s	+s	+s	++s	+s	–	–	+m	+m	++m
B 2	+s	–	CL	–	–	–	–	+m	–	–	+m	–	+m	–	+m	+m	+m	–	–	±m	±m	+s	–	+s	++s	++s	++s	++s	–
B 3	+ + +s	–	+ + +s	CL	+ + +s	+ + +m	+ +m	–	–	–	+ + +s	+ + +s	+ + +s	+ +m	+ + +m	+ + +m	+ + +m	+ + +s	+ + +s	+ + +s	+ + +s	+ + +s	+ + +m	+ + +s	+ +s	+ +s	+ +s	+ +s	–
C	±n	±n	±n	±n	CL	±m	±m	±m	±m	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n	±n
D 1	–	–	–	–	–	CL	CL	CL	+ +m	+ +m	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D 2	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D 4	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D 5	–	–	–	–	–	–	–	–	CL	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D 6	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E 1	–	–	–	–	–	–	–	–	–	–	CL	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E 2	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F 1	–	–	–	–	–	–	–	–	–	–	–	–	CL	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F 2	–	–	–	–	–	–	–	–	–	–	–	–	±n	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
G	–	–	–	–	±n	–	–	–	–	–	–	–	±n	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–
H	–	–	–	–	–	–	–	–	–	–	±s	–	±n	±n	–	CL	–	–	–	–	–	±n	±s	±m	–	–	–	–	–
J	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–
K	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	±m	–	–	–	–	–	–	–	–
L 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	CL	–	–	–	–	–	–	–	–	–
L 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±m	CL	–	–	–	–	–	–	–	–	–
M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–	–
N	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	±m	–	–	–	–	–
O	–	–	–	–	–	–	–	–	–	–	–	–	–	±m	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–
T	–	–	–	–	–	–	–	–	–	±m	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	CL	–
25 (Lie)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–	–
26 (Clark)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–	–
27 (Scholtens)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–	–
28 (Scholtens)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL	–
29 (Borman)	–	–	–	–	–	–	–	–	–	CL	–	–	–	CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	CL

CL = confluent lysis; – = no plaques; ± = a few plaques usually present; +, + +, + + + = increasingly numerous plaques; n = normal plaques; s = small plaques, visible to the naked eye; m = minute plaques, visible only by means of magnifying lens (× 10).

https://doi.org/10.1017/S0022172400044211 Published online by Cambridge University Press

The cultures from the first four patients in the Oswestry outbreak had all been isolated by blood culture, and had been submitted to the bacteriophage tests with the least possible delay. The fact that the cultures, nevertheless, were found to react as 'degraded Vi strains' did not, at first, seem to be remarkable. Similar observations have been made by one of us (A. F.) on earlier occasions, and those relating to certain outbreaks due to strains of Vi-phage Type N (originally known in England as Type 'Richmond') have been specifically mentioned in a previous paper (Craigie & Felix, 1947).

A further culture from the Oswestry outbreak, received on 13 September, clearly belonged to Type A, as did two cultures received on 15 September. Of fifteen cultures from twelve patients received from 11 to 17 September inclusive, six belonged to Vi-phage Type A; the remainder reacted as 'degraded Vi strains'. Three cultures were received from one patient, of which two gave the 'degraded' reaction and the third was Type A. Table 2 gives the result of Vi-phage typing on first examination of all cultures examined during the first week of the outbreak.

Table 2. *Results of routine phage typing of cultures isolated during the first week of the outbreak*

Case no.	Initials	Culture isolated from	Clinical case or symptomless excreter	Result of Vi-phage typing
1	Ll.H.	Blood*	Case	Degraded Vi strain
2	Mrs M.	Blood	Case	Degraded
		Faeces†	—	Type A
3	E. We.	Blood	Case	Degraded
4	W.M.	Blood	Case	Degraded
5	P.P.	Blood	Case	Type A
6	J.S.	Faeces	Case	Type A
7	G.D.	Blood	Case	Degraded
		Faeces	—	Degraded
8	E.Wi.	Blood	Case	Type A
9	M.B.	Faeces	Symptomless excreter	Degraded
		Faeces	—	Degraded
10	P.J.	Faeces	Case	Degraded
11	M.C.	Faeces	Case	Type A
12	J.D.	Faeces	Precocious carrier	Type A

* All blood cultures were grown in bile broth.

† All faecal cultures were isolated by enrichment in selenite F medium followed by plating on desoxycholate-citrate agar.

It is seen from Table 2 that it was impossible to decide at this stage whether the outbreak was due to one strain or whether two different strains were responsible.

It has been long recognized that Type A, the only Vi-phage type that is fully sensitive to all the adapted preparations of the original Vi-phage II of Craigie & Yen (1938), occupies an exceptional position in the phage-typing scheme. On the one hand, Type A strains, like all the other recognized Vi-phage types, are found to be responsible for epidemiologically well-defined outbreaks. On the other hand, Type A has been isolated as a variant of a number of specific Vi types, such as

B1, C, D5, F1, N, O and T (Craigie & Felix, 1947). This 'dissociation' was found to occur not only in cultures maintained for long periods of time in the laboratory but also in chronic carriers of long standing (Felix, unpublished observations). Craigie & Felix (1947) therefore concluded that the isolation from related cases or carriers of Vi-phage Type A, in association with one of the other types, need not necessarily signify infection with more than one strain of the typhoid bacillus. Since that paper was published, we have observed many instances of 'dissociation' of initially specific Vi-type cultures, resulting in confluent lysis with all the heterologous phage preparations, and thus leading to the emergence of Type A.

The position was complicated by the fact that epidemiological evidence suggested that sewage pumped into the cabbage field at the Oswestry Hospital might have been the source of the outbreak. If this assumption was correct, it was to be expected that more than one Vi-phage type might be concerned.

An attempt was made to adapt the standard Vi-phage A to a few of the degraded cultures that gave cross-reactions with various typing phages, but in all instances in which a fairly potent phage preparation was so produced it proved to be an unchanged Type A phage (see Table 3). This finding was not unexpected, because earlier experiments clearly indicated that once strong cross-reactions with heterologous phages have developed, it is not possible to trace the original type from which the degraded culture is derived (Anderson & Felix, to be published).

We were thus in the unpleasant position of being unable to type a number of the Oswestry cultures or to decide whether one or two different strains were responsible for the outbreak.

(*b*) *Effect of agar medium used for typing*

At the time when the Oswestry cultures were being examined we were experiencing difficulties with the agar medium employed for typing. Of the two different media recommended in the paper by Craigie & Felix (1947), the one preferred by Craigie, namely, agar made with 'Bacto' nutrient broth (dehydrated), supplied by Difco Laboratories, Detroit, Michigan, U.S.A., was not available for reasons that were beyond our control. In the work on typhoid and paratyphoid-B Vi-phage typing carried out in this laboratory since 1940 (Felix, 1943, 1948; Felix & Callow, 1943) tryptic meat-digest agar had been used almost exclusively. When wartime conditions necessitated the employment for a short period of papain agar, it was soon found unsatisfactory and had to be replaced by agar made with tryptic meat-digest.

To safeguard against the effect of the inevitable fluctuations in the composition of trypsin-digest agar the medium was always prepared in large batches, and each new batch was compared with the previous one by testing the Vi-type strains N, O and T against the whole series of standard typing phages. If the agar medium did not permit full development of the small-size plaques that characterize these three Vi-phage types the batch was not accepted for use in bacteriophage tests. The necessity for carrying out these comparative tests on each successive batch of agar medium has been emphasized in a previous paper (Craigie & Felix, 1947).

Thanks to the strict observance of this routine procedure we were aware of the fact that the agar medium we were using at the time of the Oswestry outbreak was not satisfactory. Owing to fluctuations in the quality of different batches of meat and of pancreatic extract, and to frequent changes in the staff of the media room, we were unable to obtain a regular supply of satisfactory trypsin-digest agar. The trypsin-digest broth employed for the initial growing of the cultures presents little trouble, because it is used after addition of 1 % peptone; but a good trypsin-digest agar is much more difficult to prepare. We were thus forced to employ some batches of agar medium which were below standard quality. It was soon noticed that the confusing phage-typing results with the Oswestry cultures were obtained only when the tests were carried out on agar medium known to be unsatisfactory, as judged by poor development of plaques with the test cultures of Types N, O and T. When trypsin-digest agar of good quality was procured all the Oswestry cultures were found to be fully susceptible to all the typing phages, that is to say, the cultures reacted in the way Vi-type A strains do.

Table 3. *Bacteriophage reactions of Oswestry cultures on trypsin-digest agar from two different batches*

Trypsin digest agar	Strain	Routine test dilutions of standard Vi phages			Phage A propagated on Oswestry strain, Mrs M.		Control, pooled phages I+IV
		A	F1	L1			
		1:30,000	1:30,000	1:150,000	1:10,000	1:100,000	1:100
Batch of poor quality	Oswestry symptomless excreter, M.B.	−	+m	+m	−	−	CL
	Oswestry case, Mrs M.	++s	+++s	+++s	+++s	+s	CL
	Oswestry case, Miss S.	CL	CL	CL	CL	SCL	CL
	Stock strain Vi-type A	CL	CL	CL	CL	SCL	CL
Batch of good quality	Oswestry symptomless excreter, M.B.	SCL	SCL	SCL	SCL	++s	CL
	Oswestry case, Mrs M.	SCL	SCL	SCL	SCL	++s	CL
	Oswestry case, Miss S.	CL	CL	CL	CL	CL	CL
	Stock strain Vi-type A	CL	CL	CL	CL	CL	CL

Reading after 8 hr. continuous incubation at 37·5° C. CL = confluent lysis with standard loopful of test dilution of phage; SCL = semi-confluent lysis with standard loopful of test dilution of phage; − = no plaques; +, ++, +++ = increasingly numerous plaques; s = small plaques, visible to the naked eye; m = minute plaques, visible only by means of magnifying lens (× 10).

Phages I and IV = original unadapted Vi-phage I and Vi-phage IV of Craigie & Yen (1938).

Table 3 shows, in abridged form, a comparative test of three of the Oswestry cultures on an unsatisfactory medium and a good one. On the former, only one of the three Oswestry cultures (case Miss S.) gave the reactions of a Type A strain,

https://doi.org/10.1017/S0022172400044211 Published online by Cambridge University Press

whereas all three cultures were readily typed as belonging to Type A when tested on agar of good quality. These observations indicated that the Oswestry strain was more exacting in its nutritional requirements than strains of *Salm. typhi* usually are, and this finding was confirmed by other tests described in the subsequent sections of this paper. Since most of the Oswestry cultures gave the Type A reaction when tested under suitable conditions, we felt justified in concluding that one strain only was concerned in the outbreak and that it belonged to Vi-phage Type A.

VIRULENCE OF THE OSWESTRY STRAIN FOR MICE AND FOR MAN

(*a*) *Agglutination and virulence tests with the Oswestry strain*

Because of the irregular reactions obtained with the first Oswestry cultures in the phage-typing tests, it was considered advisable to examine the cultures for their Vi-antigen content. It was found that all the cultures tested, whether isolated from the blood or from faeces, were fully agglutinable by pure O antiserum, and that the reactions with pure Vi serum were almost negligible. Most of the cultures showed in the Vi-agglutination test not more than a faint trace of agglutination, discernible only under 10-fold magnification, and in some instances clear-cut negative reactions were recorded.

It was obvious from the results of the agglutination tests that the Oswestry strain either was unusually poor in Vi antigen or was losing it rapidly on subculture. Arrangements were therefore made to obtain from a number of patients specimens of blood which were sent to us in the form of blood-clots in pure ox-bile and as blood cultures in poured agar plates. The specimens were taken as early as the third day of illness and reached our laboratory in less than 24 hr. It was thus possible to employ in the Vi- and O-agglutination tests cultures representing only the third or fourth subculture after the original isolation from the blood. There was no need to use any of the more complex selective media that might possibly have affected the result, and special care was taken to employ only the best trypsin-digest agar for growing the cultures. The technique of the agglutination tests was that described previously (Felix, 1938; Felix & Pitt, 1951). Table 4 illustrates the results obtained in these tests.

In spite of the precautions taken, the Oswestry cultures were found to be deficient in the Vi antigen. Table 4 shows that the control strains, grown on the same agar medium, developed their full quota of Vi antigen. The controls included the well-known strains Ty 2, 'Watson' and O 901, and also another strain of Vi-phage Type A, which had been recently isolated from a group of cases in Welshpool, near Oswestry, believed to be connected with the Oswestry outbreak.

The unusual finding that the Oswestry strain, immediately on isolation, appeared to be almost devoid of the Vi antigen called for an examination of the mouse virulence of these cultures. Table 5 summarizes the results of the virulence tests. The technique was the same as employed in earlier work (Felix, 1938).

Two series of virulence tests were carried out: the first within a few days after the cultures had been isolated, the second about 3 months later. During the interval

Table 4. *Comparative agglutination tests on freshly isolated cultures of the Oswestry strain of* Salmonella typhi

		Suspensions of living organisms of strains											
		Oswestry. Culture isolated from						Welshpool. Culture isolated from					
		Blood-clot in ox-bile		Blood in poured agar plate		Faeces							
Serum	Dilution	Case 1	Case 2	Case 3	Case 4	Case 5	Excreter	Faeces. Case 1	Faeces. Case 2	Faeces. Case 3	Ty 2	Watson	O 901
Pure H serum	1:1000	±	(±)	−	−	(±)	−	(±)	(±)	(±)	±	±	−
Pure O serum	1:500	+++	+++	+++	+++	+++	+++	((±))	+±	((±))	((±))	((±))	+++
	1:1000	+++	+++	+++	+++	+++	+++	−	±	−	−	−	+++
	1:5000	+++	+++	+++	+++	+++	+++	−	(±)	−	−	−	+++
	1:20,000	+++	+++	+++	+++	+++	+++	−	(±)	−	−	−	+++
Pure Vi serum	1:400	((±))	−	((±))	((±))	((±))	((±))	+++	+++	+++	+++	+++	−
	1:800	−	−	((±))	((±))	−	−	++	++±	++	+	+++	−
	1:1400	−	−	−	−	−	−	+	++±	±	((±))	++	−
	1:2000	−	−	−	−	−	−	−	+±	−	−	±	−
	1:5000	−	−	−	−	−	−	−	−	−	−	−	−

Notes. Saline controls with live and steamed suspensions are omitted from the table.

+++ = strongest degree of agglutination; supernatant fluid completely clear.

++± to + = degrees of incomplete agglutination; supernatant fluid turbid.

± = weakest degree of agglutination which could be estimated with the naked eye.

(±) = trace, ((±)) = faint trace } estimated by means of magnifying lens.

the cultures were preserved on Dorset egg medium without being subcultured. The Welshpool strain, which was responsible for a small group of typhoid cases of average clinical severity, was tested at the same time. Table 5 shows that a culture of the Welshpool strain, isolated from a faecal specimen, proved to be as virulent for mice as the classical strain Ty 2. On the other hand, the eight freshly isolated Oswestry cultures were all found to be of lower mouse-virulence. There were differences in the lethal effects of individual cultures, apparently due to the peculiar instability of the Oswestry strain, which is reflected by its irregular reaction to the specific Vi phages described in this paper. But the average death-rate among mice infected with the Oswestry cultures was considerably lower than that following infection with the Welshpool strain of Type A, or with strain Ty 2 which was isolated as long ago as 1918.

Table 5. *Comparative mouse-virulence tests*

Strain		Culture isolated: Date	Culture isolated: From	No. of organisms in dose	Lethal effects following intraperitoneal inoculation of mice: Tested 13. x. 48	Tested 25. i. 49
Oswestry	Case 1	23. ix. 48	Blood-clot in ox-bile	100×10^6	0/8	1/10
	Case 2				2/8	3/10
	Case 3	4. x. 48	Blood in poured agar plate	100×10^6	5/8	.
	Case 4				3/8	4/10
	Case 5	11. x. 48	Faeces	100×10^6	6/8	6/10
	Case 6	8. x. 48	Blood	100×10^6	4/8	.
	Symptomless excreter	15. ix. 48	Faeces (*a*)	100×10^6	1/8	1/10
			Faeces (*b*)	100×10^6	.	4/10
		Average		.	3·75/10	3·17/10
Welshpool	Case 3	8. x. 48	Faeces	100×10^6	8/8	9/10
Ty 2		1918	.	100×10^6	17/18	18/20
				80×10^6	.	9/10

Note. The numerator indicates the number of mice that died, the denominator the number inoculated.

The results of the agglutination and virulence tests thus clearly indicated that the Oswestry strain was one of low Vi-antigen content and of relatively low virulence. This finding made it possible to predict at an early stage during the epidemiological investigation that the outbreak would take a course not usually met with in a typhoid epidemic due to an organism of average virulence. The situation was considered to resemble an outbreak of paratyphoid-B fever, accompanied by numerous symptomless excreters, and a large secondary wave was to be expected. The accounts of the Oswestry outbreak published by Bradley *et al.* (1951) and by Jones (1951) show that this prediction was fulfilled.

(*b*) *Unusual features of the Oswestry outbreak*

As a result of the careful epidemiological and laboratory investigations conducted during the Oswestry outbreak the following facts were established.

(1) *Unusually high incidence of symptomless excreters and 'precocious carriers'.* In the course of repeated examination of the faeces of 435 staff and patients at the hospital nineteen symptomless excreters and fifteen incubating cases ('precocious carriers') were found. Thus thirty-four (i.e. 25·2 %) of the total of 135 cases and excreters in this outbreak were detected by isolation of the bacilli before the onset of symptoms. Twenty-five of these excreters were nurses or food handlers found at work (Jones, 1951).

(2) *Unusually long incubation periods.* In two of the cases with fully developed clinical symptoms the incubation periods were 25 and 30 days respectively (Bradley *et al.* 1951).

(3) *Low case-fatality rate.* There were seven deaths in this outbreak, i.e. the case fatality was 5·2 %. Because of insufficient accommodation at the local hospital the patients were admitted to a number of widely scattered hospitals and there was no uniformity in treatment. Chloramphenicol was not then available. In a group of twenty-two patients treated in one hospital with Felix's anti-typhoid (Vi + O) serum there were no fatalities. In other hospitals treatment with various drugs was employed, including penicillin, sulphathiazole and aerosporin, all of which are now known to be ineffective in typhoid fever.

(4) *Low incidence of the chronic-carrier state.* So far as is known only one chronic carrier developed among the 135 persons infected with the Oswestry strain (Jones, 1951). The follow-up of recovered typhoid patients in this country now includes also testing for the presence of Vi agglutinins in the convalescents' serum (Felix, 1944; Ministry of Health, 1945), in addition to the customary examination of the excreta. Careful follow-up was facilitated in this outbreak by the fact that 103 of the total of 135 cases and excreters were members of the hospital staff.

It is evident from these facts that the low Vi-antigen content and the relatively low mouse virulence of the Oswestry cultures truly reflected the low virulence of this strain for man.

(c) *Vi-agglutination tests with sera of convalescents and suspected carriers*

In view of the low Vi-antigen content of the Oswestry cultures it is of interest to note that patients who contracted clinical typhoid fever showed Vi-agglutinin titres of the same order of magnitude as those in a typhoid outbreak of average severity. Table 6 gives a few examples of the TVi-agglutinin titres recorded in recovered patients from the Oswestry outbreak.

Table 6. *Vi-agglutinin titres observed in recovered typhoid patients from the Oswestry outbreak*

Case	Date of onset	Specimen of serum collected	Standard TVi-agglutinin titre
1	21. ix. 48	19. xii. 48	1 in 40
2	24. ix. 48	22. xii. 48	1 in 40
3	3. x. 48	29. xii. 48	1 in 25
4	9. ix. 48	1. xii. 48	1 in 10
5	22. ix. 48	1. xii. 48	1 in 120

Note. 'Standard' titres are based on the 'Provisional Standard Anti-typhoid Serum' (Felix, 1938).

https://doi.org/10.1017/S0022172400044211 Published online by Cambridge University Press

It is seen from Table 6 that the TVi titres observed in convalescence were sufficiently high to serve as a base-line for comparison with subsequent specimens of serum that were taken at intervals of about 6 weeks in the course of the routine examination for final clearance. On the other hand, few of the symptomless excreters developed significant TVi-agglutinin titres (Jones, 1951). Serological examination was, in fact, extensively employed during the investigation of this outbreak in order to prove that no chronic carrier was present among the hospital staff.

Table 7. *Exceptionally high TVi agglutination in a case of typhoid cystitis*

Specimen of serum collected	Titre of agglutination		
	TH	TO	TVi (Standard)
Onset 7. ix. 48	.	.	.
21. ii. 49	20,000	1,000	1,000
20. iv. 49	20,000	500	600
8. viii. 49	10,000	500	400

The case of the Oswestry patient listed in Table 7 is of particular interest because of his exceptionally high titre of Vi agglutination. After a fairly severe attack of typhoid fever this patient suffered two attacks of cystitis and excreted typhoid bacilli in the urine for several months. The decreasing TVi titre, although still far above the average, afforded further proof that the patient had ultimately freed himself of the infection.

DIFFERENTIATION OF THE OSWESTRY STRAIN FROM OTHER TYPHOID STRAINS OF Vi-PHAGE TYPE A

It is well known that the epidemiological assistance given by typhoid Vi-phage typing is of a high order. Nevertheless, circumstances may arise in the course of an epidemiological investigation which call for the employment of additional laboratory methods of identification of the epidemic strain. This necessity is rarely encountered in practice, but the Oswestry outbreak provided such an instance owing to the fact that Vi-phage Type A is the most common typhoid Vi-phage type indigenous to Great Britain.

Table 8. *Percentage distribution of typhoid Vi-phage types in Great Britain*

(Average for 8 years 1942–9 (Felix, 1951).)

Vi-phage types ...	A	B2	C	D1	D2	D4	D5	D6	E1	F1	F2	G
Percentage	27·3	0·2	11·3	5·8	0·4	0·9	0·2	0·5	24·0	4·2	0·6	0·1

Vi-phage types ...	H	J	L2	N	O	T	Untypable Vi strains	Degraded Vi strains	Vi-negative cultures
Percentage	0·1	0·6	0·2	2·9	4·5	2·2	9·4	3·0	1·6

The figures shown in Table 8 are calculated from the number of foci from which the strains were derived, and not from the number of patients or cultures examined (Felix, 1951). It is seen from the table that Type A is the most prevalent of the

Vi-phage types occurring in this country, with Type E 1 following next. It may be mentioned here that a preliminary survey of the geographical distribution of Vi-phage types of *Salm. typhi*, based on reports from fifteen countries in all five continents, showed that Type E 1 apparently is the most common typhoid Vi-phage type throughout the world. But in Britain, Type A held the first place during seven out of the eight years under observation (International Committee for Enteric Phage Typing, 1951).

During the investigation of the Oswestry outbreak it became necessary to compare the epidemic strain with other strains belonging to Vi-phage Type A that had been isolated in the vicinity. These strains came from the following sources:

(i) From three cases of typhoid fever due to Vi-phage Type A that occurred at Welshpool, about 20 miles from Oswestry, between April and September 1948. The last case fell ill during the Oswestry outbreak.

(ii) From a laboratory technician in an adjoining county who contracted a fatal attack of typhoid fever while handling typhoid cultures from Oswestry and from another group of typhoid cases due to Vi-phage Type A.

(iii) From a chronic carrier who had contracted typhoid fever during an outbreak at Donnington Camp (Shropshire) in 1940. This man was a gardener living on a farm which supplied milk to the Oswestry hospital and it was possible that sewage from his cottage had polluted the milk (Bradley *et al.* 1951; Jones, 1951). Twenty-five single-colony cultures of the carrier strain, isolated by direct plating of faecal specimens on selective media, were examined; of these twenty-four were 'untypable Vi strains', that is, they were resistant to all Vi-typing phages, but one single-colony culture gave the reactions of a Type A strain. Although a 'dissociation' of this kind has been observed previously in chronic carriers of long standing (Felix, unpublished observations), it was, nevertheless, felt unsafe to exclude a relationship between the carrier and the Oswestry outbreak without further investigations.

It was obviously important to find some means whereby a decision could be made whether any of these strains were related to the Oswestry strain. Four ancillary tests were used for this purpose.

(*a*) *Colony size on agar*

Agar plate cultures of the Oswestry strain constantly yielded smaller colonies than those of the three other strains mentioned. On a trypsin-digest agar of poor quality the Oswestry strain grew slowly, the colonies being little more than 1 mm. in diameter after 24 hr. incubation at 37° C. Colonies of the three other strains were about double this size. On a good trypsin-digest agar the Oswestry strain showed better growth, colonies reaching about 2·0 mm., whereas those of the other strains were 2·5 mm. in diameter. This difference in colony size was observed throughout a large number of test platings repeated in the course of over two years.

(b) Colonial variation on agar

The majority of colonies of Oswestry cultures were perfectly smooth when grown on trypsin-digest agar for 18–24 hr. at 37° C., but a few colonies invariably showed small surface and marginal buds. These were papillary when limited to the surface, but formed projecting sectors when they encroached upon the colonial margin. Such sectors were less opaque and less smooth than the remainder of the colony. The loss of opacity indicated absence of the Vi antigen (Craigie & Brandon, 1936; Giovanardi, 1938; Felix, 1938).

If incubation was continued, all apparently smooth colonies developed these outgrowths, which increased rapidly in size so that the original colonial outline was distorted into an irregular polygon. The 'budding' was apparent on both good and bad batches of medium, but the phenomenon was more obvious on trypsin-digest agar of poor quality, because growth of the buds was not inhibited to the same extent as that of the mother colony. The fully developed picture was present after 48 hr. incubation at 37° C. It was found in all cultures from the Oswestry outbreak, irrespective of whether they reacted with the phages as 'degraded Vi strains' or as phage Type A.

The strains isolated from Welshpool cases, from the technician and from the chronic carrier, did not show this 'budding' although they were examined for it on many occasions.

(c) Biochemical reactions

Kristensen & Henriksen (1926) and Kristensen (1938) classified strains of the typhoid bacillus on the basis of differential fermentation of L-arabinose and xylose. More recently, Jude & Nicolle (1949) employed this method as an ancillary test in the classification of different strains of the typhoid bacillus belonging to the same Vi-phage type.

The Oswestry strain and the strains from Welshpool and from the technician were found to belong to Kristensen's Type I (xylose positive). The carrier strain, on the other hand, belonged to Type II (xylose negative). The single-colony culture isolated from the gardener that reacted as a Vi-phage Type A strain also failed to ferment xylose. This was further proof that the gardener could not be incriminated as the carrier responsible for the Oswestry outbreak.

A number of cultures isolated during the typhoid outbreak at Donnington Camp in 1940, in which the gardener contracted his infection, were still available in the collection of stock cultures maintained in this laboratory. These cultures were all 'untypable Vi strains' and were also found to belong to Kristensen's Type II. The strain had therefore retained this biochemical characteristic during more than eight years' maintenance in the laboratory.

(d) Sensitivity to anti-O bacteriophage

One of the anti-O bacteriophages described by Felix & Callow (1943) has been employed for the past ten years in this laboratory as a control reagent in the routine Vi-phage typing of *Salm. typhi* and *Salm. paratyphi B*. It is known that

salmonella anti-O phages are much less specific than anti-Vi phages. Even minor O-antigenic components that are common to many diverse salmonella species, though they are not listed in the Kauffmann-White diagnostic scheme, provide an adequate point of attack for anti-O bacteriophages (Felix & Callow, 1943).

Most of the first twenty-four recognized typhoid Vi-phage types and subtypes are, as a rule, fully sensitive to the high-titre anti-O phage employed, although strains belonging to any of these Vi-phage types are occasionally encountered that are resistant. On the other hand, strains of Types D6, F1, F2, H, J, K, L1, L2 and M usually give no lysis with anti-O phage, or only a few micro-plaques. On several occasions the test with anti-O phage proved useful in the differentiation of 'untypable Vi strains' from different outbreaks, when Vi-phage typing could give no further assistance. One such instance of the use of anti-O phage has been described previously (Marmion & Martin, 1946).

All the Oswestry cultures, whether reacting as Type A or as 'degraded Vi strains', were fully sensitive to anti-O phage; so also were the cultures from Welshpool and from the technician. On the other hand, the 'untypable Vi strain' isolated from the gardener and those from the 1940 outbreak in Donnington were all resistant to anti-O phage. The single-colony culture isolated from the gardener that reacted as a Type A strain was, however, lysed by anti-O phage. This is in keeping with observations we have made on 'degraded' Type A variants derived from other strains of the typhoid bacillus.

DISCUSSION

The most interesting point arising from the foregoing paper is the correlation between mouse virulence of the typhoid bacillus and its virulence for man. The fact that the relative virulence of *Salm. typhi* for mice runs *pari passu* with virulence for man was known to one of us (A. F.) from unpublished work carried out during the war of 1939–45. Findlay (1951) has now published the results of those experiments, showing that properly conducted virulence tests in mice do, in fact, reflect the relative virulence for man of typhoid strains isolated from severe or mild outbreaks of typhoid fever.

The finding that the Oswestry strain was one of low Vi-antigen content and low mouse virulence enabled us to predict at an early stage during the investigation that the outbreak would resemble a paratyphoid-B outbreak more closely than an average typhoid outbreak. The unusual features of the Oswestry epidemic, described by Bradley *et al.* (1951) and by Jones (1951), showed that this prediction was correct. The high incidence of symptomless excreters and 'precocious carriers' among the food handlers at the hospital caused the protracted course of the outbreak, which was not brought under control until all kitchen staff were suspended from work.

The Oswestry typhoid outbreak also resembled a paratyphoid-B outbreak in that it could not be traced to a chronic carrier. In all the recent outbreaks of paratyphoid-B fever in Britain, the persons identified as responsible for the spread of infection were later proved to be temporary excreters. The true culprits, namely

the chronic carriers, escaped detection (Felix, 1944, 1951). The same difficulty arose in the investigation of the unusual typhoid outbreak in Oswestry. It is probable that the epidemic originated from a symptomless excreter or 'precocious carrier' who had become infected outside the hospital.

Some workers believe that the fact that certain typhoid strains are virulent for man and for mice may be merely coincidental; that the presence or absence of the Vi antigen is not associated with the degree of virulence of the strain; and that other factors, possibly another, yet unknown, antigen or a non-antigenic substance, or substances, might be at play. If additional evidence is needed to show that these speculations are unwarranted the present findings and those of Findlay (1951) would appear to provide it.

Another point of interest was the difficulty in the routine phage-typing procedure caused by the instability of the Oswestry strain and by its failure to develop its Vi antigen on certain batches of agar. Cultures isolated at the same time from different patients, or even from blood and faeces of the same patient, did not react in identical manner to the standard Vi-typing phages. Differences in the sensitivity of individual cultures to the typing phages were noticeable even on the best trypsin-digest agar, although it was ultimately possible to assign all the cultures to Vi-phage Type A.

The paramount importance of using for routine phage typing an agar medium of high nutritive value has been emphasized by Craigie & Felix (1947). If the organism does not grow readily on the medium and form its full quota of Vi antigen, it is not possible to reproduce the reactions with the standard preparations of the Vi-typing phages on which the typing scheme is based. As a result of the difficulties experienced with the typing of the Oswestry strain the use of trypsin-digest agar for routine Vi-phage typing was discontinued and the medium now employed is agar containing not less than 20 g. 'Bacto' dehydrated nutrient broth per litre (Craigie & Felix, 1947).

Because the Oswestry strain belonged to Type A, which is the most common typhoid Vi-phage type indigenous to Britain, it was necessary to employ other tests in order to distinguish the epidemic strain from other Type A strains isolated in the vicinity. The value of any such test of identity, ancillary to Vi-phage typing, depends on the constancy of the character tested. The danger lies in the choice of a single character which, because of the ease with which micro-organisms vary, may disappear from one strain or appear in another. For this reason it is important to use as many criteria as possible when attempting to distinguish between different strains of the same Vi-phage type, since several characters are less likely to be lost or gained simultaneously in a variant than is a single character. The four ancillary tests used in this investigation have been discussed in some detail, and the results obtained are summarized in Table 9.

Table 9 shows that the Oswestry strain differed in at least two respects from each of the other strains with which it was compared, and it could reasonably be concluded from this that it was not epidemiologically related to any of them.

Table 9. *Summary of results of ancillary tests of identity*

Strain	Vi-phage type	Average colony size after 24 hr. at 37° C. on trypsin-digest agar of: Poor quality (mm.)	Good quality (mm.)	'Budding'	Fermentation of: Arabinose	Xylose	Sensitivity to anti-O phage
Oswestry	A	1	2·0	+	−	+	+
Welshpool	A	2	2·5	−	−	+	+
Laboratory technician	A	2	2·5	−	−	+	+
Gardener (chronic carrier)	Untypable Vi strain	2	2·5	−	−	−	−
	A	2	2·5	−	−	−	+

SUMMARY

1. The low Vi-antigen content and low mouse virulence of freshly isolated cultures of the Oswestry strain of *Salm. typhi* reflected its low virulence for man. This is additional evidence of the association between the degree of virulence of *Salm. typhi* and its Vi-antigen content.

2. The prediction that the epidemic caused by this typhoid strain of low mouse virulence would resemble a paratyphoid-B outbreak more closely than an average typhoid outbreak proved to be correct.

3. Sera from convalescent patients in this typhoid outbreak showed significant Vi-agglutinin titres in spite of the low Vi-antigen content of freshly isolated cultures.

4. The nutritional requirements of the Oswestry strain were unusual, and its sensitivity to the specific Vi-typing phages was variable. This caused considerable difficulty in the typing of the cultures.

5. Additional tests of identity had to be employed in order to distinguish the epidemic strain from other strains of the same Vi-phage type isolated in the vicinity. Colony size, colonial morphology, biochemical reactions and sensitivity to anti-O bacteriophage served as useful ancillary criteria.

We are glad to acknowledge the kind co-operation of Dr A. C. Jones of the Public Health Laboratory, Shrewsbury, who supplied most of the cultures and sera. Our thanks are also due to Dr A. L. Peeney of the Queen Elizabeth Hospital, Birmingham, for sending us a number of cultures.

REFERENCES

Bradley, W. H., Evans, W. & Taylor, I. (1951). *J. Hyg., Camb.*, **49**, 324.
Craigie, J. & Brandon, K. F. (1936). *J. Path. Bact.* **43**, 233.
Craigie, J. & Felix, A. (1947). *Lancet*, **1**, 823.
Craigie, J. & Yen, C. H. (1938). *Canad. publ. Hlth J.* **29**, 448, 484.

FELIX, A. (1938). *J. Hyg., Camb.*, **38**, 750.
FELIX, A. (1943). *Brit. med. J.* **1**, 435.
FELIX, A. (1944). *Brit. med. Bull.* **2**, 269.
FELIX, A. (1948). *Proc. 4th Int. Congr. trop. Med. & Malaria*, p. 283. Washington.
FELIX, A. (1951). *Brit. med. Bull.* **7**, 153.
FELIX, A. & CALLOW, B. R. (1943). *Brit. med. J.* **2**, 127.
FELIX, A. & PITT, R. M. (1951). *J. Hyg., Camb.*, **49**, 92.
FINDLAY, H. T. (1951). *J. Hyg., Camb.*, **49**, 111.
GIOVANARDI, A. (1938). *Zbl. Bakt.* Abt. I, Orig., **141**, 341.
INTERNATIONAL COMMITTEE FOR ENTERIC PHAGE TYPING (1949). *Proc. 4th Int. Congr. Microbiol.* (1947), p. 621. Copenhagen.
INTERNATIONAL COMMITTEE FOR ENTERIC PHAGE TYPING (1951). *Proc. 5th Int. Congr. Microbiol.* (1950) (in the Press). Rio de Janeiro.
JONES, A. C. (1951). *J. Hyg., Camb.*, **49**, 335.
JUDE, A. & NICOLLE, P. (1949). *Ann. Inst. Pasteur*, **77**, 550.
KRISTENSEN, M. (1938). *J. Hyg., Camb.*, **38**, 688.
KRISTENSEN, M. & HENRIKSEN, H. C. D. (1926). *Acta path. microbiol. scand.* **3**, 551.
MARMION, B. P. & MARTIN, A. E. (1946). *Mon. Bull. Minist. Hlth & Emerg. Publ. Hlth Lab. Serv., Lond.*, **5**, 205.
MINISTRY OF HEALTH (1945). *Mon. Bull. Minist. Hlth & Emerg. Publ. Hlth Lab. Serv., Lond.*, **4**, 224.

(*MS. received for publication* 4. v. 51.)