This paper summarizes the results of a study of 500 Salmonella cultures and offers some observations having theoretic and practical bearings on phage typing.

Observations on the Types and Typing of *Salmonella paratyphi B* Cultures in the United States

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FELIX and Callow [1] summarized the results of phage typing of strains of *Salmonella paratyphi B* isolated in Great Britain for the years 1942–1950. Nicolle, Jude, and Hamon [2] reported similar data for France, Algiers, Tunis, Morocco, Madagascar, and the French occupied zone of Berlin. Desranleau and Martin [3] summarized the results of phage typing in the Province of Quebec. No similar statistics have been published for the United States. During the past three years 493 cultures of *S. paratyphi B* isolated in the United States and its territories during the period from 1934 to 1952 have been studied at the National Reference Laboratory for phage typing.

SOURCE AND CHARACTERISTICS OF CULTURES

In Table 1, 505 cultures of *S. paratyphi B* are classified as to their sources, their flagellar antigen components, and their reactions in D-tartrate medium. The cultures from human beings are divided further on the basis of the clinical conditions of the individuals from whom they were derived. The category labeled "unknown" is large because in many instances precise information could not be obtained from the sender. Table 1 includes 250 of the 262 cultures of Edwards, Bruner, and Moran,[4] since these were phage typed during the course of the present work. The remaining 243 cultures were received subsequently. The relatively large number of cultures from sewage reflects the results of large-scale surveys to determine the extent of contamination with enteric pathogens of irrigation water in the western part of the United States. The majority of the cultures from sewage were diphasic but tartrate-positive, in contrast to the strains from human sources which were largely diphasic and tartrate-negative. The literature dealing with the distribution of biochemical and serological varieties of *S. paratyphi B* has been reviewed.[4] Attention is called to the appearance of 15 cultures of *S. paratyphi B* var. *java* among the cultures of animal origin. None had been encountered previously among cultures of *S. paratyphi B* from lower animals.

An analysis of the geographical sources of the cultures revealed that they had been received from 36 states, the District of Columbia, and four foreign countries; but there was no correlation between any of the phage types and their geographical sources. The

TABLE 1

Sources of Cultures of Varieties of Salmonella paratyphi B

Source	Number of Diphasic Cultures		Number of Monophasic Specific Cultures		Total
	D-tartrate +	D-tartrate −	D-tartrate + (var. java)	D-tartrate −	
Man					
Enteric Fever	3	125	3	7	138
Gastroenteritis	9	11	45	2	67
Carriers	13	26	23	2	64
Miscellaneous	0	4	0	0	4
Unknown	9	23	29	2	63
Subtotal	34	189	100	13	336
Turkeys	3	0	2	1	6
Chickens	3	3	2	0	8
Cattle	0	3	0	0	3
Sheep	0	4	0	0	4
Swine	3	5	3	0	11
Dogs	11	1	5	4	21
Mice	1	0	0	0	1
Spider Monkey	0	0	0	2	2
Mink	2	0	1	0	3
Duck	0	0	2	0	2
Egg Powder	1	0	0	0	1
Sewage	67	13	13	0	93
Unknown	2	5	5	2	14
Subtotal	93	34	33	9	169
Total	127	223	133	22	505

desirability of analyzing phage type distribution on the basis of numbers of outbreaks or foci of infection encountered rather than on the basis of number of cultures obtained is obvious. However, the necessary epidemiological information was not always supplied. Table 2 shows the distribution of phage types based both on the number of cultures studied and on the number of foci of infection. The latter were determined as accurately as possible from the data received after all cultures from sewage were eliminated. It is evident that regardless of whether cultures or outbreaks are considered, untypable cultures (not lysed by any of the typing phages used) constituted a large percentage of the total number involved. The phage type encountered most often in outbreaks of *S. paratyphi B* infection was 3aI, followed in order by 3b, Beccles, 1, 3a, and Jersey. The Taunton, B.A.O.R., and Dundee types were found much less frequently. Neither the monophasic nor diphasic state of the flagellar antigens, the reactions of the organisms in D-tartrate medium, nor the zoological

TABLE 2

Frequency Distribution of Phage Types of S. paratyphi B in the United States

	Phage Types											
	1	2	3a	3aI	3b	Jersey	Beccles	Taunton	B.A.O.R.	Dundee	Untypable	Total
	Cultures of Each Type											
Number	50	0	19	70	135	8	45	4	2	4	156	493
Per cent	10		4	14	27	2	9	0.8	0.4	0.8	32	100
	Outbreaks Due to Each Type											
Number	27	0	12	53	42	8	27	4	2	4	108	287
Per cent	9		4	18	15	3	9	1.4	0.7	1.4	38	99.5

TABLE 3

Phage Typing Reactions of Cultures of S. paratyphi B var. odense (I, IV, XII: b-1, 2)

	Phages										
Cultures *	*1*	*2*	*3a*	*3aI*	*3b*	*Jersey*	*Beccles*	*Taunton*	*B.A.O.R.*	*Dundee*	*Type*
1031	—	—	CL	CL	< CL	±	< CL	< CL	CT	++++	3a
1162 †	CL	CL	CT	CT	—	CL	—	—	—	< CL	1
3771	—	—	—	—	—	—	++++	++++	—	< CL	Beccles
5538	—	—	CP	< CL	++++	—	++++	++++	< CL	++++	3a
8085	—	—	CL	CL	CT	±	< CL	< CL	CT	++++	3a
8653	—	—	CL	CL	< CL	±	< CL	< CL	CT	++++	3a
9864	—	—	—	—	—	—	++++	++++	—	< CL	Beccles
Hval	—	—	—	—	—	—	—	< CL	—	< CL	Taunton
Oxrud-Tesdal	CL	CL	CL	CL	—	CL	++++	++++	—	++++	1

* All cultures failed to ferment D-tartrate. All diphasic except 1162.
† Nonmotile
See legend of Table 4 for explanation of symbols

source of the strains was correlated with the results obtained by phage typing. The proportions of typable and untypable strains in all categories were approximately the same.

ACTION OF S. PARATYPHI B TYPING PHAGES ON ODENSE VARIANTS

An unfortunate controversy[5, 6] concerning the nomenclature of the heat-labile somatic antigenic component, usually found in *S. paratyphi B* cultures, has resulted in confusion in regard to the host-phage relationship. Since most workers view Vi antigens as components serologically identical with, or very closely related to, the antigen so labeled in the typhoid bacillus, it is distressing to find that the serologically unrelated V (five) antigen of *S. paratyphi B* is sometimes referred to as Vi antigen.[5] Evidence is offered here that the V antigen of *S. paratyphi B* (or "Vi" antigen of Felix and Callow) has very little if any connection with the results obtained by phage typing. Moreover, it is shown that the typing phages are not specific for *S. paratyphi B* but attack other Salmonella serotypes which may or may not have similar somatic antigens. Table 3 shows the results of phage typing of nine strains of *S. paratyphi B* var. *odense* (I, IV, XII: b-1, 2) which are devoid of V (five) antigen.[7] * All were successfully typed. In addition, nine cultures, free of V (five) antigen, were received among those sent in for routine serological typing. Six of these proved to be untypable; one was type 3b; one was type 3aI; and the third was type 1. Thus, the absence of V (five) antigen, detectable by serological means, does not preclude the successful application of phage typing procedures to *S. paratyphi B.*

SUSCEPTIBILITY OF OTHER SALMONELLA SEROTYPES TO THE PHAGES OF S. PARATYPHI B

The typing phages are not specific for the V (five) antigen or even for *S. paratyphi B.* Two hundred and thirty-seven cultures, representing 23 serotypes and seven somatic groups, were tested by applying five typing phages at 10 times the concentrations recommended for

* The nine cultures of *S. paratyphi B* var. *odense* were supplied by Dr. F. Kauffmann.

TABLE 4

Action of Typing Phages for S. paratyphi B on other Salmonella Types

Cultures	Phages at 10 × CTC				
	1	*2*	*3a*	*3aI*	*3b*
S. typhi murium 4146–49	—	—	—	—	CT *
S. typhi murium 2212–49	—	—	—	—	+ *
S. typhi murium 2410–49	—	—	+ *	+	—
S. abortus equi 2278–49	CP	CP *	CP	CL	—
S. miami 2813–49	CT	CT *	CT *	CT *	—
S. gallinarum 170–50	CL *	CL	CL	CL	CL
S. gallinarum 2923–49	CL	CL	CL	CL	CL *
S. pullorum 2308–49	—	2 +	—	+	CT *
S. pensacola 4482–50	—	—	—	—	2 + *
S. blegdam 127–49	—	—	—	—	± *

Legend:
± to 4 + = Increasing number of plaques
CP = Confluent Plaques
CT = Confluent Translucency
* = Selected for adaptation to respective culture
< CL = Less than confluent but more than 4 + lysis

routine identification. Seventy cultures, representing 13 serotypes and four somatic groups, were lysed by one or more phages to degrees ranging from a few plaques to confluent lysis.

Table 4 shows the degree of lysis of 10 of these cultures by standard typing phages applied at 10 times their critical test concentrations. As indicated in the table, certain phages were selected for further adaptation to the heterologous serotypes using the methods described by Craigie and Yen.[8] Preparations were obtained which produced confluent lysis at dilutions of phage ranging from 10^{-1} to 10^{-5}. Each adapted phage was adjusted to its critical test concentration for the organism on which it had been propagated. The reactions given by these phages on the type cultures of *S. paratyphi B* were then compared with those given by the original typing phages. The data which are presented in Table 5 illustrate, for the most part, a close relationship between the adapted and unadapted phages in respect to lysis of the type cultures. The evidence supports the contention that the adapted phages actually are the typing phages and not symbionts which became predominant during the course of the experiments. The identity of the adapted phages and the typing phages is substantiated by the results of inactivation tests with antiphage sera prepared for each of the *S. paratyphi B* typing phages. Parallel tests on adapted and unadapted phages, following the method of Craigie and Yen,[9] were performed simultaneously with control tests in which each phage was titered on its homologous host.

DISCUSSION

A comparison of the distribution of phage types of *S. paratyphi B* in the United States with that reported from other countries reveals several interesting points. For example, no type 2 cultures were identified among the 493 cultures typed, a finding which is in agreement with the reported absence of

TABLE 5

Lysis of Type Cultures of S. paratyphi B by Adapted and Unadapted Typing Phages

Phage *	Adapted to	Phage Type Cultures						
		1	2	3a	3aI	3b	B	T
1	*S. paratyphi B* Type 1	CL †	—	—	—	—	—	—
1	*S. gallinarum* 170–50	SCP	—	—	—	—	—	—
2	*S. paratyphi B* Type 2	CL	4 +	—	—	—	—	—
2	*S. abortus equi* 2278–49	—	—	—	—	—	—	—
2	*S. miami* 2813–49	SCP	3 +	—	—	—	—	—
3a	*S. paratyphi B* Type 3a	CL	—	CL	CT	—	—	—
3a	*S. typhi murium* 2410–49	4 +	—	SCP	CT	—	—	—
3a	*S. miami* 2813–49	4 +	—	CL	SCT	—	—	—
3aI	*S. paratyphi B* Type 3aI	CL	—	CL	CT	—	—	—
3aI	*S. miami* 2813–49	±	—	CP	SCT	—	—	—
3b	*S. paratyphi B* Type 3b	—	—	CT	—	CT	—	—
3b	*S. typhi murium* 4146–49	—	—	+	—	+	—	—
3b	*S. typhi murium* 2212–49	—	—	2 +	—	2 +	—	—
3b	*S. gallinarum* 2923–49	—	—	—	—	—	—	—
3b	*S. pullorum* 2308–49	—	—	—	—	—	—	—
3b	*S. pensacola* 4482–50	—	—	2 +	—	2 +	—	—
3b	*S. blegdam* 127–49	—	—	4 +	—	4 +	—	—

* Used at critical test concentration for host
† See legend of Table 4 for explanation of symbols not given below
SCP = Semi-Confluent Plaques
SCT = Semi-Confluent Translucency

this type among 1,372 cultures from France and Algiers [2] and among 520 cultures from Canada.[3] Felix and Callow [1] found type 2 in only 6.4 per cent of the foci from which cultures were received in Great Britain. Types Taunton and Dundee were reported to be the etiological agents in 86 per cent of the foci of infection studied by Nicolle, *et al.*,[2] but were present to the extent of only 18.5 per cent in Great Britain. In our series, the Taunton, Jersey, B.A.O.R., and Dundee types were rarely encountered.

Nicolle, *et al.*, were unable to type about 0.65 per cent of their cultures with eight phages, while Felix, *et al.*, reported that 11.6 per cent of the outbreaks in Great Britain during the period 1942 to 1950 were produced by organisms untypable by the five phages then in use. In the Canadian series, 15 per cent of persons examined were excreting untypable cultures and 20 per cent of all cultures were untypable with five phages. In the United States about 32 per cent of all cultures were untypable with 10 phages, and untypable cultures were obtained from approximately 38 per cent of the outbreaks. The percentage of typable cultures among recently isolated strains and among cultures which had been kept in the laboratory for several years was approximately the same. Thus, it appears that a far greater number of refractory *S. paratyphi B* cultures are found in the United States than occur

in Great Britain or France. Type 1 was encountered only rarely by Nicolle, *et al.*, in France, whereas this is the type most frequently found in Great Britain and ranks in intermediate positions in the United States and Canada. Types 3aI and 3b occurred most frequently in our series, and were common also in Canada. Type 3b has been rarely reported in Great Britain, and neither type 3aI nor type 3b is commonly found in France. As was expected, the frequency distribution of phage types of *S. paratyphi B* in the United States resembled more nearly that of Canada than that of Great Britain or of France.

The application of the standard phages to the type identification of *S. paratyphi B* cultures devoid of V (five) antigen (referred to by Felix as "Vi" antigen) demonstrates that these phages are not specific for this antigen. The ability of some of these phages to lyse other Salmonella serotypes and to be propagated to a high titer on these cultures offers additional evidence of the lack of specificity for *S. paratyphi B*. Phages prepared by one to five serial single-plaque selections on the new culture largely retain the original pattern of lysis for *S. paratyphi B* type cultures and are serologically identical with, or very closely related to, the parent phage. These facts seem to us to constitute sufficient evidence that we were not dealing with symbiotic phages which had replaced the original ones during adaptation.[1, 10, 11]

SUMMARY

Over 500 cultures of *Salmonella paratyphi B* from man and animals were subjected to complete serological analysis and were classified as to source, state of flagellar antigens, and ability to ferment D-tartrate. The human cultures were classified, as far as possible, on the basis of the clinical condition of the individual from whom they were isolated.

These cultures were subjected to phage typing using 10 phages distributed by the International Central Reference Laboratory. About 32 per cent of these were untypable. The decreasing frequency of occurrence of the various types was as follows: 3b, 3aI, 1, Beccles, 3a, Jersey, Taunton, Dundee, and B.A.O.R. Type 2 was not found.

Attention is called to the confusion resulting from the use of the term "Vi" to describe the V (five) antigen of *S. paratyphi B*. Evidence is presented to show that the typing phages for this organism are not specific for strains containing V (five) antigen nor even for *S. paratyphi B*. These phages were propagated to high titer on other Salmonella serotypes, and such preparations not only retained their serological characteristics, but, with minor exceptions, retained also the patterns of reaction associated with the parent phages. It is concluded that these phages do not have the high degree of specificity for *S. paratyphi B* which exists between the Vi typing phages of *Salmonella typhi* and their host cells.

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The Journal 25 Years Ago

NO LONGER OLD AT FIFTY

With the lengthening of the human life span in the last quarter of a century, there has been a corresponding lengthening of the definition of youth and middle age. Thus it is a bit surprising to find that only 25 years ago it was necessary to object to 50 years as retirement age.

In an article in the *Journal* of December, 1928, on "Industrial Fatigue," the late Eugene Lyman Fisk, M.D., medical director of the Life Extension Institute, said, "It is impossible to consider this question of fatigue without being at once led into the question of the longevity of the worker, his physical efficiency at the various decades of life, and therefore of the final problem of why the worker in industry is assumed to be more or less a liability at middle life and later. There is a certain naivete in so carefully discussing all of these fatigue states as a reflection of work itself and then accepting the dictum that the worker suddenly, without previous ill health, becomes at age 50 (on the average) a liability in the plant.

"Secretary of Labor Davis has bitterly deplored this traditional attitude toward the man of 50 and speaks of the time when at that age it was customary to give a man a gold-headed cane and gracefully bow him out of the plant. Of course a broader view is taken of this question now and in many plants there is a real effort made to adjust every kind of work to the older workers who have been highly trained and who have served their employers faithfully. This is a very wise policy as far as it goes, but a very unwise policy if it stops there. Through a right approach the need for adjustment of working conditions to the elderly man will be lessened, or rather the age at which such adjustment is considered important will be pushed forward and the liability point fixed nearer 60 than 50." And today we are objecting to 65 as the "liability point."

Dr. Fisk's entire article foreshadows some of today's thinking. He suggests that fatigue is not a wholly unmixed blessing but a great stabilizer of health. He believes the intrinsic factors of fatigue should be given more study. Says he, "So comparatively negligible a factor as the color of the walls in a work room has been given serious consideration, while the possible influence of such definite conditions as septic tonsils or defective vision as a factor in fatigue has been given little attention. If more than half of the people in such a room have faulty vision, uncorrected—as we find to be true in average groups—which is more important, to seek out this faulty vision and correct it or merely to mitigate the fatigue of these strained eyes by changing the color of the walls?

". . . The possible effect of bowel sluggishness in inducing fatigue is worthy of more comprehensive study. Yet constipation is only one among manifold possible intrinsic factors influencing the efficiency and productivity of the employee."