EPIDEMIOLOGICAL INVESTIGATIONS OF TYPHOID AND PARATYPHOID FEVER IN JAPAN WITH AID OF THE PHAGE TYPING METHOD

I. DISTRIBUTION OF PHAGE-TYPES OF *SALMONELLA TYPHI* AND *SALMONELLA PARATYPHI B* IN JAPAN, 1956-1965

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SUMMARY : In order to elucidate the typhoid and paratyphoid B phage type distributions in Japan, during the period of 1956-1965, a total of 1,808 typhoid cases from 1,002 foci and 114 paratyphoid B cases from 87 foci were submitted for phage typing of their infected organisms, and the following results were obtained.

1. The typhoid strains tested fell into at least 30 Vi-phage types. The sequence of frequency as counted by foci was as follows : types D2 (20 %), M1 (13 %), E1 (12 %), A (7.8 %), N+D1 (7.4 %), D1 (2.9 %), D6 (2.8 %), B2 (2.7 %), 39 (1.5 %), H (1.1 %), J1 and 46 (0.80 %); C5, L1 and 41 (0.50 %); B1, B3, C4, D4, D10, E2, E4, E9, L2, T, 28, 29, 36, 38 and 47 (less than 0.50 %). Distribution of typhoid Vi-phage types in Japan was characteristic of preponderance of types D2 and M1.

2. Of the foci studied, 5.3 % could not be assigned that their causative organisms belonged to any hitherto recognized Vi-phage type.

3. The prevalent typhoid Vi-phage types, D2, M1, E1, A and N+D1 were evenly encountered throughout the last ten years and distributed among almost throughout the country.

4. Some of the rarely recognized typhoid Vi-phage types were indigenous to the relatively limited areas.

5. Three new Vi-phage types, 39, 41 and 47 were identified.

6. Paratyphoid B phage types encountered were as follows : Types 3a (49 %), 1 (33 %), 3b (5.7 %), Dundee (2.3 %), 3aI (1.1 %) and Beccles (1.1 %). The remainders could not be assigned to any known type.

INTRODUCTION

Craigie and Yen (1938a, 1938b) reported that *Salmonella typhi* could be differentiated by means of certain specific Vi-phage preparations, and that the types thus differentiated were stable even after repeated natural passages through human beings. They also suggested that the method might, therefore, be of value as one of the epidemiological tools. Thereafter, the method was extended by Felix and Callow (1943) to

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Salmonella paratyphi B. The phage typing methods of *S. typhi* and *S. paratyphi* B for epidemiological purposes are now generally recognized to be employed as valuable aids for the control of typhoid and paratyphoid fevers.

In 1947, the need for the standardization of the technique was suggested by Craigie and Felix and the proposal was adopted by the International Committee for Enteric Phage Typing which was organized as one of the special committees of the International Association of Microbiological Societies. Since then, the survey of the distribution of typhoid and paratyphoid B phage types has been conducted on a worldwide scale by the Committee with the cooperation of the national laboratories for enteric phage typing of various countries participating in the International Scheme for Enteric Phage Typing by the internationally standardized technique. The authors have also been participating in this program in Japan since 1956, when the National Laboratory for Enteric Phage Typing was established in the National Institute of Health, Tokyo. During the past ten years, epidemiological investigations of typhoid and paratyphoid fevers have also been performed in our laboratory with the aid of phage typing procedure of the causative organisms, and the results achieved are considered to have somehow contributed to the control of enteric fever epidemics in Japan especially to the detection of the infective sources of some outbreaks.

The purpose of the present paper is to present the status of distribution of the phage types of *S. typhi* and *S. paratyphi* B in Japan, as a background of the epidemiological investigation of enteric fever. This result is concerned with the cultures obtained during the period from 1956 to 1965. Descriptions are also made of the three new typhoid phage types which have been isolated during the course of this study.

MATERIALS AND METHODS

Cases investigated and strains tested: Cultures of *S. typhi* and *S. paratyphi* B were collected throughout the country with cooperation of the public health laboratories and hospitals for infectious diseases. During the period from 1956 to 1965, a total of 1,808 typhoid cases, 1683 patients and 125 carriers, from 1,002 foci were investigated, and the cultures isolated from them were tested by the phage typing. At the same time, some relevant data concerned with the cultures, such as the onset of the disease, the name, age, occupation and address of the patient, the date of isolation of the cultures and the source of the isolation was carefully collected.

These cultures were submitted to phage typing as soon as possible after they had been received. If necessary, they were kept on Dorsett's egg-medium or in the lyophilized state after finishing phage typing.

One hundred and fourteen paratyphoid B cases from 87 foci were also gathered to be examined.

Typing phages used and typing technique: The typing phage set which was received from Dr. Anderson, International Reference Laboratory for Enteric Phage Typing, London, were used throughout the investigations. Because the preparations received were of the concentration 100 times stronger in lytic titer than that of the routine test dilution (R.T.D.) as indicated by Anderson, they were diluted to 1 R.T.D. with broth when used routinely. The titer of diluted phage preparation was frequently checked according to the lytic activity against the cultures type A and the corresponding type.

The typing technique described by Anderson and Williams (1956) was followed. In the early stage of the investigations all the 45 typing phages on hand were used for

routine typing procedure, but since at the present, the number of phage type for *S. typhi* is as large as 78 so far, it is very time-consuming to apply the full set of the phage preparations to each test culture. In order to simplify the routine work, therefore, cultures were first tested with the set of selected phage preparations. The set now in use consists of the following phages: A, B2, C2, C5, D1, D2, D5, D6, E1, E2, E4, H, J1, L1, L2, M1, N, O, T, 28, 39, 41, 46, O-1, O-2, O-3, Vi-I and Vi-IV. Most of them are the types known to be prevalent in this country according to our experience obtained during the early part of the investigation, while some of them were used in order to distinguish the prevalent types from the ones which showed confusing cross-reactions. More than 73 % of the cultures tested were identified with one of these types by using this combination of the phage preparations, while all the phage preparations were employed for testing the remaining ones. There were some cultures which were not identified with any of the known types, though all the phage preparations were applied. Such cultures have been sub-divided into three groups. The cultures of the first group were totally refractory to all the Vi-phage II derivatives available, though they were sensitive to Vi-phages other than II. The cultures were referred to as "untypable Vi-strains". Secondly, some cultures were more or less sensitive to a large number of the typing-phage preparations and showed various degrees of lytic reactions to them, so that it was unable to identifying them with one of the known phage types. Typhoid strains reacting to the typing phages in this way were designated as "degraded Vi-strains". Among them, the strains which showed the heavy cross-reactions of wide range approaching the lytic pattern of type A were especially designated as "A degraded". Finally, if a culture did not possess the Vi antigen, the type of the strain was of course unable to be determined by this method. The term "Vi negative strains" was reserved for such strains.

The cultures of type N which showed heavy cross-reaction with the D group of phage were called as type "N+D1" according to Nicolle, Hamon and Edlinger (1953).

Phage typing of *S. paratyphi* B was conducted according to the procedures described by Anderson and Williams (1956). Typing phage preparations which had been forwarded to this laboratory from the International Reference Laboratory were used at a dilution of R. T. D.

Calculation of the frequency distribution of phage-types: The frequency distribution of phage types was calculated by counting the number of foci due to the infection with a given phage type, and not by the number of cases or cultures examined. As Felix (1951, 1955) had repeatedly emphasized, a true picture of the relative frequencies of types in various localities, or in a given locality over a period of years can be obtained only by counting the number of foci of infection. However, it was not always possible to decide whether or not the cases occurred in a given district within a limited period of time having the same focus. Such cases, of which epidemiological relationship from each other was unknown or doubtful were counted as a separate focus.

Identification of a new Vi-phage type: Attempts were made to adapt Vi-phage II to the untypable Vi-strains. Logarithmically growing phase of the culture, on which the phage was to be propagated, was spread on the surface of infusion agar plate and allowed to dry. Typing phage A was applied to this culture in decimal dilutions. The plate was incubated overnight at 38.5 C, and several discrete plaques, if any, were cut and immersed into 2 ml of broth. The host cells were then killed by heating at 56 C for 40 minutes, after which the suspension was titrated on the same host strain. Further purification by picking up a single plaque was made and titrated again as before.

Finally, a single plaque was picked up together with a small amount of the surrounding culture and emulsified in 10 ml of prewarmed broth, then the suspension was incubated at 38.5°C with an agitation until lysis occurred. It was then centrifuged, and the supernatant was heated to kill the remaining host cells. The titration of the newly propagated phage was carried out to determine its R. T. D. on the type A culture and on the corresponding Vi-type strain of *S. typhi* on which the phage had been propagated. Then the lytic pattern of the phage was tested against all the representative of Vi-phage type strains including the corresponding host strain, using a dilution of R. T. D. In order to detect the contaminated non-Vi-phages in the lysate, the phage was also tested in a much a higher concentration on *S. typhi* strain O 901 and the other several strains which were expected to be resistant to all the Vi-typing phages.

If the culture in question produced no plaque with phage A, similar attempts were made with other typing phages, especially with those which had shown some indication of lysing it.

RESULTS

Frequency of the Occurrence of Phage-types of Salmonella typhi

The frequency of occurrence of typhoid Vi-phage types in each of the ten years under observation is shown in Table 1, together with the total of the yearly sums. Totally, 30 Vi-phage types were detected among 1,002 foci examined during the investigations. The most prevalent Vi-phage type in this country was D2, which amounted to 200 foci and covered 20 % of the total foci examined. Types M1 (128 foci, 13 %), E1 (124 foci, 12 %), A (77 foci, 7.7 %) and N+D1 (74 foci, 7.4 %) was also encountered frequently. Following them were types D1 (29 foci, 2.9 %), D6 (28 foci, 2.8 %), B2 (27 foci, 2.7 %), 39 (15 foci, 1.5 %), H (11 foci, 1.1 %), J1 and 46 (8 foci, 0.80 %), C5, L1, and 41 (5 foci, 0.50 %). The types rarely encountered were as follows: B1, B3, C4, D10, E2, E4, E9, L2, T, 28, 29, 36, 38 and 47 (less than 0.50 %). Sixty foci, including 198 cases, were classified as "Degraded Vi-strains", and represented 6.0 % of the total foci examined. Most of them were "A degraded". Although the frequency of occurrence of this group was considerably high, the several subgroups of strains which respectively showed the characteristic lytic patterns were included in this group. Such lytic patterns of some of these were constant for strains that were epidemiologically related to each other. Description of the epidemic due to the type of this sort will be made in the following paper of this series.

The relative frequency of occurrence of "untypable Vi-strains" was 5.3 %. The number of internationally recognized Vi-phage types of *S. typhi* have been rapidly increasing during our investigations. Therefore, the stock cultures of the strains that had been untypable at the time of isolation, were later submitted to typing again whenever the preparations of newly recognized typing phages were received from the International Reference Laboratory. If a culture had been identified with one of the new typing phages, the culture was removed from the untypable group and put in the type subsequently identified. Types 39, 41 and 47 which had been found first in this laboratory were represented in the corresponding figures of 1956, 1957 and 1957, respectively, in Table 1, though these types were lately ratified by the International Committee. Full descriptions of these new types will be made later. The specific type which had provisionally been designated as "Kito" by the authors and appeared in the report of Nicolle (1962) have now been classified as type 46, because it was confirmed later in

Table 1. Frequency of occurrence of typhoid phage-types in Japan, 1956-1965

Vi-phage type	1956			1957			1958			1959			1960			1961			1962			1963			1964			1965			Total (1956-1965)			Vi-phage type
	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Average Per-centage*	
A	5	5	4.8	23	23	14	16	16	19	13	13	8.7	4	6	6.7	4	4	7.3	1	1	2.6	2	2	1.9	5	5	4.7	4	6	3.1	77	81	7.7	A
B1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	8	1.8	—	—	—	1	1	0.93	—	—	—	2	2	1.6	4	11	0.40	B1
B2	—	—	—	7	18	4.2	4	9	4.7	2	2	1.3	—	—	—	—	—	—	4	4	11	2	2	1.9	6	6	5.7	2	5	1.6	27	46	2.7	B2
B3	3	3	2.9	1	1	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4	0.40	B3
C4	—	—	—	1	1	0.60	—	—	—	1	1	0.67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	0.20	C4
C5	1	1	0.96	—	—	—	3	3	3.5	—	—	—	—	—	—	—	—	—	1	1	2.6	—	—	—	—	—	—	—	—	—	5	5	0.50	C5
D1	—	—	—	9	9	5.4	4	4	4.7	4	4	2.7	2	2	3.3	2	2	3.6	—	—	—	3	3	2.8	1	1	0.94	4	4	3.1	29	29	2.9	D1
D2	20	26	19	31	32	18	10	10	12	32	48	21	12	21	20	12	14	22	8	8	21	27	130	25	24	28	23	24	43	19	200	360	20	D2
D4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1.8	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	D4
D6	4	4	3.8	5	22	3.0	1	2	1.2	10	11	6.7	1	1	1.7	1	1	1.8	1	1	2.6	1	1	0.93	—	—	—	4	4	3.1	28	47	2.8	D6
D10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	2.6	—	—	—	—	—	—	—	—	—	1	2	0.10	D10
E1	10	10	9.6	23	23	14	9	14	11	20	23	13	3	3	5.0	5	8	9.1	5	5	13	17	17	16	14	18	13	18	22	14	124	143	12	E1
E2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.78	1	1	0.10	E2
E4	—	—	—	—	—	—	1	1	1.2	—	—	—	—	—	—	1	1	1.8	—	—	—	—	—	—	—	—	—	—	—	—	2	2	0.20	E4
E9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.94	—	—	—	1	1	0.10	E9
H	—	—	—	1	1	0.60	—	—	—	1	1	0.67	—	—	—	1	1	1.8	—	—	—	3	3	2.8	1	2	0.94	4	15	3.1	11	23	1.1	H
J1	—	—	—	2	2	1.2	3	5	3.5	—	—	—	—	—	—	3	3	5.5	—	—	—	—	—	—	—	—	—	—	—	—	8	10	0.80	J1
L1	—	—	—	3	3	1.8	1	1	1.2	1	1	0.67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	5	0.50	L1
L2	—	—	—	—	—	—	—	—	—	1	1	0.67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	L2
M1	24	40	23	19	115	11	7	8	8.2	18	20	12	6	6	10	6	6	11	1	1	2.6	10	10	9.3	12	30	11	25	28	19	128	264	13	M1
T	1	1	0.96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	T
28	—	—	—	1	1	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	28
29	1	1	0.96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	29
36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.94	—	—	—	1	1	0.10	36
38	—	—	—	2	2	1.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.94	—	—	—	3	3	0.30	38
39	1	4	0.96	1	1	0.60	—	—	—	2	2	1.3	1	13	1.7	1	1	1.8	1	5	2.6	2	34	1.9	1	60	0.94	5	119	3.9	15	239	1.5	39
41	—	—	—	4	4	2.4	—	—	—	1	1	0.67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	5	0.50	41
46	3	3	2.9	—	—	—	—	—	—	1	1	0.67	4	4	6.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	8	0.80	46
47	—	—	—	1	1	0.60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.10	47
N+D1	7	7	6.7	12	12	7.1	7	20	8.2	18	18	12	6	9	10	3	4	5.5	2	2	5.3	8	10	7.5	6	49	5.7	5	6	3.9	74	137	7.4	N+D1
Degraded Vi-strains	1	1	0.96	5	5	3.0	3	3	3.5	6	6	4.0	10	54	17	6	11	11	3	6	7.9	11	29	10	3	22	2.8	12	61	9.3	60	198	6.0	Degraded Vi-strains
Untypable Vi-strains	2	2	1.9	5	5	3.0	3	3	3.5	3	3	2.0	2	2	3.3	2	2	3.6	4	4	11	10	10	9.3	13	14	12	9	10	7.0	53	55	5.3	Untypable Vi-strains
Vi negative strains	21	21	20	12	12	7.1	13	13	15	16	16	11	9	9	15	6	6	11	6	6	16	10	10	9.3	17	17	16	10	10	7.8	120	120	12	Vi negative strains
Total number tested	104	129		168	293		85	112		150	170		60	130		55	73		38	46		107	262		106	255		129	336		1,002	1,808		Total number tested
Carrier		9			19			2			2			—			—			2				15			36			40		125		Carrier
Patient		120			274			110			170			130			73			44				247			219			296		1,683		Patient

* Percentage distribution counted by the number of foci.

this laboratory that these two types were indistinguishable from each other by our experiment using the Vi-II phage adapted to strain "Kito" and phage 46¹⁾. Therefore, this type was given an account in the column 1957 of Table 1, though the type was formally added in 1961.

The strains isolated from 120 foci turned out to be "Vi negative strains", which represented 12 % of the total foci tested.

As far as the fluctuation of occurrence rate of phage types is concerned, it could be stated that it appears to be very small if there is any. Especially, in the case of type D2, the commonest one, this tendency was clearly seen. The order of the types M1, E1, A and N+D1, which are also very common in our country have shown some yearly fluctuations. However, all these types were always prevalent as compared with other types. The other types which were found rarely appeared and disappeared from year to year.

Geographical Distribution of Phage-types of Salmonella typhi in Japan

Geographical occurrence of Vi-phage types of *S. typhi* is illustrated in Fig. 1. In the figure, the specific types detected in various administrative districts are marked using differentiable symbols for each type. As clearly seen there, the common types, viz. D2, M1, E1, A and N+D1, were widely distributed all over the country. Furthermore, these types predominated in every district, though their frequencies in various districts appeared somewhat different from each others.

Some of the infrequent types, D6, D1, 39, H and 46, also showed relatively wide-spread distribution, while many others were found in a considerably limited area or areas. For example, types E4, L2, T, 28 and 29 have not been encountered except in Tokyo; types E2, E9, D10 and 36 have been isolated only in Hyogo, Niigata, Mie and Osaka Prefecture, respectively. According to informations available, however, some patients or carriers from whom these types were isolated had a story of having had some relation with foreign countries or foreigners. Therefore, the possibility of some of these rarer types having been imported from a foreign country could not be excluded.

Table 2 shows a detailed comparison of the frequency distribution of typhoid phage-types in the two representative urban areas in Japan, Tokyo and Osaka, where survey of typhoid phage typing have been done relatively well because in these areas a collection of *S. typhi* isolates reached a significantly large number for comparative analysis. As is seen from the table, types D2, M1, E1 and N+D1 were also prevalent in both the areas, and the frequency of occurrence of these types coincided closely. However, the frequency of occurrence of type A in Osaka was significantly higher than in Tokyo. Types B2 and 39 were encountered in frequencies of 6.2 and 3.1 %, respectively, in Tokyo, while they have not been detected at all in Osaka.

Phage-types of Salmonella typhi Newly Isolated in Japan

Three new Vi-phage types of *S. typhi* were isolated in Japan during the period of

1) In addition to this conversion, the type designated as "Shigaraku" which also appeared in the Nicolle's report have been classified as "degraded Vi-strains" in this report. Furthermore, the figures presented in Table 1 concerning the results obtained during the years 1956 and 1957 are somewhat different from these which had been appeared in the Nicolle's report. These differences derived from the increases in the number of tested cases and from the further epidemiological informations concerning the foci under observation.

Fig. 1. Geographical distribution of phage-types of *Salmonella typhi* in Japan, 1956-1965.

the present investigation.

Type 39: The type to be described was isolated from a sporadic case in Hokkaido, in 1956; the strain was first designated as "Yakumo" because of its isolation place. The phage type of this strain could not be determined by the set of typing phages available at that time. The strain, however, showed minor reactions against phage T and several other typing phages. An attempt was made, therefore, to adapt Vi-phage to strain "Yakumo" according to the above mentioned procedure. A titration of the phage thus obtained by adaptation which would be designated hereafter "phage Yakumo" for the sake of convenience of description was then carried out. The lytic spectrum of the "phage Yakumo" against all the representative type strains was next examined in R. T. D. As shown in Tables 3 and 4, the phage showed a confluent

Table 2. Frequency of occurrence of typhoid phage-types in Tokyo and Osaka, 1956-1965

Vi-phage types	Tokyo			Osaka		
	Number of foci	Number of cases	Per-centage*	Number of foci	Number of cases	Per-centage*
A	20	24	6.2	28	28	17
B1	—	—	—	2	2	1.2
B2	20	39	6.2	—	—	—
B3	4	4	1.2	—	—	—
C4	—	—	—	1	1	0.62
C5	3	3	0.93	—	—	—
D1	6	6	1.9	6	6	3.7
D2	69	80	21	26	63	16
D4	1	1	0.31	—	—	—
D6	11	12	3.4	4	4	2.5
E1	39	51	12	14	17	8.7
E4	1	1	0.31	—	—	—
H	2	2	0.62	3	3	1.9
J1	2	3	0.62	5	6	3.1
L1	1	1	0.31	3	3	1.9
L2	1	1	0.31	—	—	—
M1	46	52	14	25	44	16
T	1	1	0.31	—	—	—
28	1	1	0.31	—	—	—
29	1	1	0.31	—	—	—
36	—	—	—	1	1	0.62
38	1	1	0.31	1	1	0.62
39	10	232	3.1	—	—	—
41	1	1	0.31	—	—	—
N+D1	24	72	7.5	11	11	6.8
Degraded Vi-strains	9	60	2.8	3	4	1.9
Untypable Vi-strains	13	13	4.0	8	9	5.0
Vi negative strains	35	35	11	20	23	12
Total number tested	322	697		161	226	

* Percentage distribution counted by the number of foci.

lysis on type A and "Yakumo", and produced various degrees of confluent lysis on the type cultures of B-group and types 0, 28 and 32; of course, the culture "Yakumo" was confluent only by its corresponding phage in R. T. D. These findings were confirmed by the International Reference Laboratory, and this type was reported to be called as type 39 internationally (Anderson, 1962).

Some time after the isolation of the "Yakumo" strain, three strains of *S. typhi* were recovered from carriers seemingly related to the patient from whom the strain "Yakumo" had been isolated. All of them proved to be of type 39. Besides these

cases, the strains of this type had been isolated only from one sporadic case in Tokyo in 1957 and from two carriers in Niigata Prefecture in 1959. In 1960, however, an epidemic caused by this type occurred in a northern part of Tokyo, Takinogawa area, and about 20 cases were found by the end of the year. After silence for two years, it recurred in 1963 and showed no tendency to subside until the end of 1965. In 1965, furthermore, the epidemic spread to a neighboring area, Ikebukuro. Thus, at present, type 39 have become one of the predominant types in Tokyo, although it was assumed that the patients due to type 39 had probably been infected from a common source. Two typhoid patients of type 39 were notified in Saitama and Ibaragi Prefecture in 1963 and 1964, respectively. Both the cases were also assumed to be epidemiologically associated with the infective source in Takinogawa. Descriptions concerning the epidemic in Takinogawa will be made in a following paper of this series.

Type 41: A strain was isolated from a sporadic case of typhoid fever in Hiroshima, in 1957, and tentatively designated as strain "Murakami". The culture showed no confluent lysis with any of the typing phage preparations. However, it produced a few plaques in 1 R. T. D. of many of the typing phage preparations, though the lytic pattern of the strain was not compatible to any of the hitherto recognized types. Then the Vi-II phage was adapted to the strain according to the method described above. The phage "Murakami" thus obtained caused the confluent lysis on the cultures type A and "Murakami", and some minor reactions on type B1 and B2 in 1 R. T. D. The lytic patterns of strain "Murakami" against typing phage set and the lytic spectrum of phage "Murakami" are shown in Tables 3 and 4, respectively.

The results were confirmed in the International Reference Laboratory, and the designation of "type 41" as new Vi-phage type was ratified by the International Committee for Enteric Phage Typing (Anderson, 1962).

Besides the strain "Murakami", four strains of the same type of *S. typhi* had been isolated, one in Hiroshima, two in Fukuoka in 1957, and one in Tokyo in 1959. No outbreak due to the type has been encountered in the country as yet.

Type 47: Another strain seeming to be of a new Vi-phage type was encountered, and named strain "Hasumi". It was isolated from a sporadic case in Gumma Prefecture, in 1957. As shown in Table 3, this strain had been refractory to all the typing phage preparations in 1 R. T. D. except to phage L1 to which it showed several scattered plaques. The adapted phage was prepared, therefore, according to the technique described above. As presented in Table 4, the newly adapted phage had the following host range: full reactions on the culture of type A and strain "Hasumi", major reactions on type B1, B3 and O, and trace reaction on type B2 in 1 R. T. D. The type was adopted as a new Vi-phage type "47" by the International Committee for Enteric Phage Typing (Anderson, 1963). As pointed out by Anderson the type strain of type 47 has lost its specificity rapidly during the preservation on Dorsett's egg medium, then the stock culture of the strain shows relatively wide-ranged susceptibility against the typing phages. No other strain of this type has been encountered as yet.

Frequency of Occurrence of Phage-types of Salmonella paratyphi B

The findings shown in Table 5 which indicates the frequency of occurrence of phage types of *S. paratyphi B* have been derived from a total of 87 foci, including a total of 114 patients, carriers or foods examined during the ten years, 1956-1965. Only the following four types seemed indigenous to Japan: types 3a (43 foci, 49%), 1 (29

Table 3. Lytic patterns of strain Yakumo, Murakami and Hasumi

Vi-phage preparation (R. T. D.)	Vi-phage strain		
	Yakumo (type 39)*	Murakami (type 41)*	Hasumi (type 47)*
A	—	—	—
B1	— (3)	—	—
B2	—	—	—
B3	— (1)	—	—
C1	—	—	—
33 (C2)*	—	—	—
30 (C3)*	—	—	—
C4	— (2)	+	—
C5	—	—	—
C6	ND	ND	—
C7	ND	ND	—
C8	ND	ND	—
D1	—	—	—
D2	—	—	—
D4	—	±	—
D5	—	±	—
D6	— (2)	±	—
D7	ND	ND	—
D8	ND	ND	—
E1	—	—	—
E2	—	—	—
E3	—	±	—
E4	—	±	—
E5	—	—	—
E6	—	—	—
E7	— (1)	±	—
31 (E8)*	—	—	—
T 4904 (E9)*	—	+	—
F1	— (2)	—	—
F2	— (3)	—	—
G	—	±	—
H	— (1)	—	—
J1	—	—	—
K (K1)*	—	—	—
L1	— (1)	—	+
L2	—	—	—
M (M1)*	—	—	—
N	—	—	—
O	— (1)	—	—
T	±	—	—
25	— (1)	—	—
26	—	—	—
27	— (2)	—	—
28	—	+	—
29	—	—	—
32	— (1)	—	—
34	— (1)	—	—
35	—	—	—
36	—	±	—
37	— (1)	—	—
38	ND	ND	—
Yakumo (39)*	CL	±	—
40	ND	ND	—
Murakami (41)*	—	CL	—
42	ND	ND	—
Hasumi (47)*	ND	ND	CL

Degrees of lysis: CL=confluent lysis; SCL=semi-confluent lysis; ≡=>120 plaques; ≡=120-81 plaques; +=80-61 plaques; +=60-41 plaques; +=40-21 plaques; ±=20-6 plaques; —=5-0 plaques (numbers in brackets indicates the numbers of discrete plaque); ND=tests were not done, because the corresponding typing phage preparations and specific type strains had not been available; * Formal designation at present.

Table 4. Lytic spectra of the Vi-II phage phenotypes produced from the strains in the preceeding table

Vi-phage strain	Vi-phage preparation (R. T. D.)		
	Yakumo (39)*	Murakami (41)*	Hasumi (47)*
A	CL	CL	CL
B1	SCL	±	±
B2	±	+	±
B3	±	—	CL
C1	—	—	—
33 (C2)*	—	—	—
30 (C3)*	—	—	—
C4	—	—	—
C5	—	—	—
C6	ND	ND	—
C7	ND	ND	—
C8	ND	ND	—
D1	—	—	—
D2	—	—	—
D4	—	—	—
D5	—	—	—
D6	—	—	—
D7	ND	ND	—
D8	ND	ND	—
E1	—	—	—
E2	—	—	—
E3	—	—	—
E4	—	—	—
E5	—	—	—
E6	—	—	—
E7	—	—	—
31 (E8)*	—	—	—
T4094 (E9)*	—	—	—
F1	—	—	—
F2	—	—	—
G	—	—	—
H	—	—	—
J1	—	—	—
K (K1)*	—	—	—
L1	—	—	—
M (M1)*	—	—	—
N	—	—	—
O	±	—	SCL
T	±	—	—
25	—	—	—
26	—	—	—
27	—	—	—
28	±	—	—
29	—	—	—
32	— (2)	—	—
34	—	—	—
35	—	—	—
36	—	—	—
37	—	—	—
38	ND	ND	—
Yakumo (39)*	CL	—	—
40	ND	ND	—
Murakami (41)*	—	CL	—
42	ND	ND	—
Hasumi (47)*	ND	ND	CL

For definition of reactions, see Table 3.

Table 5. Frequency of occurrence of paratyphoid B phage-types in Japan, 1956-1965

Phage type	Number of foci	Number of cases	Percentage*
1	29	47	33
2	—	—	—
3a	43	52	49
3aI	1	1	1.1
Jersey	—	—	—
Beccles	1	11	1.1
Taunton	—	—	—
B. A. O. R.	—	—	—
Dundee	2	2	2.3
Worksop	—	—	—
Untypable strains	6	6	6.9
Total number tested	87	114	
Carrier		11	
Patient		103	

* Percentage distribution counted by the number of foci.

foci, 33 %), 3b (5 foci, 5.7 %) and Dundee (2 foci, 2.3 %). The strains of types 3aI and Beccles were isolated from horse-meat imported from Argentina, in 1965. No patients or carriers infected with these types, however, were encountered up to the present in this country. The relative frequency of foci from which the "untypable strains" were isolated was 6.9 %.

DISCUSSION

It may be stated that the Vi-phage types of *S. typhi* which is found to be most commonly distributed in the world have already appeared among the collection first encountered by Craigie and Yen (1938a). According to the reports summarized by Felix (1955) and Nicolle (1962), the survey concerning the geographical distribution of typhoid Vi-phage types actually shows that the types E1 and A are the commonest types throughout the world. Nevertheless, Felix (1959) described in his report that "certain of the more recently identified types that are rare, or do not occur at all, in some parts of the world are of great importance in others". These facts were also confirmed by the findings presented in this paper. In Japan, the types E1 and A have also been encountered frequently, while the types C1 and F1 which have been frequently found in nearly all the European and North-American countries have not been detected in Japan so far as tested in this laboratory. More characteristic feature in the distribution of typhoid Vi-phage types in Japan was preponderance of the types D2 and M1. According to Felix (1955) and Nicolle (1962), the type D2 has already been known to exist almost all over the world in relatively minor frequency. Consequently, among the countries participating in the International Scheme for Enteric Phage Typing, Japan is the only country in which this type holds its place as the most prevalent typhoid Vi-phage type. On the other hand, it has also been shown in their reports that the

type M1 is more frequently found in some Asian countries, especially in Vietnam and Japan. In addition, Aoki *et al.* (1965) and Suh (1965) reported the predominance of the type M1 among the typhoid strains isolated in Korea. The value of frequency in Korea is hardly comparable with that in Japan presented here, because they calculated the frequency distribution on the basis of the number of cultures examined and not the foci found. Their findings, however, suggest that type M1 is also one of the common types in Korea. The authors have also encountered the two typhoid patients infected by the type M1 during their stay in Korea. Recently ten cultures isolated in Taiwan have been tested by the authors at an Aoki's request (1965), and strains of types D2 and M1 have been found among them. From these facts, it may be concluded, therefore, that preponderance of type M1 is highly characteristic among certain Far Eastern countries. Aoki *et al.* (1965) have also obtained the same conclusion with the birds'-eye view of the literature recorded by many workers. According to Nicolle (1962), and Dhayagud and Banker (1951), however, the type M1 has not been isolated in Philippines, Indonesia or India.

The types 39, 41 and 47 which had been identified first by the authors were also characteristic in Japan, though these types did not show so frequent occurrence. These types have not been known to exist in the other countries as yet.

On the basis of these geographical observations it could be stated, therefore, that the closeness of international relations are reflected in the appearances of typhoid phage type distributions. Moreover, it would be reasonable to consider from these observations that typhoid fever has, generally considering, a rather endemic characteristic and not so frequently imported from abroad.

Prior to establishment of the cooperation of the International Committee, phage typing of *S. typhi* had been carried out in Japan by Kawabe (1942) and Kodama, Hata and Ichibayashi (1948), using the typing phage preparations received from Yen, one of the originators of Vi-phage typing. Kawabe reported that 83, 29, 22, 14 and 10 strains out of 223 strains examined were types D2, E1, M, A and H, respectively. Kodama *et al.* showed that among 887 typhoid strains isolated in Tokyo-Yokohama area during a 3 year period, 1941-1943, the most prevalent type was D2 which amounted to 231. Besides this, types E1 (153 strains), B2 (125 strains), M (113 strains), A (69 strains) and H (50 strains) also encountered them. In addition to the above, Imamura and Brandis (1955) and Brandis (1955) also presented data of frequency distribution of Vi-phage types of *S. typhi* in Japan. Unfortunately, none of these investigators gave regard of as to whether their findings were based on the number of foci or cultures, and a considerable number of strains obviously originated from one focus may have possibly been included in their collections of the strains tested. From their data, therefore, it is difficult to know a true picture of the relative frequencies of phage types in Japan up to the time when the National Laboratory for Enteric Phage Typing was established in this Institute. However, on the basis of their findings and the results presented here, it could be supposed that types D2, M1, E1 and A continued holding their predominance at least for the past twenty-five years in this country.

The present investigations could not cover all the notified cases of typhoid fever in this country. The cultures examined, however, may be considered to present a reliable feature of frequency distribution of typhoid Vi-phage types. At least 30 of the known typhoid Vi-phage types could be found in such a sea-surrounded island country as Japan during the period of the present investigations. Furthermore, geographical comparison of the frequency distribution among some divided parts within the country suggests

that some difference exists among the different parts of the country, although it might be due partly to a relatively small number of cases tested in some districts. This fact supports the consideration that typhoid fever is, in principle, indigenous to a considerably limited area. As a matter of course, however, the outbreak could occur more extensively, if food contaminated by typhoid carrier was on a sales chart covering a wide area. The authors experienced in 1957 an outbreak which had a wide-spread distribution of infected cases in the area covering Hiroshima, Fukuoka and Osaka Prefectures. This outbreak was found to be due to contaminated oysters sent for sale to the three Prefectures mentioned above (1959).

All these findings in the present investigations will confirm that the phage typing method is very useful to trace the infective source in an outbreak of typhoid fever and to obtain a good feature of the epidemics.

The proportion of untypable strains is not so high and compares well with figures reported by others (Felix, 1955; Nicolle, 1962), it may still be expected that further new Vi-phage types will be found in future among such untypable strains. Virtually, identifications of the two strains which seemed to be hitherto unrecognized types is in progress.

The investigation for paratyphoid B phage type distribution also revealed that the relative frequency of the various types occurring in Japan was quite different from those found in Europe. Type "Taunton", the most prevalent type in Europe has not been encountered in this country as far as tested in this laboratory. The common types in this country, 3a and 1 have also been encountered frequently in Canada (Desranleau, 1950; Felix, 1955; Nicolle, 1962). However, it is uncertain whether or not the similarity in paratyphoid B phage type distribution between the two countries has any reasonable explanation.

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