THE SIGNIFICANCE OF Vi-PHAGE TYPES F1 AND F2 OF *SALMONELLA TYPHI*

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(With Pl. 16 and 1 Figure in the Text)

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INTRODUCTION

The subdivision of the typhoid bacillus into a number of types and subtypes by means of the adapted Vi phages introduced by Craigie & Yen (1938), has provided a reliable means of establishing the identity of strains responsible for outbreaks of typhoid fever and of tracing the origin of the outbreaks. The proved epidemiological value of this technique has led to its adoption by many countries as a routine aid in the control of typhoid fever.

The revised typing scheme of *Salmonella typhi* suggested by Craigie & Felix (1947) contained twenty-four types and subtypes. Five new Vi-phage types were identified by workers in different countries during the past 3 years (International Committee for Enteric Phage Typing, 1950). The present Vi-typing scheme, comprising twenty-nine types and subtypes, was published in full in a recent paper (Felix & Anderson, 1951*a*). Table 1 is an abbreviated version of this scheme.

It will be seen from Table 1 that there are various groups of the typhoid bacillus, each headed by a member which is sensitive to all the other typing phages of the group, in addition to being lysed by its own phage. Such groups are the D group, headed by Type D1, and the E and F groups. The least specific member of each of these groups, that is, the subtype reacting with the greatest number of phages,

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occupies a position in relation to the remaining member or members of the group analogous to that of Type A in relation to all other Vi types of the typhoid bacillus.

Types F1 and F2 were included in the original scheme of Craigie & Yen in 1938. Helmer, Kerr, Dolman & Ranta (1940) isolated both types from an acute case of typhoid fever. They concluded that the patient might have been infected from two sources, or that the change from Type F1 to Type F2 had occurred during the course of the infection. They attempted, unsuccessfully, to convert Type F1 into F2 by mouse passage.

Craigie & Felix (1947) pointed out that the epidemiological significance of subtypes F1 and F2 was still uncertain. Obviously, if they are readily interconvertible, there is nothing to be gained from their recognition as separate subtypes. If, however, they are stable under field conditions, useful information can be derived from their separate identification.

Table 1. *Reactions of certain Vi-phage types of* Salmonella typhi

Vi-type strains	Vi-phage preparations												
	A	D1	D2	D4	D5	D6	E1	E2	F1	F2	G	H	K
A	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL	CL
D1	—	CL	CL	CL	SCL	SCL	—	—	—	—	—	—	—
D2	—	—	CL	—	—	—	—	—	—	—	—	—	—
D4	—	—	—	CL	—	—	—	—	—	—	—	—	—
D5	—	—	—	—	CL	CL	—	—	—	—	—	—	—
D6	—	—	—	—	—	CL	—	—	—	—	—	—	—
E1	—	—	—	—	—	—	CL	CL	—	—	—	—	—
E2	—	—	—	—	—	—	—	CL	—	—	—	—	—
F1	—	—	—	—	—	—	—	—	CL	CL	—	—	—
F2	—	—	—	—	—	—	—	—	—	CL	—	—	—
G	—	—	—	—	—	—	—	—	—	—	CL	—	—
H	—	—	—	—	—	—	—	—	—	—	—	CL	—
K	—	—	—	—	—	—	—	—	—	—	—	—	CL

CL = Confluent lysis. SCL = Semiconfluent lysis. — = No plaques.

The Vi-phage reactions of Types F1 and F2 suggest that they are closely related to each other. If they had no ancestral relationship, they should not occur together in an outbreak with a frequency higher than that of any two of the other unrelated phage types of the typhoid bacillus endemic in the same area. It is known that, with the exception of certain well-defined associations such as that of Type D1 with Type D4, and of Type A with a number of specific types (see Craigie & Felix, 1947; Henderson & Ferguson, 1949; Felix & Anderson, 1951*a*), one type only is found in the overwhelming majority of typhoid outbreaks.

If Types F1 and F2 are found together with a higher frequency than that of any other two phage types, it can reasonably be inferred that this association is not accidental, but is due to a true hereditary affinity.

In examining the evidence available, it seemed likely that Type F1 arose by mutation from Type F2. This assumption was based on the observation by Craigie & Felix (1947) that mutation in the direction of non-specificity (i.e. the develop-

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ment of cross-reactions with heterologous typing phages) occurred in cultures of specific phage types. A study of Table 1 will show that the change Type F2→ Type F1 would conform to this pattern if its occurrence could be confirmed.

The approach to the problem was twofold: first, the phage-typing of cultures isolated in the field; secondly, attempts to induce the change Type F2→Type F1 or vice versa, in the laboratory.

I. EXAMINATION OF CULTURES ISOLATED FROM CASES AND CHRONIC CARRIERS INFECTED WITH TYPE F1 OR F2

This was conducted as follows:

(*a*) The direct examination of cultures of Types F1 and F2 submitted for phage-typing by outside laboratories.

(*b*) The examination of at least six single-colony subcultures from a plating of each strain on 'Difco' agar (Craigie & Felix, 1947).*

(*c*) The investigation of single-colony subcultures from patients or carriers.

The cultures in the last group were picked by pathologists in various parts of the country, from primary platings of faeces or urine on selective media such as deoxycholate-citrate or Wilson-Blair agar. It seemed probable that, if the two types were present in a patient, the selection of primary colonies in this way would provide a better chance of yielding both types than the examination of colonies from platings following enrichment in tetrathionate or sodium selenite broth, since, if one type had a survival advantage over the other during active growth, it might completely overgrow the other during enrichment.

(*a*) *Direct examination of Type F1 and F2 cultures*

In all cases where successive cultures were received from individual patients without the exercise of special precautions for the selection of single colonies from primary platings, the type, whether F1 or F2, remained the same throughout.

During the summer of 1948, an outbreak occurred at Kilcreggan, in Scotland, which gave a great impetus to the work. In the early phase of this outbreak, Type F1 was isolated from two and F2 from eight patients. As there was no reason to suspect more than one source of infection, it was assumed that the two types had originated from the same carrier. It was finally possible to incriminate a chronic carrier, Miss C., who had suffered from typhoid fever in 1895 and was now found to be excreting *Salm. typhi* in both urine and faeces. According to information received from Dr J. S. M. Gray, County Medical Officer, Renfrewshire, two girls—a niece and her friend—who were visiting Miss C. in 1926 contracted typhoid fever, from which the niece died. From then until the outbreak at present under discussion, no further cases could be attributed to Miss C. The majority of cases in the Kilcreggan outbreak—the total was under forty—were infected by drinking water from a stream contaminated by sewage from Miss C.'s cottage. The first two cultures from the faeces of Miss C. belonged to Type F1. However, it was obvious that such a finding could not be accepted as final, and, as will be described, extended investigations later led to the isolation of both types from this carrier.

* 'Bacto' dehydrated nutrient broth, 20·0 g.; sodium chloride, 7·5 g.; powdered New Zealand agar, 13·0 g.; distilled water to 1000 ml.

(*b*) *The examination of single colonies of Type F1 and F2 strains*

Most strains belonging to Type F1 or F2 received during 1948 and 1949 were plated on Difco agar, and six single colonies of each strain were picked at random and examined with the typing phages. Sixteen strains originating in different parts of the world were examined in this way. They comprised fifteen Type F1 and one F2. With each strain, the examination of single colonies yielded the same phage type as the original culture.

A number of possible conclusions could be drawn from these findings. Firstly, the cultures may have been homogeneous in type. Secondly, the number of colonies tested may have been inadequate, because in each culture the population density of the heterologous type may have been small. Thirdly, the culture as received at the Central Enteric Reference Laboratory may have been descended from a single colony picked from a plate, and therefore not truly representative of an infecting population of mixed types.

One exception to the findings quoted above occurred, but the circumstances involved were somewhat unusual, and judgement was reserved until the general problem had been more exhaustively scrutinized. Dr Z. Buczowski, of the State Institute of Hygiene, Branch Laboratory in Gdynia, Poland, sent to the Central Enteric Reference Laboratory a subculture of the type strain of Type F2 in 1949. He had received this strain from Prof. G. Olin of Stockholm in 1946, and the latter had received Craigie's original type strain from Dr Felix in 1945. Dr Buczowski had observed that his culture of this F2 strain reacted strongly with phage F1 as well as phage F2; that is, it seemed to behave as Type F1 rather than Type F2 (see Table 1). We were able to confirm this finding on the unplated culture.

The strain was plated on Difco agar, and six colonies were picked and tested individually with the typing phages. Four of the six were Type F1 and two Type F2. This strain, therefore, consisted of a mixture of Types F1 and F2, with a predominance of the former, which explained the heavy cross-reaction given by the unplated culture with phage F1. It was tempting to conclude that Type F1 had arisen spontaneously in the Type F2 culture, but as the possibility of laboratory contamination with Type F1 could not be excluded with certainty, a decision on the phenomenon was withheld until further work had been completed.

(*c*) *The examination of single-colony subcultures from patients and carriers*

When cultures received in the Enteric Reference Laboratory proved to belong to Type F1 or F2, laboratories sending these strains were asked to obtain at least six single-colony subcultures from primary platings on deoxycholate-citrate or Wilson-Blair agar. As has been indicated earlier, it was thought that substrains isolated in this way would be more likely to show whether the two types were present.

Table 2 illustrates such an investigation applied to the Kilcreggan outbreak; it shows that primary single-colony subcultures from fifteen patients were examined. Eleven yielded pure cultures of Type F1, and four Type F2.

This finding, that primary colonies all belonged to the same type, was repeated in all instances in which it was possible to obtain single-colony subcultures from

patients infected in Great Britain, and from four carriers of Type F 1—one infected in Germany, two in Poland, and one in England.

When primary colonies from the Kilcreggan carrier, Miss C., were examined, however, a different picture presented itself. A total of fifty-nine such colonies from her faeces and urine were examined. The result is shown in Table 3.

Of the fifty-nine single-colony cultures isolated from this carrier, twelve (20 %) belonged to Type F 1 and forty-seven (80 %), to F 2 (see Table 3).

Table 2. *Typing of single colonies isolated from patients in Kilcreggan outbreak*

Patient	No. of primary single-colony subcultures received	Source	Vi-phage type F1	Vi-phage type F2
H.	2	Faeces	—	2
McC.	3	Faeces	3	—
M.	5	Faeces	—	5
Mont.	4	Faeces	—	4
Hur.	3	Faeces	—	3
McD.	8	Faeces	8	—
	6	Urine	6	—
Mon.	3	Urine	3	—
Han.	2	Urine	2	—
W.	3	Urine	3	—
C.	8	Urine	8	—
O'H.	2	Urine	2	—
Sw.	10	Urine	10	—
D.	5	Urine	5	—
Hol.	4	Urine	4	—
G.	3	Urine	3	—

Table 3. *Typing of single colonies isolated from Kilcreggan carrier, Miss C.*

Source	No. of primary single-colony subcultures tested: Vi-phage type F1	Vi-phage type F2	Total
Faeces	11	31	42
Urine	1	16	17
Total	12	47	59

II. *IN VITRO* ATTEMPTS TO EFFECT A TYPE F1↔F2 TRANSFORMATION

Because most patients, even when infected by a carrier excreting both types, seemed to excrete only one or other type, it was assumed that conditions favouring the change from one type to the other were found only in the chronic carrier.

Although survival and multiplication of the typhoid bacillus are favoured by the constant exchange and replenishment of the bile in the gall-bladder of a faecal carrier, it is probable that there is a considerable population of organisms resident in the hypertrophied mucosa, in the granulation tissue and on the surface of calculi, whose environment is not optimal, and whose growth is not at all, or only spasmodically, logarithmic. Nevertheless, these organisms will contribute to the

total population of typhoid bacilli excreted by the carrier. It was conjectured that, if the change from Type F2 to F1 occurred spontaneously, the maintenance of Type F2 organisms under unfavourable conditions, such as those obtaining in the wall of the gall-bladder, might facilitate its detection.

Working on this premise, it seemed that the examination of ageing cultures offered the best possibility of detecting the phenomenon *in vitro*.

Experimental methods

Media. Two media were used, Difco broth* and sterile ox bile. These were bottled in 3 ml. quantities in 25 ml. screw-cap containers. Two series of cultures were maintained in parallel. In one, the containers were sealed with the screw-cap; in the other, with a cotton-wool plug. The two series were still further duplicated, one complete set of cultures being incubated at 37·5° C., while the other remained at room temperature.

When wool-plugged containers were used, the contents, which were depleted by evaporation, were periodically restored to the original volume by the addition of distilled water.

Six strains of the typhoid bacillus were used to examine the Type F2↔F1 change. A culture of each type isolated from Miss C. was included in these experiments, but the remaining four strains were of separate origin. Each strain was plated on Difco agar and twelve perfectly smooth colonies were selected and phage-typed individually. A pool of the twelve colonies was simultaneously seeded on to a Difco agar slope which was incubated at 37·5° C. for about 8 hr., and on two Dorset egg slopes, one of which was incubated at 37·5° C. for an indefinite period, the other at 37·5° C. overnight and stored thereafter at room temperature in the dark.

When it had been confirmed that all twelve descendant colonies of each strain belonged to the same phage type as the parent, the previously prepared Difco agar slopes of the pooled cultures of these colonies were seeded into the various liquid media, and maintained under the conditions already described. Platings were made from these media at intervals, and six single colonies were picked at random and phage-typed.

The experiments in this section were carried out in two groups. Group 1 began in November 1948 and continued until June 1949; group 2 began in February 1950.

Results of experiments in group 1

Table 4 summarizes the findings on these cultures. In all the tests listed in this table, therefore, the phage type of the single colonies tested remained the same as that of the parent strain from which they were descended.

The same was found in tests of six colonies, from platings after storage for 180 days, of the Dorset egg cultures inoculated with the same twelve colonies of each strain as were used for the inoculation of the liquid media.

The possibility that the change Type F2↔F1 had taken place in these cultures cannot, of course, be excluded, but if it had done so the proportion of organisms

* Nutrient composition identical with Difco agar.

of the heterologous type in each culture must have been low, and the sampling method of testing six colonies (amounting on each day of test to an examination of a minimum of forty-eight colonies of each strain) was not sufficiently sensitive to detect the change.

Results of experiments in group 2

The Dorset egg pooled 12-colony subcultures prepared at the commencement of the group 1 experiments and maintained at room temperature in the dark were plated on Difco agar after 444 days. As this series was originally designed as a simple repetition of group 1, twelve smooth colonies were phage-typed individually, and were simultaneously pooled on to Difco agar and Dorset egg slopes as described earlier. The agar slope cultures were subcultured into the broth and ox-bile bottles before the typing results of the single colonies were known. However, in the preliminary typing tests, one culture, no. 6 of Table 4, originally Type F2, proved by this time to be a mixture of Types F1 and F2. Of the twelve colonies phage-typed, two belonged to F1 and the remainder to F2. A further

Table 4. *Tests of six single colonies of strains of Types F1 and F2 maintained in Difco broth and ox bile at* 37° C. *and at room temperature*

Culture no.	Country of origin	No. of days of plating after original inoculation	Original phage type of strain	Phage type of single colonies
1	Poland	4, 18, 175	F1	F1
2*	Scotland	4, 18, 175	F1	F1
3	Poland	5, 19, 176	F1	F1
4	Germany	5, 19, 176	F1	F1
5*	Scotland	6, 20, 177	F2	F2
6	France	6, 20, 177	F2	F2

* Kilcreggan carrier.

plating of this culture gave a similar result, one out of twenty-four colonies belonging to Type F1, and the remainder to Type F2. A test of the Dorset egg culture from which these platings were made, showed that, instead of the negative result with phage F1 characteristic of Type F2, a heavy cross-reaction occurred, suggesting that the culture had partly changed into Type F1.

Subcultures were prepared of the Type F1 colonies isolated from the parent F2 culture, and were tested for type stability by methods later to be described; it was shown that the F1 subcultures did not revert to F2.

The remaining five strains were maintained in the various media and under the varying conditions described in the experiments in group 1; they were plated, and colonies were phage-typed, after 8 days. No change of type was found in any of these subcultures. Work on them was then suspended, as the observations on strain no. 6 had focused special attention on it.

Of the original twelve colonies selected for preparing the pooled culture of strain no. 6 for maintenance in the various media employed in the test, two belonged to Type F1 and the remainder to Type F2. It was known from the start, therefore, that a considerable proportion of Type F1 organisms were seeded into the media, and it was to be expected that platings from these media would yield

a mixture of both types. However, Type F1 was found only in the ox-bile culture in a wool-plugged container maintained at room temperature. Table 5 summarizes the findings in this test.

The colony designated '1?' under Type F1 in Table 5, gave a heavy cross-reaction with phage F1. This was the only occasion on which a single colony gave a reading of this nature, and it was concluded that the line it represented was either actively mutating from Type F2 to F1, or stemmed from at least two organisms, one of each type, lying sufficiently close to each other on the plate to give rise to a single colony. Platings from a Difco agar subculture of this colony (hereafter referred to as line M) yielded one-third of Type F1 colonies and two-thirds Type F2, which suggested that it had been formed from a population contributed by organisms of both types and not from a line intermediate in phage reactions between Types F1 and F2. Indeed, it was later possible to show that the change F2→F1 occurred in a single step and had no intermediate stage.

Table 5. *Phage type of single colonies of strain no.* 6 *after maintenance in the various media for* 8 *days*

(Original type of strain, F2.)

	Type of single colonies	
Medium and maintenance	F1	F2
Difco broth screw-cap; 37° C.	—	6
Difco broth wool-plug; 37° C.	1?	5
Ox bile screw-cap; 37° C.	—	6
Ox bile wool-plug; 37° C.	—	6
Difco broth screw-cap; room temperature	—	6
Difco broth wool-plug; room temperature	—	6
Ox bile screw-cap; room temperature	—	6
Ox bile wool-plug; room temperature	3	3

A Dorset egg culture of Craigie's original type strain of Type F2, prepared in London in 1940, and kept thereafter in the dark at room temperature without subculture until 1950, was tested without preliminary plating. It gave a heavy cross-reaction with phage F1. Of six colonies picked from a plating of this culture, four were Type F1 and two F2. The culture therefore consisted of a mixture of Types F1 and F2 and, incidentally, the observation by Dr Buczowski that the type strain of F2 in his possession had reacted strongly with phage F1 was explained.

A strain of Type F2, isolated from the Kilcreggan carrier, showed a similar change in a Dorset egg culture 22 months old.

It was thought that, if the change from Type F2 to F1 took place in a random fashion, it might be possible, by the serial subculture of a mixture of the two types descended from the same strain, to show a periodic increase in the proportion of Type F1 organisms as the multiplying population of F1 already present was added to by further mutation. An experiment of this nature was carried out as follows:

Line M of strain no. 6 was subcultured on to a Difco agar slope, and serial subcultures were carried out twice daily on this medium. Transfer to fresh slopes was effected by a very small loop. Platings were made each evening from the agar slope inoculated in the morning of the same day. The following morning,

twenty-four colonies were picked at random and tested for phage-type. At the same time, serial subcultures were made in an identical way of a pure Type F1 line and a pure Type F2 line descended from the common parent strain no. 6, and twenty-four colonies tested in the same way as colony M. Serial subcultures were designated by Roman numerals. The time interval between platings was 24 hr. The Difco agar slope inoculated in the morning was incubated for 8 hr., that inoculated in the evening for 16 hr. The results of these experiments are shown in Table 6.

Table 6. *Serial subculture of Types F1 and F2, mixed and separately*

(Origin: strain No. 6.)

Culture	Serial no. of subculture at time of plating	Type of single colonies	
		F1	F2
Colony M (=F1+F2)	I	8	16
	III	3	21
	VII	0	24
Pure F1 colony	I	24	—
	III		
	VII		
Pure F2 colony	I	—	24
	III		
	VII		

Table 6 shows that the proportion of Type F1 to F2 organisms in the mixed population of both types steadily diminished until, from a ratio of 8:24 in the first subculture, they constituted less than 1:24 in the seventh. Pure cultures of Types F1 and F2, derived from the same strain as line M, maintained their stability and purity during serial subculture. This experiment was repeated on a number of occasions with identical results; i.e. the proportion of Type F1 organisms in mixtures with Type F2 always diminished until F1 could not be found among twenty-four colonies from any one plating. The same phenomenon showed itself in serial subcultures of the 10-year-old culture of the original type strain of F2 which contained both types, and of the mixed culture of Types F1 and F2 which had appeared in the 22-month-old Dorset egg culture of an F2 strain isolated from the Kilcreggan carrier. It was also observed that Type F1 was eliminated whenever it was mixed with Type F2 and serially subcultured, even if the two types originated from different strains. Changing the medium in which propagation was carried out from Difco agar to Difco broth did not affect the disappearance of Type F1 from these mixtures, except to make it more rapid.

An alternative, rather shorter method than examination of single colonies with the typing phages was devised to demonstrate the proportion of organisms of each type present in cultures. Difco agar plates were prepared containing either phage F1 or phage F2 in a final dilution equal to the routine test dilution (R.T.D.) used for phage-typing. Cultures were first diluted with broth to contain about 1000 organisms per ml., and 10 drops seeded on to the phage-impregnated plate with a 50-dropper pipette and spread with a glass spreader. The plate was incubated overnight at 37° C. The inoculum yielded about 200 colonies to the plate.

Cultures of pure Type F1 plated on a medium containing phage F1 showed nibbling of all colonies by the phage, whereas pure Type F2 showed no nibbled colonies on such a plate. On plates containing phage F2, both types showed nibbling of all colonies. Mixed cultures of the two types, plated on a medium containing phage F1, presented a mixture of nibbled and entire colonies, the proportion of the former depending on the proportion of Type F1 variants present in the culture used.

Pl. 16 shows the effectiveness of this method.

III. THE QUANTITATIVE EXAMINATION OF THE ELIMINATION OF TYPE F1 FROM MIXED CULTURES OF TYPES F1 AND F2

It seemed possible that the disappearance of Type F1 from these mixtures was due to Type F2 growing more rapidly than F1, either because it had a shorter generation time, or because it possessed an advantage over Type F1 in competition for an essential metabolite. A growth experiment was devised to test this possibility.

Media

Difco broth in 2·85 ml. amounts was used throughout as the medium for maintaining the cultures in a logarithmic phase of growth. All broths were pre-warmed to 37° C. before inoculation.

Difco agar plates were used for bacterial counts, which were carried out by the method of Miles & Misra (1938), serial dilutions being performed in half-strength Ringer solution.

'Phage plates' consisted of Difco agar containing either phage F1 or phage F2 in a final dilution equal to the R.T.D.

Methods

The cultures used belonged to Types F1 and F2, isolated from strain no. 6. These were grown separately in Difco broth and standardized to an opacity of 200×10^6 organisms per ml.; equal quantities were mixed to form the starting-point for the serial subcultures of the mixture of F1 and F2. The remainder of the pure type cultures served to initiate the serial subcultures of the separate types. The method of subculture was to transfer 0·15 ml. of the incubated culture to 2·85 ml. of fresh broth at 37° C., thus giving a 1:20 dilution at each laboratory generation. The mixed culture and the separate cultures of the two types were counted at the start of the experiment, and at the termination of each 4 hr. period of incubation of subcultures. Incubation was carried out from 9 a.m. to 1 p.m., and on the next subculture from 1 to 5 p.m. The latter culture was then placed in the ice-box overnight, to be subcultured at 9 a.m. on the following day.

'Phage plates' were inoculated in the manner described earlier from dilutions prepared for the viable counts, the inoculum being so adjusted as to give 200–300 colonies per plate after overnight incubation at 37° C.

As the number of organisms present at the beginning and end of each subculture was known from the counts, it was possible, assuming the generation indices of the two types to be 2 during the logarithmic phase of growth, to establish the

number of generations traversed during each subculture. Making the further assumption that the organisms underwent no significant metabolic change after their overnight sojourn in the refrigerator, it was thought reasonable to regard the experiment from beginning to end as a continuous logarithmic phase of growth, and it was thus possible to estimate the total number of generations covered by the experiment.

Seventy-two generations were covered in this way. The actual counts are not given here, as it was impossible to show any difference between the generation times of the separate types and the mixture. However, inspection of the colonies of mixtures on the 'phage plates' revealed that the elimination of Type F1 from the mixture of F1 and F2 was extremely rapid (see Table 7).

Table 7. *The disappearance of Type F1 from a broth culture of a mixture of F1 and F2*

Serial no. of culture	Time of examination	Types (%) F1	Types (%) F2	No. of colonies inspected
I	(*a*) Immediately after preparing mixture	47	53	134
	(*b*) After 4 hr. incubation at 37° C.	12	88	154
II	0·15 ml. of I(*b*) to 2·85 ml. broth; incubated for 4 hr. at 37° C.	0	100	>200

As no difference could be found between the growth rates of Types F1 and F2 separately, and as a subsequent examination of the mixture after Type F1 had been eliminated showed that the growth rate of Type F2 had not been increased by its contact with Type F1, it could be assumed that the disappearance of Type F1 from these mixtures was not due to a detectable inequality in multiplication rates.

The puzzle was solved when further work showed that Type F2 always carries a phage to which F1 is highly susceptible. This phage, which we have called phage f2, can produce lysis of F1 and can also transform it into F2; both processes lead to the disappearance of F1. A preliminary note on this subject has already been published (Felix & Anderson, 1951*b*) and a fuller exposition will be given in a subsequent paper (Anderson & Felix, to be published).

DISCUSSION

The observation by Helmer *et al.* (1940) that Types F1 and F2 could occur in the same patient, suggested two possibilities to these workers. Firstly, the patient might have been infected from two different sources. Secondly, the transformation of Type F1 into F2 might have occurred during the course of the disease. A third possibility, that the patient might have been infected by a carrier excreting both types, is not mentioned by Helmer and his co-workers.

It seemed to us, as the phage-typing method suggested that there was a close relationship between the two types, that it was most likely that one type could mutate into the other, and for reasons given in the Introduction it was expected that F2 would change spontaneously into F1. There was a suggestion that the

change had already taken place in the subculture of the type strain of F2 sent from Poland by Dr Buczowski, but it was not until confirmatory evidence had been adduced that this finding was admitted to be genuine.

The point under consideration has more than academic importance; it has a direct epidemiological application. If it can be shown that under ordinary conditions both types are stable, their retention in the typing scheme is justified, irrespective of whether or not one type can be derived from the other. Not all carriers of Type F strains are excreting both subtypes; indeed, we have encountered this phenomenon on one occasion only (Miss C.). No instance has so far been encountered of the spontaneous change from Type F2 to F1 or vice versa in actively growing cultures consisting originally of one type. It would appear, therefore, that the two types are epidemiologically stable in most instances, and, as the information to be derived from their separate recognition is more than could be gained if they were merged into one, they should retain their claim to type rank.

The evidence so far available suggests that a carrier infected with a pure Type F1 strain will continue to excrete this type without change, while one originally infected with F2 will tend, after an unpredictable interval, spontaneously to produce F1 variants. The rare finding that a patient suffering from the active disease is excreting both types, does not suggest infection from more than one source. The isolation of F1 from one group of patients in an outbreak and F2 from the remainder again does not suggest infection from two different sources. If, however, a carrier is excreting F1 only, the occurrence in his neighbourhood of an F2 infection would certainly suggest a different source of infection and it could safely be assumed that another excreter existed who was carrying Type F2.

The experiments described have shown that conditions obtaining in old cultures of F2 are favourable for the appearance of the F1 variant. Interest lies in the fact that the difference between the two types is controlled by a phage, as was recently shown in a brief communication (Felix & Anderson, 1951*b*). This phage, it may be added, is an O- and not a Vi-phage. The Vi-typing phages render possible the detection of the phage-free F1 variants in an F2 culture.

The cycle connecting Types F1 and F2 is summarized in Fig. 1.

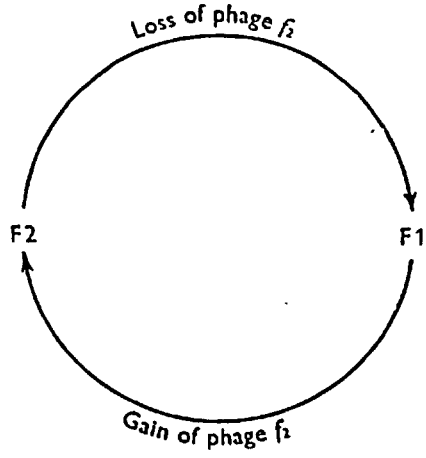

Fig. 1.

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SUMMARY

1. Type F1 will appear in old Dorset egg cultures of Type F2 after storage for months or years at room temperature in the dark.

2. It is probable that a similar change is responsible for the appearance of Types F1 and F2 in the same carrier and outbreak of typhoid fever.

3. The two types are stable when growing logarithmically in the pure state.

4. There is also evidence that the two types are stable under ordinary field conditions.

5. Logarithmic growth of F1 in the presence of F2 results in the disappearance of F1.

6. This change is caused by a phage carried by F2, which converts F1 into F2.

7. The significance of these observations is discussed. It is suggested that the two types retain their status in the typing scheme.

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REFERENCES

ANDERSON, E. S. & FELIX, A. (to be published).
CRAIGIE, J. & FELIX, A. (1947). *Lancet*, **1**, 823.
CRAIGIE, J. & YEN, C. H. (1938). *Canad. publ. Hlth J.* **29**, 448, 484.
FELIX, A. & ANDERSON, E. S. (1951*a*). *J. Hyg., Camb.*, **49**, 288.
FELIX, A. & ANDERSON, E. S. (1951*b*). *Nature, Lond.*, **167**, 603.
HELMER, D. E., KERR, D. E., DOLMAN, C. E. & RANTA, L. E. (1940). *Canad. publ. Hlth J.* **31**, 433.
HENDERSON, N. D. & FERGUSON, W. W. (1949). *Amer. J. Hyg.* **50**, 340.
INTERNATIONAL COMMITTEE FOR ENTERIC PHAGE TYPING (1950). *Proc., 5th Int. Congr. Microbiol.* Rio de Janeiro. (In the Press.)
MILES, A. A. & MISRA, S. S. (1938). *J. Hyg., Camb.*, **38**, 732.

EXPLANATION OF PLATE 16

Platings of Types F1 and F2, separately and mixed, on Difco agar containing phage F1.

Fig. 1. Type F1.
Fig. 2. Type F2.
Fig. 3. Types F1+F2.

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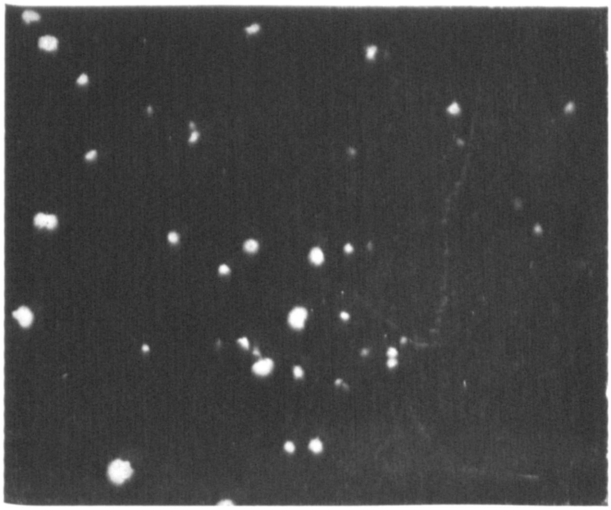

Fig. 1.

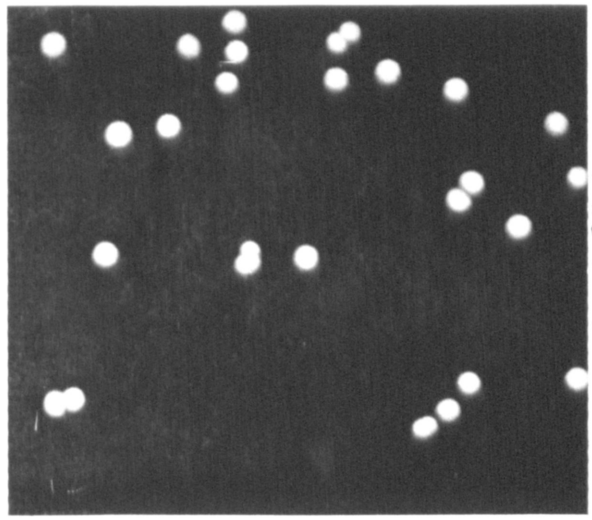

Fig. 2.

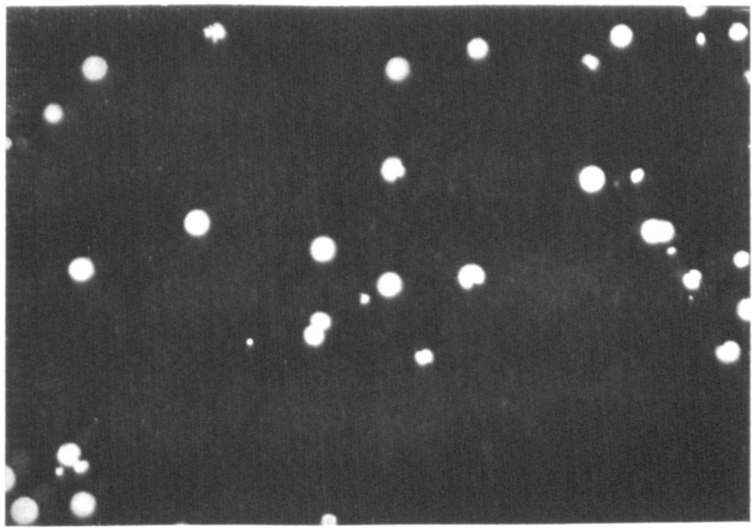

Fig. 3.

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