

CRAIGIE'S Vi PHAGE METHOD AND SOUTH AFRICAN STRAINS OF *B. TYPHOSUS*, WITH SPECIAL REFERENCE TO TYPHOID CARRIERS*

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In Pretoria for many years interest has been taken in epidemiology of typhoid fever and detection of carriers, and now includes typing of strains of *B. typhosus* by Craigie's Vi phage method (Craigie & Yen, 1938*a*). After fruitless attempts to isolate a type II Vi phage from Pretoria sewage, typing was undertaken by means of phages and type strains gratefully received from Dr J. Craigie and Dr A. Felix.

TECHNIQUE

Most strains of *B. typhosus* isolated from patients were grown from blood sent for routine diagnostic tests. The clot was placed in bile broth and incubated for 2 days. If bacilli grew, a loopful was planted out in a Petri dish on de Loureiro's modified Wilson and Blair medium (de Loureiro, 1942). The same medium was used for the inoculation of stool and urine specimens from all suspect carriers possessing Vi agglutinins (Pijper & Crocker, 1937, 1943; Pijper, Crocker & Todd, 1943). Strains of *B. typhosus* were identified by their biochemical reactions and by agglutination tests with a pure Vi and an O serum. For the typing of strains, the technique laid down by Craigie was followed (Craigie & Yen, 1938*a*; Craigie, 1941). The work was begun with phages and type strains for types C, E, F, G, H, J and L. Phages were prepared for some South African strains, and in this way the classification was extended. In March and August 1944 further material was received from Dr Felix. This included phages and type strains for types T, D 1, D 2, D 4, D 5, L 2 and 91 and phages for types D 3, B 1, B 2 and B 4.

TYPES OF STRAINS OF *B. TYPHOSUS* FOUND

Table 1 gives the number of strains subjected to typing, and the results.

Table 1

A	C	E	F	B 2	B 4	D 1	T	J.S.	Un- Rho- classi- desia	Total
195	2	41	13	63	2	8	3	3	21	144
									144	495

It is seen that strains from 495 individuals have been typed. Typing was begun in 1942. Out of the 495 strains, 246 were isolated by me from Pretoria cases or carriers, 104 strains were received from Cape Town, six from Durban, sixty-nine from the South African Institute for Medical Research in Johannesburg, and seven from their Port Elizabeth Branch. Fifty-four strains were received from Northern Rhodesia, five from Bulawayo, two from Nigeria and two from the Middle East.

Type A strains were isolated from five urinary carriers, from three intestinal carriers, twice from bile collected at a gallbladder operation, and from the blood of 185 cases. One of the urinary carriers was discovered and handed over to us by the Hygiene Section of the Union Defence Force. He has now been kept under observation for 3 years, and strains of *B. typhosus* were isolated from his urine on nine different occasions. Seven of these were typed and all proved to be type A strains. Two urinary carriers excreting type A strains were discovered during our routine Vi testing of dairy and other employees. One was seeking employment at a dairy and the other at the Pretoria Water Works.

In January 1943 there was a small outbreak of typhoid in Pretoria and twenty cases were notified. Strains were isolated from the blood of seven of these cases. The tracing of the carriers responsible for this outbreak will be described later. Another very large typhoid outbreak caused by a type A strain occurred at Brakpan in the Transvaal in November and December 1945. During this epidemic over 300 cases were reported. Sixteen strains were isolated from Brakpan cases and sent to me for typing. All proved to be type A.

Many of the cases from which type A strains have been isolated were sporadic and no carriers have been found. The two strains sent to me from Nigeria, by the late Dr Smith, proved to be type A. These had been isolated from native cases.

Type A is evidently widely spread in Southern Africa.

No type C strains have been isolated from any cases or carriers in South Africa. Strains isolated and

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handed to me by Dr Finlayson of the Baragwanath Hospital in Johannesburg from two patients who developed typhoid fever on a voyage from Egypt to the Union, proved to be type C strains. They had been infected outside the Union, probably in Egypt.

Of the forty-one type E strains typed since 1942, two were obtained from intestinal carriers, one from a urinary carrier, one was isolated at the S.A. Institute for Medical Research from a spinal fluid and the remainder were isolated from blood cultures or stool specimens from typhoid patients. One of the carriers had had typhoid 30 years ago, when living in England. Two persons in her household at that time had contracted typhoid and this led to the discovery that she was a carrier. She came to South Africa and intended to reside in Durban, but first visited Pretoria. She wished to know whether she was still a carrier and so a specimen of stool was examined. It still yielded a growth of typhoid bacilli. The other intestinal carrier excreting a type E strain was a native nurse who was discovered during the routine testing of applicants for employment at the Pretoria General Hospital. She gave no history of having had typhoid and no cases have been traced to her, but she had to abandon her profession. A type E strain sent from Port Elizabeth was isolated from a carrier who lived at Uitenhage, in the Cape Province. He had had typhoid many years ago, but it is not known whether he had caused any cases.

In February 1946 a small outbreak caused by a type E strain occurred at a Child Welfare Centre in Pretoria. In January some of the children from this centre, with the members of the staff, had camped at Parys, in the Free State Province. They returned to Pretoria on 15 January and during the first week in February eight of the children developed typhoid. Type E strains were isolated from the stools of five of the children and from one blood culture. Although the view was held that they had been infected from a local Parys source, routine blood tests were done on members of the staff, with the result that one European and one native gave positive Vi tests. Three stool and urine samples were examined from each, but typhoid bacilli were not isolated.

Type E seems to be widely spread over South Africa.

Thirteen type F strains have been isolated since 1942, of which nine were from Pretoria cases, three were from Port Elizabeth and one from Johannesburg. Seven of the type F strains isolated from Pretoria cases were biochemically atypical, in that they did not produce acid in maltose. Five of these strains were isolated during the period November 1942 to March 1943 from apparently sporadic and unconnected cases. The sixth strain was isolated in July and the seventh in November, both from patients in the Pretoria General Hospital.

I have traced only one article in which reference was made to a strain of *B. typhosus* atypical in maltose. This is a note by Coulthard (1939) on the maintenance of cultures for the Rideal-Walker test. He states that the Lister Institute culture which is supplied for the test does not ferment maltose, but that it appears capable of giving rise to colonies which produce acid in maltose. Dr Coulthard was asked for further information about this Lister Institute strain, which appeared to be similar to the strains isolated in Pretoria. He replied that it was a Rawlings strain. The Rawlings strain was the strain chosen by Craigie as the type F strain. Therefore, so far, all strains of which it is known that they do not ferment maltose belong to the F group. The strain however supplied to me by Craigie as the type F strain is a typical strain and does produce acid in maltose.

Obviously typhoid bacilli which can be identified by means of Craigie's method as type F, fall into two groups, those that ferment maltose and those that do not. It is remarkable that such aberrant strains which do not ferment maltose have only been found in group F. It is suggested that this finding, which allows further differentiation of F strains by a simple biochemical test, may prove useful in the epidemiological investigation of typhoid fever. Olitzki, Olitzki & Shelubsky (1945) have shown that type C strains can be divided into three subgroups by the fermentation of xylose and arabinose and the utilization of citrate and *d*-tartrate.

The F strain does not appear to occur frequently in South Africa.

When I started typing South African strains I could only identify strains belonging to types A, C, E and F. I encountered many Vi forms at that time, which did not fall into any of these types. Phages were prepared by me for some of these strains, the majority of which could be definitely identified later, when phages and strains for types B and D were received from Dr Felix. Strains from Port Elizabeth and Cape Town were then found to belong to type B2. One strain isolated from a chronic urinary carrier was found to belong to type B4. Felix has stated (private communication) that in Dr Craigie's revised scheme all subtypes of B may disappear and be included in A. Further advice regarding the position of these strains is awaited. Another phage was likewise prepared by me for a strain isolated from the blood of a patient living in the Ermelo district, in the Transvaal. This strain has now been found to belong to type D1. Six other strains belonging to this type have since been encountered.

Felix has reported a small outbreak of typhoid in Britain, caused by a carrier infected in South Africa more than 40 years ago. This strain was provisionally labelled type T (Felix, 1944), and had not been found in England before. The fact that it is indigenous in

South Africa, and had been transferred to England by the responsible carrier 40 years ago, was proved by the isolation of the same type strain by me from three cases in South Africa.

My strains labelled J.S. and 'Rhodesian' have not yet been identified with any of the established types. Some of the strains shown in the table as unclassified are imperfect Vi forms or W forms. Others are Vi forms and must be new types not found elsewhere. Phages are being prepared for some of these strains, in the hope that an exchange will be found possible later on.

PRACTICAL APPLICATION OF PHAGE TYPING

(1) During August 1941, there were three cases of typhoid at boarding house X in Pretoria. These were traced to a native waiter, J.S., who was found to be a urinary carrier. He was transferred to the Municipal carrier camp (Pijper & Crocker, 1937) but absconded after a few months. In November 1941 during the investigations of a case which occurred at another boarding house Y, J.S. was once again found, employed there as a waiter. He was sent back to the carrier camp, but disappeared for the second time. In July 1942 he was found again at a third boarding house Z where a case of typhoid had been notified. A phage was prepared for this strain J.S., which gave confluent lysis with its own strain, but not with any of the Craigie type strains.

Nothing more was heard of this carrier for several months and it was presumed that he was still in the carrier camp. In November 1942 there were three cases at a fourth boarding house P. Investigations were made by the Health Department and blood specimens were taken from the native employees. One native waiter gave a positive Vi test and he was removed to the carrier camp. Stool and urine specimens from him were examined on three occasions, but all were negative for *B. typhosus*. As will be shown, this man in all probability had nothing to do with the three cases. Strains were isolated from the blood of two of the cases and these on typing proved to be the same exceptional type as the strains isolated from the old carrier J.S. As this was such a rare type, inquiries were made about this carrier and it was found that he had again disappeared from the carrier camp. The Medical Officer of Health was warned that the carrier J.S. was probably the cause of the three cases at the boarding house P and a special effort was made to find him. The proprietor was questioned, but denied ever having employed a native with the name J.S. Then places where he had previously been employed were visited. Information was at last received that this carrier was now working at a private hotel R. This was visited by the Health Inspector, but this owner

also denied having a native J.S. on the staff. The waiters were inspected and J.S. was found among them, now known by another name. When questioned, he admitted that he had also worked at the boarding house P where the identical type cases had occurred. He had left 8 days before the cases were notified and was without doubt the source of the infection. It seemed possible that he had already spread infection at the hotel R where he had been found, but no cases occurred. His urine still contained the same type typhoid bacilli. Without our typing of strains the investigations of the Health Department would never have reached him, and an outbreak would sooner or later have occurred. His urine has since become negative.

(2) In January 1943 there was an outbreak of fourteen cases of typhoid at the boarding house X where the carrier J.S. previously mentioned had been found in August 1941. Although he had been removed from there by the Health Department at the time, this native had visited the premises of this boarding house on Christmas Day 1942 and when the first case of typhoid was reported 2 weeks later, he was naturally considered by the Health Department to be the cause.

As a routine measure, blood specimens were taken from the members of the staff, and the cook and one waiter gave positive Vi tests. They were transferred to the carrier camp and stool and urine specimens were examined, but were negative. Strains of *B. typhosus* were isolated from the blood of four of the cases and all were type A. Thus the native J.S. was excluded by me from being the causative carrier, as the strain which had been isolated from his urine was not type A. The Medical Officer of Health was informed of this fact and further steps were then taken to find the responsible carrier. In the meantime, six more cases were notified in families which received their milk from the same dairy that supplied the boarding house. Strains were isolated from three of these cases and these again were all type A. It now seemed likely that the dairy was the source of the infection. This dairy was outside the Pretoria Municipal area, but sold milk to eighty families within the Pretoria area. Blood samples were taken from all the dairy employees and three natives gave positive Vi tests. They were removed to the carrier camp and the proprietor was temporarily forbidden to sell milk in the Pretoria area. Stool and urine specimens were examined from these three natives and typhoid bacilli were isolated from the urine of one and the stool of another. These strains were typed and both were type A, the same type as the strains isolated from the patients. No further cases occurred after the removal of these natives to the carrier camp. In this outbreak the typing of strains was again of great value. Without this test the native J.S. would have been regarded as the

responsible carrier, further investigations would have been put off and many more cases would have occurred.

(3) In August 1944 there was a small outbreak of typhoid in Pretoria, consisting of seven cases. Five of these cases were boarders at a girls' school and the other two were members of families which obtained their milk from the dairy which also supplied the school. All employees of this dairy had previously been subjected to the Vi test and found negative, but at the time of the outbreak there had been a shortage of milk and this dairy had obtained additional supplies of milk from two outside dairies which had not participated in the Pretoria voluntary scheme for Vi testing of dairy employees.

When it was established that there was a connexion between all the cases through the milk supply, all employees of the two outside dairies were subjected to the Vi test and nine natives gave positive blood tests. These natives were removed to the carrier camp and stool and urine specimens were examined. The first samples to be examined proved to be negative, but investigations were continued for 4 months and the carrier was eventually discovered. This was a native whose stool and urine had been examined four times with negative results, but on the fifth occasion an imperfect Vi strain of *B. typhosus* was isolated from his urine. Such an imperfect Vi strain had already been isolated from three cases at the school and from the two other cases. Therefore it was concluded that this native was an intermittent urinary carrier and was responsible for the outbreak, although no actual typing could be done. No further cases occurred after his removal to the carrier camp in August. Further investigations were made about his previous occupation and it was considered likely that he had previously been the cause of five cases. However no cultures had been isolated from any of these cases. This native has been kept under observation and his urine has been examined eight times since December, when the strain was isolated, but has been negative each time. The story illustrates the difficulties connected with outbreaks due to such very intermittent carriers.

(4) In April 1945 a case of typhoid occurred at a house in Pretoria and a native servant L. S. was found to be a urinary carrier. Imperfect Vi strains were isolated from the blood of the patient and from L. S.'s urine. In May and August L. S.'s urine was re-examined, but was negative on these two occasions. His blood still gave a strongly positive Vi test and further stool and urine specimens were requested. These were examined on 12 September and a type A strain was isolated from the stool and also from the urine. On 24 September stool and urine specimens were again both positive, type A strains again being isolated. On 31 October, the stool and urine were positive, but on this occasion, although the strain

from the urine was type A, an imperfect Vi strain was obtained from the stool. Several individual colonies from the stool culture were then examined, and eventually a type A strain was recovered. Curiously enough a Vi bacteriophage was isolated from the culture that had behaved as an imperfect Vi form. In December the stool and urine were both negative and in January the stool was negative and a type A strain was isolated from the urine. In June 1946, an imperfect Vi form was once more isolated from the urine. The question arises whether the repeated isolation of an imperfect Vi form was due to the presence of a bacteriophage in the carrier.

THE VALUE OF THE PHAGE TYPING TEST

It has been shown in this paper how the typing of strains by Craigie's phage method has led to the identification of carriers in outbreaks of typhoid in Pretoria. In the first only three cases occurred, but a much larger outbreak was probably prevented by the tracing of the carrier concerned. In another there were twenty cases. Many more people might have become infected if the tracing of the carrier had been delayed.

Since 1938 when Craigie and Yen introduced the phage typing method, with the typing of strains from Canada and elsewhere (Craigie & Yen, 1938*b*), many other workers have used it. The typing of strains has been undertaken by Coleman in Albany, New York State (Coleman, 1941, 1942; Coleman & Wilson, 1944), and in Los Angeles County by Vener & Stevens (1940), Lazarus (1940), and Ward (1944). Yen (1939) has studied forty-nine strains isolated during 1937-9 in Peiping. Schlesenger (1943), Menzies (1944), Gunther (1945), and Gell, Hobbs & Allison (1945) have used the method to trace typhoid carriers.

Boyd (1943) has given the phage types of strains isolated during three outbreaks in Prisoner of War camps in the Western desert. In the outbreak of December 1940 to January 1941, only type G was found. In the summer of 1941, type B 4 and type C were present. So far, types C and G have not been found in South Africa, but they may have been introduced recently by carriers infected in Egypt or the Western Desert. In the same way, strains indigenous to South Africa may have been carried by army personnel to England and elsewhere.

Felix (1943, 1944) and Bradley (1943) have published the results obtained by the use of this test in Britain. There the method has been employed by the Emergency Public Health Laboratory Service since 1940. Felix states that the value of the typing test cannot be emphasized too strongly. With the typing of the T strain common to England and South Africa, a beginning has been made in the exchange of

cultures advocated by him. Some of the strains found in South Africa according to my findings must be new types because they do not fit into the present scheme and it is hoped that a further exchange of material will soon be possible.

SUMMARY

The work began with efforts to isolate a type II Vi phage from local sewage. When this attempt failed, typing material was obtained from Dr J. Craigie and Dr A. Felix.

The typing method was applied to 495 strains of typhoid bacilli, the technique laid down by Craigie being followed throughout.

It was found that in South Africa the distribution of the various types of typhoid bacilli is different from that obtaining in other countries where this typing method has so far been applied.

During further investigations of type F strains the fact came to light that by means of a simple biochemical test type F strains can be subdivided into two sub-types, which subdivision will be useful in further epidemiological work. No such biochemical subdivision was found possible in any other type.

It did not prove possible to type all South African strains with the available phage preparations. Some of these strains must represent new types. Workers in other countries have had similar experiences and it will become necessary to compare all these unusual strains directly with one another in order to reach uniformity in nomenclature. It has already been possible to co-operate with workers in England, and they have been assisted by my finding that a strain, unique in England and supposed to have been brought there by a carrier, who had typhoid fever in South Africa 40 years ago, was endemic in South Africa.

During the course of the work, two outbreaks of typhoid fever occurred in which the typing method proved most useful, not only for linking up a particular carrier with a particular outbreak, but also for excluding an already known carrier who was under grave suspicion.

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REFERENCES

- BOYD, J. S. K. (1943). *Brit. Med. J.* **1**, 719.
 BRADLEY, W. H. (1943). *Brit. Med. J.* **1**, 438.
 COLEMAN, M. B. (1941). *Ann. Rep. Div. Lab. Res., Albany*, p. 58.
 COLEMAN, M. B. (1942). *Ann. Rep. Div. Lab. Res., Albany*, p. 52.
 COLEMAN, M. B. & WILSON, M. S. (1944). *Ann. Rep. Div. Lab. Res., Albany*, p. 49.
 COULTHARD, C. E. (1939). *J. Hyg., Camb.*, **39**, 268.
 CRAIGIE, J. (1941). *Piersol's Cyclopaedia of Medicine, Surgery and Specialties*, Service vol. 5.
 CRAIGIE, J. & YEN, C. H. (1938a). *Canad. Publ. Hlth J.* **29**, 448.
 CRAIGIE, J. & YEN, C. H. (1938b). *Canad. Publ. Hlth J.* **29**, 484.
 FELIX, A. (1943). *Brit. Med. J.* **1**, 435.
 FELIX, A. (1944). *Brit. Med. Bull.* **2**, 12, 269.
 GELL, P. G. H., HOBBS, B. C. & ALLISON, V. D. (1945). *J. Hyg., Camb.*, **44**, 120.
 GUNTHER, C. B. (1945). *J. Bact.* **50**, 126.
 LAZARUS, A. S. (1940). *Bull. Los Angeles City Bd Hlth Commissioners*, **65**, 31.
 DE LOUREIRO, J. A. (1942). *J. Hyg., Camb.*, **42**, 224.
 MENZIES, D. B. (1944). *Canad. Publ. Hlth J.* **35**, 419. Abstracted in *J. Amer. Med. Ass.* **127**, 487, 24 Feb. 1945.
 OLITZKI, L., OLITZKI, Z. & SHELUBSKY, M. (1945). *Trans. Roy. Soc. Trop. Med. Hyg.* **39**, 167.
 PIJPER, A. & CROCKER, C. G. (1937). *J. Hyg., Camb.*, **37**, 332.
 PIJPER, A. & CROCKER, C. G. (1943). *J. Hyg., Camb.*, **43**, 201.
 PIJPER, A., CROCKER, C. G. & TODD, J. (1943). *S. Afr. Med. J.* **17**, 175.
 SCHLESENGER, E. R. (1943). *Amer. J. Publ. Hlth*, **33**, 1257.
 VENER, H. I. & STEVENS, G. M. (1940). *Bull. Los Angeles City Bd Hlth Commissioners*, **65**, 1.
 WARD, W. E. (1944). *J. Bact.* **48**, 379.
 YEN, C. H. (1939). *Proc. Soc. Exp. Biol., N.Y.*, **41**, 162.

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