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Temperate bacteriophages of *Escherichia coli* O119:B14

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SUMMARY

Lysogeny was detected in 98·8% of the 343 *Escherichia coli* O119:B14 strains. A suitable indicator strain *E. coli* KS was selected to demonstrate the presence of temperate phages in this serotype. A great diversity in the temperate population was observed based on their lytic patterns and neutralization studies. No definite relationship could be established between the biochemical reactions and the flagellar antigens of the lysogenic strain and its temperate phage though some temperate phages released by *E. coli* O119:B14 strains with certain flagellar antigens did give specific lytic patterns and were serologically identical. Lysogenic strains, which did not release temperate phages spontaneously, were u.v. inductible. Cross-reactions with lysogenized colonies which were immune to corresponding phages also confirmed diversity of temperate phages in *E. coli* O119:B14.

INTRODUCTION

Escherichia coli serotype O119:B14 was first isolated from a case of calf diarrhoea and was reported as 034W by Wramby (1948). Smith (1953) was the first to demonstrate its importance in cases of infantile gastro-enteritis. Since then many authors have isolated it in different countries. Chambon (1955); Coetzee & Pretorius (1955); Banerjee, Chatterji & Praminik (1956); Quilligan *et al.* (1957); Cooper, Walters & Keller (1956); Gamble & Rowson (1957); Sacrez, Levy, Minck & Poirier (1958); Lutz, Grad & Bass (1959); Ayoma (1960); McCallum, Le Minor, Le Minor & Chabbert (1961) and Hiroki (1961) isolated these strains from epidemic or sporadic cases of infantile gastro-enteritis. This serotype has been studied in detail by many authors from the point of view of antigenic structure, fermentation reactions and susceptibility to antibiotics. The present study was undertaken first to demonstrate the presence of temperate phages in *E. coli* serotypes O119:B14 in order to establish a phage typing system useful for epidemiology and secondly to see the relationship between the biochemical characters and antigenic structures of *E. coli* O119:B14 and the temperate phages derived from them.

MATERIAL AND METHODS

Bacterial strains

In total 343 strains of *E. coli* O119:B14 from the collection of Institut Pasteur, Paris, were studied. These strains were isolated from epidemic or sporadic cases of gastro-enteritis in Germany, France, Belgium, United States, Great Britain and

Mexico. Biochemical, serological and antibiogram studies of these strains were done by McCallum *et al.* (1961) who divided the strains into flagellar types H_4, H_5, H_6, H_8, H_{27}, H_{32} and H_{40}. Non-motile strains were designated as H-.

Bacteriophages

The lysogenic property of *E. coli* strains was demonstrated by classical techniques on an agar plate inoculated with an indicator strain, using either a drop of broth culture, or a drop of supernatant fluid of a broth culture to which a few drops of chloroform had been added, followed by centrifugation at 6000 rev./min. for 10 min. *Shigella paradysenteriae* Y 6R, *E. coli* Bordet, *E. coli* B, *E. coli* 36 and *E. coli* K (S and R used as suffix for smooth and rough variants respectively) were used as indicator strains. Mostly *E. coli* KS was used as the indicator strain of choice for detection of temperate phages in cultures of *E. coli* O119:B14. In cases where the above two techniques failed, the presence of temperate phages was demonstrated either by 'mixed culture' technique or by u.v. induction. In the former case *ca.* 10^4 bacteria/ml. of the suspected lysogenic strain were mixed with an equal number of cells of the indicator strain. The mixed culture was incubated at 37° C. for 4–5 hr. in a water bath agitator and then filtered through a Chamberland L3 filter. The filtrate was tested for the presence of bacteriophages.

For u.v. induction experiments (Lwoff, Siminovich & Kjeldgaard, 1950) an ultra-violet lamp developing an energy of 4000 ergs/mm.^{2}/min. at a distance of 50 cm. was used. Irradiation of diluted log. phase liquid culture (about 10^3 cells/ml.) in thin layers was done in Petri dishes. Immediately after irradiation, the culture was incubated for 3 hr. at 37° C. in a water bath, at the end of which a few drops of chloroform were added and the culture centrifuged at 6000 rev./min. for 10 min. The supernatant fluid was titrated on the indicator strain for the presence of phage.

Purification of bacteriophages

Cultures were sometimes polylysogenic. Purification of phage was done by cutting out a piece of agar showing a well-isolated plaque in the centre with a scalpel and transferring it to a flask containing 30 ml. of nutrient broth. After incubation at 37° C. for 3–8 hr., the broth was filtered and spotted in serial dilutions so as to get isolated plaques on nutrient agar plates seeded with the propagating strain. Single plaque propagation was repeated at least three times before obtaining pure bacteriophage suspension. Different plaques showing different morphology from the same polylysogenic strain were propagated separately.

Preparation of high-titred bacteriophages and titration

Preparation of high-titred bacteriophages and titration were usually done according to Adams (1959). In some cases a high titre was obtained by incubating phage–host suspension at 37° C. for a few hours and then leaving it at laboratory temperature or even at 4° C. until lysis occurred. The lysate was treated with a few drops of chloroform to lyse the bacterial cells and centrifuged to get rid of

suspended bacteria and debris. The supernatant fluid was filtered through a Chamberland L3 filter and stored at 4° C.

Serology of bacteriophages

For purposes of identification and antigenic classification, phage antisera were prepared in rabbits. Phage suspensions in broth containing *ca.* 10^9 particles/ml. were diluted 1/5 in peptone water and injected two or three times into rabbits intraperitoneally in doses of 50 ml. each at weekly intervals. The rabbits were bled 15 days after the third injection and the serum was tested against the corresponding bacteriophage by neutralization tests (Adams, 1959). Usually high-titred antisera were obtained except in a few cases where even the addition of adjuvants like Freund, ascorbic acid, pantothenic acid and nicotinic acid did not help. In neutralization experiments 0·5 ml. of 1×10^7 p.f.u./ml. of a bacteriophage in nutrient broth was mixed with 0·5 ml. of antiphage serum in varying dilution. The mixture, after incubation at 37° C. for 30 min., was diluted 1/10 in broth and titrated on the indicator strain to get the p.f.u./ml. at each serum dilution. The maximum dilution of the serum was recorded where phage was neutralized completely.

Phage resistance by lysogenization

The resistant colonies appearing in a lytic area were picked and subcultured two or three times on agar slants to remove any carry over of the original bacteriophage. These lysogenized colonies were then verified as resistant to superinfection by the corresponding bacteriophages and able to release the lysogenizing phage on the appropriate indicator strains. The lysogenized colonies were designated by the name or number of the *E. coli* indicator strain followed by the number of the phage in parentheses, e.g. KS (6719).

Designation

A temperate phage was either designated by the strain number of *E. coli* O119:B14 from which it was derived or, in case this phage was propagated to get a high titre, it was designated by the number of the *E. coli* strain from which the phage was derived with the name of the propagating strain as denominator, e.g. phage 6719/KS isolated from *E. coli* O119:B14 number 6719 and propagated on *E. coli* KS.

RESULTS

Demonstration of lysogeny by homologous indicator strains

Temperate phages of *E. coli* O119:B14 very rarely show lysis on strains of the same serotype. In cross-culture technique only seven out of 343 strains gave lysis on seven other indicator strains of *E. coli* O119:B14. These seven temperate phages could be divided into four groups based on their lytic pattern (Table 1). The first group consisted of phage 6719 derived from the strain *E. coli* number 6719 with flagellar antigen H_4. Further studies proved that serologically this phage was different from other temperate phages. The second group which included phages

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13485, 967 and 16180 derived from *E. coli* strains with flagellar antigens H_6, H_6 and H_{32} respectively showed same lytic pattern. The third group consisting of phages 16114 and 16115 derived from lysogenic *E. coli* with H_8 and H_{40} respectively gave similar lytic pattern. They were found to be related in their antigenic properties (Table 6). The fourth group was represented by two phages derived from the same strain 15755 with flagellar antigen H_5, one giving clear plaques (C) and the other turbid (T). They also gave more or less similar lytic pattern. In neutralization studies they were observed to be identical. The clear plaque-producing phage was derived as virulent mutant of the wild-type turbid plaque-producing phage.

Table 1. *Lysogeny within strains of* Escherichia coli O119:B14 *employing homologous indicator strains*

Indicator strains			Phages from lysogenic strains with flagellar antigens							
									15755 H_5	
Nos.	Flagellar antigen	Bio-type	6719 H_4	13485 H_6	967 H_6	16180 H_{32}	16114 H_8	16115 H_{40}	T	C
15766	H_6	2	4 pl	–	–	–	–	–	6 pl	++
14001	H_8	1	–	–	–	–	+	CL	–	–
16167	H_8	2	–	–	–	–	+	CL	–	–
18867	H_{32}	1	–	SCL	+++	+++	–	–	+++	SCL
15664	H-	2	–	–	–	–	–	–	–	SCL
968	H_6	2	+	++	1 pl	8 pl	–	–	+++	+++
15379	H_6	2	–	–	–	–	–	5 pl	–	–

T, turbid plaques; C, clear plaques; CL, confluent lysis; SCL, semi-confluent lysis; pl, actual number of plaques.

Table 2. *Frequency of lysogeny in* Escherichia coli O119:B14

		Phage detection by indicator strains				
		E. coli K			Y 6R	
Technique	No. of strains tested	Colony S	Colony R	*E. coli* B	Colony S	Colony R
Chloroform and centrifugation	343	297* (86·6 %)	198 (57·7 %)	131 (38·2 %)	188 (54·8 %)	236 (68·8 %)
U.v. irradiation	–	34 (9·9 %)	6 (1·7 %)	–	19 (6·4 %)	22 (5·5 %)
Mixed culture with *E. coli* KS	–	8 (2·3 %)	–	–	–	2 (0·6 %)
Total	343	339 (98·8 %)	–	–	–	–

* Represents the number of strains found lysogenic.

Demonstration of lysogeny by heterologous indicator strains (frequency of lysogeny)

Forty-eight strains representing different species in the family *Enterobacteriaceae* were tested as indicator strains to temperate phages of *E. coli* O119:B14. Amongst them, *Shigella paradysenteriae* Y 6R (F. M. Burnet), *E. coli* K (R. Legroux), *E. coli* B (Demerec and Fano) and *E. coli* 36 (F. M. Burnet) were found to be very

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sensitive. In total 297 out of 343 strains, i.e. 86·6 % were found to be spontaneously lysogenic as demonstrated by the ordinary method of chloroformed culture filtrate (Table 2). Out of the remaining 46 strains, 42 strains were found to be lysogenic either by u.v. induction or by mixed culture technique or by both methods. Only four strains were found to be non-lysogenic or were defective lysogenic.

Table 3. *Optimum time for u.v. induction of* Escherichia coli O119:B14 *strain* 15755

U.v. irradiation time (sec.)	Phage titre assayed on *E. coli* KS—p.f.u./ml.	Observation
0	0	
15	$1{\cdot}6 \times 10^5$	1⁰ With increase of u.v. irradiation dose, plaques diminish in size
30	$4{\cdot}4 \times 10^5$	
60	$4{\cdot}4 \times 10^6$	
90	5×10^6	2⁰ The number of plaque-forming units increase up to 90 sec. of u.v. irradiation and then a u.v. killing effect is obtained
120	$1{\cdot}6 \times 10^6$	
180	$2{\cdot}8 \times 10^5$	
240	$2{\cdot}8 \times 10^5$	

U.v. induction of lysogenic Escherichia coli

Table 3 shows the optimum time of u.v. irradiation to induce lysogeny in a strain 15755 which did not show any lysogeny either by the ordinary classical method of chloroformed culture filtrate or by mixed culture technique. The optimal time of irradiation to get the maximum plaque formers from a lysogenic strain was found to be 90 sec.

Lytic pattern

Forty-five lysogenic strains were selected on the basis of their differences in flagellar antigen, biotype and geographical origin; 45 phages isolated from these strains were purified, propagated and titrated on *E. coli* KS. These temperate phages employed in routine test dilution gave nine lytic patterns when tested on seven different indicator strains. As shown in Table 4, lytic patterns produced by temperate phages of H_4, H_5 and H- *E. coli* strains were all different from each other and from other groups. The phages derived from different strains of *E. coli* O119:B14:H6 gave different lytic images. A number of temperate phages derived from *E. coli* strains with H6 gave similar lytic patterns. Different lytic patterns were obtained with the phages derived from *E. coli* strains in the same geographical site, e.g. *E. coli* strains isolated in lower Alsace gave phages that produced four different lytic patterns on a set of indicator strains.

Cross-immunity between lysogenized colonies produced by different phages

A lytic pattern for the lysogenized colonies was obtained employing corresponding lysogenizing phages. As shown in Table 5, each lysogenized strain was immune to lysis by the corresponding phage but was lysed by other phages. Most of the lysogenized strains showed a specific lytic pattern which in certain cases was related to the flagellar antigen of the temperate phage-producing strain, e.g.

lysogenizing phage derived from *E. coli* H_4 gave specific pattern for K (6719). On the contrary, phages derived from *E. coli* strains with same flagellar antigen H_6 formed lysogenized colonies which gave different lytic patterns, e.g. phages 7629, 13076, 966, 21003 and 17575.

Table 4. *Lytic pattern obtained by* 45 *phages derived from* Escherichia coli *strains with different flagellar strains*

	Lysogenic strains			Phages giving	Lytic pattern of indicator strains						
Lytic pattern	Flagellar antigen	Biotype H_6	Origin	similar pattern	*E. coli* KS	*E. coli* KR	*E. coli* Y 6 R	*E. coli* Bordet	*E. coli* B	*E. coli* K 125	*E. c* Lis bon
1	H_4	—	Pennsylvania	1	+	+	+	+	+	+	+
2	H_5	—	Lower Alsace	1	+	+	+	+	+	+	−
3	H_6	1 (3)	Berlin (3)	9	+	+	+	+	+	−	−
		2 (5)	Lower Alsace (4)								
			Aberdeen (2)								
4	H_6	2	Strasbourg	2	+	+	+	−	+	+	+
			Aberdeen								
5	H_6 (26)	1 (25)	Lower Alsace (13)	27	+	−	+	−	−	+	+
	H_{32} (1)	3 (1)	Liege (4)								
			Lyon (1)								
			Berlin (9)								
6	H_6	2	Lower Alsace	1	+	+	+	−	−	−	+
7	H_6	2	Berlin	1	+	+	−	−	−	−	−
8	H_6	2	Berlin	2	+	+	+	−	+	+	−
			Lyon								
9	H-	2	Lower Alsace	1	+	+	+	−	−	−	−

( ) Indicates the number of strains.

Table 5. *Cross-reactions between the lysogenized* Escherichia coli *KS colonies and the corresponding phages*

	Phages with flagellar antigens of the lysogenic strains											
Lysogenized colonies resistant to lysis	6719 H_4	15755 H_5 C	15755 H_5 T	7629 H_6	13076 H_6	11235, 974, 15379 H_6	966 H_6	21003 H_6	17575 H_6	16443, 18807 H_{32}	16180 H_{32}	15764 H-
KS (6719)	−	CL	CL	CL	CL	CL	CL	CL	CL	+ + +	CL	CL
KS (15755)	CL	CL	−	CL	SCL	CL	+ + +	CL	CL	+ + +	CL	CL
KS (7629)	CL	CL	CL	−	CL	CL	CL	CL	SCL	+ + +	CL	CL
KS (13076)	SCL	CL	CL	CL	−	CL	+ + +	CL	SCL	−	SCL	CL
KS (11235-974-15379)	CL	CL	CL	−	−	—	+ + +	CL	SCL	−	SCL	CL
KS (966)	CL	CL	CL	CL	−	CL	−	CL	−	−	SCL	CL
KS (21003)	CL	CL	CL	SCL	CL	CL	CL	−	SCL	−	+ + +	CL
KS (17575)	CL	CL	CL	CL	CL	CL	CL	CL	−	SCL	CL	CL
KS (16443)	CL	CL	CL	CL	CL	CL	SCL	CL	SCL	−	CL	CL
KS (18807)	CL	CL	CL	CL	CL	CL	SCL	CL	SCL	−	CL	CL
KS (16180)	CL	CL	CL	CL	SCL	CL	SCL	CL	SCL	SCL	−	CL
KS (15764)	−	CL	−	SCL	SCL	CL	CL	CL	SCL	+ + +	SCL	−

CL, confluent lysis; SCL, semiconfluent lysis; + + +, above 100 plaques; T, turbid plaques; C, clear plaques.

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Serology

The great diversity amongst lysogenic phages of *E. coli* O119:B14 was confirmed by neutralization tests. Antisera against 11 phages isolated from lysogenic strains with different flagellar antigens were prepared. Antisera against phages derived from the clear and the turbid plaques of *E. coli* strain 15755 were also prepared. Certain phages (6719, 7629, 15106, 15755, 15764, 16114 and 16115) were excellent antigens and produced high titred neutralizing antisera. On the other hand phages 9623, 13076 and 16180 gave very poor antisera in different lots of rabbits even in the presence of various adjuvants. The antisera produced against the first two phages were removed from Table 6 because of the complete lack of

Table 6. *Cross-neutralization of* 27 *temperate phages by antiphage sera*

	Phages														
Anti-phage serum	6719 H_4*	15106 H_6	16114 H_8	16115 H_{40}	15755 H_5 C	15755 H_5 T	15764 H-	7629 H_6	16180 H_6	14178 H_6	77251 H_6	20850 H_4	9623 H_6	13076 H_6	13 phages selected at random
6719	**8000**†	—	—	—	—	—	100	—	—	8000	1200	8000	—	—	—
5106	—	**3000**	—	100	—	—	—	—	—	—	—	—	—	—	—
6114	—	—	**3000**	3000	—	—	—	—	—	—	—	—	—	—	—
6115	—	—	4000	**4000**	—	—	—	—	—	10	10	10	—	—	—
5755 C	10	—	—	20	**8000**	8000	1000	—	—	10	10	10	—	—	—
5755 T	10	—	—	10	8000	**8000**	1000	—	—	10	10	10	—	—	—
5764	—	—	—	—	100	100	**1000**	—	—	10	10	10	—	—	—
7629	—	—	—	—	—	—	—	**2000**	—	—	—	—	—	—	—
6180	—	—	—	—	—	—	—	—	**10**	—	—	—	—	—	—

Flagellar antigens of *E. coli* strain that produced the corresponding phages. T, turbid plaque-forming phage.
† Highest dilution of serum which neutralized the phage completely. C, clear plaque-forming phage.

specific phage antibodies. With the help of nine antiphage sera, 27 different temperate phages were divided into six groups by neutralization experiments. The first group consisted of temperate phages 6719, 14178 and 20850 which were neutralized completely at a serum dilution of 1/8000. Phage 7251 was neutralized partially up to 1/1200 which showed its partial antigenic relationship with the first three phages. The second group was represented by a single phage 15106 which was neutralized by its homologous antiserum at a dilution of 1/3000. The third group consisted of phages 16114 and 16115 which cross-neutralized each other completely by their homologous antisera. The fourth group was represented by two phages derived from *E. coli* strain 15755. There was a complete cross-neutralization of these phages by their homologous antisera. The antisera produced against these phages also neutralized phage 15764 at a dilution of 1/1000 which indicated that phage 15764 was partially related to phage 15755. The fifth group consisted of one phage 7629; phages 16180, 9623, 13076 and 13 other phages which were not neutralized by the antisera available were grouped together temporarily in the sixth group.

DISCUSSION

Most bacteria carry certain temperate bacteriophages but frequently it is impossible to demonstrate the presence of these phages because of the lack of the suitable indicator strain. The data represented in Tables 1 and 2 indicate that the frequency of lysogeny (2 %) was poor in *E. coli* O119:B14 when different strains of the same serotype were used as indicator strains; on the contrary, after having selected 45 different heterologous indicator strains, spontaneous lysogeny in the same serotype could be detected in 86·6 % of the strains. All the remaining strains, except four, were also detected as lysogenic by u.v. induction or mixed culture technique.

It has been extremely difficult to establish a relationship between the nature of the temperate phage carried by a lysogenic strain and its biochemical or antigenic character. The phages 6719 and 15755 derived from *E. coli* strains with flagellar antigens H_4 and H_5 respectively gave specific lytic patterns with homologous *E. coli* O119:B14 indicator strains (Table 1), heterologous *E. coli* indicator strains (Table 4) and a set of immune lysogenized colonies (Table 5). This led to the conclusion that the phages 6719 and 15755 were different from each other and from other temperate phages. It was further confirmed by neutralization studies that the antigenic structure of these phages was different. Similar lytic patterns (Table 1) were obtained for phages 16114 and 16115, with similar antigens associated in neutralization tests (Table 6), though derived from lysogenic *E. coli* with two different flagellar antigens H_8 and H_{40} respectively. Owing to the lack of more *E. coli* H_4, H_5, H_8 and H_{40} strains, no definite relationship could be established between their flagellar antigens, the specific lytic pattern obtained by their temperate phages and the phage antigens involved in neutralization test.

Studies with lysogenized colonies (Table 5) proved that the temperate phages of *E. coli* O119:B14 offered specific self-immunity to lysogenized strains and that these lysogenizing phages, though very diversified, could still be grouped, e.g. phages 11235, 974, 15379 and 16443 and 18807 based on the specific lytic pattern of the lysogenized strains. Here it was interesting to observe the similarity in the lytic patterns of *B. coli* KS lysogenized by phages derived from *E. coli* with the same flagellar antigens. The above first three phages were derived from *E. coli* H_6 and the last two from *E. coli* H_{32}.

In spite of the fact that almost all of the *E. coli* O119:B14 strains were lysogenic and that the temperate phages released by these strains were very diversified, the strains of *E. coli* O119:B14 were rarely sensitive to the temperate phages of other strains of the same serotype. This may be explained by the fact that probably these strains were polylysogenic and that immunity to lysis exists as an interaction between the phages or may be due to the presence of few common prophages within the cell. Because of this lack of sensitivity of the serotype towards its temperate phages, it was not possible to establish a rational phage typing scheme based on the temperate phages of the same serotype as was possible in *Salmonella typhi* and *S. paratyphi B*. However, an empirical phage-typing system was established with the help of the virulent phages isolated from sewage (Kasatiya, 1963).

Phage serological studies also supported the diversity of temperate phages in this serotype. In one case antigenic similarity between the temperate phages was related with the flagellar antigen of the corresponding lysogenic *E. coli* strain, e.g. phages 6719 and 20850, both derived from strains with flagellar antigens H_4, were serologically identical. On the other hand temperate phages of strains with the same flagellar antigens did show different antigenic structure in neutralization experiments (phages 16180, 7629, 15106 and 14178). The data proved that the temperate phages of *E. coli* O119:B14 were serologically independent, except in few cases, of the flagellar antigens of the corresponding lysogenic strain.

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REFERENCES

Adams, M. H. (1959). *Bacteriophages*: New York: Interscience Publishers, Inc.

Ayoma, I. (1960). Studies on the pathogenic *E. coli*. I. Distribution of pathogenic *E. coli*. II. Infantile diarrheal disease and isolation rate of pathogenic *E. coli* in a suckling's home. III. A mass incidence of pathogenic *E. coli* O 26 in an asylum of Utsunomyia city. *Journal of the Japanese Association for Infectious Diseases* **33**, 1004–22.

Banerjee, A., Chatterji, D. N. & Praminik, K. (1956). Studies on the bacteriology of gastro-enteritis in infants and older age group with particular reference to certain serotypes of *E. coli*. *Annals of Biochemistry and Experimental Medicine* **16**, 49–56.

Chambon, L. (1955). Recherches sur l'infection par des souches d'*E. coli* pathogènes à Saigon. *Bulletin de la Société de pathologie exotique* **48**, 296–8.

Coetzee, J. N. & Pretorius, H. P. J. (1955). Endemic infantile gastro-enteritis in the Pretoria area. The aetiological role of *E. coli*. *South African Journal of Laboratory and Clinical Medicine* **1**, 188–96.

Cooper, M. L., Walters, E. W. & Keller, N. M. (1956). *Escherichia coli* associated with infantile diarrhea. *Annals of the New York Academy of Sciences* **66**, 78–89.

Gamble, D. R. & Rowson, K. E. (1957). The incidence of pathogenic *E. coli* in routine faecal specimens. *Lancet* ii, 619–21.

Hiroki, F. (1961). Biological and immunological studies on the pathogenic *E. coli* O 112 and O 119 detected in Japan. *Hirosaki Medical Journal* **12**, 666–81.

Kasatiya, S. S. (1963). Thèse du doctorat, Université de Paris.

Lutz, A., Grad, Ch. & Bass, J. P. (1959). Sur la fréquence et la diversité d'entérobactéries pathogènes isolées dans le département du Bas-Rhin en 1958. *Strasbourg médical* **10**, 654–61.

Lwoff, A., Siminovich, L. & Kjeldgaard, N. (1950). Induction de la production de bactériophages chez une bactérie lysogène. *Annales de l'Institut Pasteur* **79**, 15–59.

McCallum, M., Le Minor, L., Le Minor, S. & Chabbert, Y. (1961). Etude des caractères biochimiques et antigéniques des *E. coli* O 119:B 14 et de leurs antibiotypes prédominants. *Annales de l'Institut Pasteur* **101**, 898–913.

Quilligan, J. J., Biddle, M., Jr., Debrick, J. W., Scott, M. L. & Burnett, I. (1957). Recovery of enteropathogenic types of *E. coli* from infants with diarrhea. *American Journal of Diseases of Children* **93**, 420–4.

Sacrez, R., Levy, J. M., Minck, R. & Poirier, G. (1958). Infections entérales à *Escherichia coli* O 119:B 14 chez le nourrisson. *Strasbourg médical* **9**, 465–71.

Smith, J. (1953). The association of serological types of *B. coli* with infantile gastro-enteritis. *Journal of Pathology and Bacteriology* **66**, 503–11.

Wramby, G. (1948). Investigations into the antigenic structure of *Bacterium coli* isolated from calves. Uppsala: *Appelbergs Baktryckeriaktiebolog*.