OBSERVATIONS ON L-FORMS OF *YERSINIA ENTEROCOLITICA*

PHYLLIS PEASE

Department of Medical Microbiology, University of Birmingham, Birmingham B15 2TJ

PLATES XVII-XXI

IN recent years *Yersinia enterocolitica* has attracted a great deal of attention, especially in Scandinavian countries, mainly because of its association with pathological conditions such as terminal ileitis, enteritis and reactive arthritis (Larsen, 1976). Numerous studies have been conducted upon the cultural, biochemical, serological and pathogenic properties of the organism, but so far there has been no report of the occurrence of L-forms.

In cultures of *Y. enterocolitica* in this laboratory, L-form-like organisms were observed to occur spontaneously, and it is the purpose of this paper to describe them.

MATERIALS AND METHODS

Organisms. Three strains of *Y. enterocolitica*, NCTC nos. 10460, 10461, and 10462, were obtained from the National Collection of Type Cultures, Colindale, London.

Culture media. Cultures were made on Difco Tryptose Blood Agar Base, Difco MacConkey Agar without crystal violet, and in Difco Brain Heart Infusion Broth.

Phase-contrast microscopy. Blocks of agar bearing colonies were cut out and placed on slides with colonies uppermost and observed directly, at low magnification, by means of a Wild phase-contrast microscope. For oil-immersion examination, coverslips were placed over the colonies.

Biochemical reactions were performed according to the methods of Cowan and Steel (1974). Where more than one method is described, the following were chosen: catalase, method 1; oxidase, 1; nitrate reduction, 1; urease, 1; H₂S, 3; indole, 2; Voges Proskauer, 2; gelatin hydrolysis, 5; arginine, ornithine, and lysine decarboxylase, 2.

Agglutination tests. Slide and Widal-type tests were performed. Antigens for agglutination tests were prepared from organisms grown at 37°C or room temperature overnight. They were steamed for 30 min. and washed three times in saline. The deposits were then resuspended in 5 ml of saline and heated at 100°C for 10 min. These suspensions were then further diluted in saline to the opacity, as measured by eye, of a standard suspension of *Y. enterocolitica*, which had been made up previously and was known to give satisfactory results in agglutination tests. The tests were done in haemagglutination trays with doubling dilutions of antiserum, and readings were taken after 4 h at 22°C. Slide-agglutination tests were examined by phase-contrast microscopy.

Gel-diffusion tests. Antigens for use in gel-diffusion tests were prepared as for agglutination, except that the suspensions were 200 times stronger, and were subjected to ultrasonic disintegration for 5–10 min. at 20 kc/sec. Tests were by double-diffusion precipitation in ion-agar gel containing sodium azide 1 g, Ion Agar No. 2 (Oxoid) 10 g, and saline (0.85% w.v. in water) 1000 ml.

Fluorescent-antibody tests. Impression preparations of colonies or smears of the organisms

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were made on slides and heat-fixed. They were covered with antiserum or pre-immunisation serum, left for 15 min., washed in Oxoid Phosphate Buffered Saline (PBS) for 15–30 min., covered with sheep anti-rabbit globulin fluorescein conjugate (Progressive Laboratories Baltimore Inc., Baltimore, USA) for 15 min., washed in PBS for 15–30 min. and then mounted in 50% (v/v) glycerol in PBS.

Antisera. Organisms from 500-ml amounts of broth culture were resuspended in 10 ml of saline after heat treatment at 100°C for 10 min. or treatment with 0.2% formalin for a minimum of 48 h; 2×1 ml of the suspension were injected into rabbits subcutaneously at weekly intervals for 5–6 weeks.

Electron microscopy (EM). Organisms were scraped from the surface of agar or centrifuged from broth cultures; they were fixed at room temperature for 30 min. in 2.5% glutaraldehyde in 0.05M phosphate buffer at pH 7.2–7.4, containing 0.05M sucrose, then post-fixed in 1.0% osmic acid in the same buffer for 2 h at 4°C, followed by routine dehydration and embedding in Epon. Sections were cut on a Sorval-Porter Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Philips EM301 electron microscope.

RESULTS

Isolation of L-forms

In cultures of *Y. enterocolitica* strain NCTC10460, papillary secondary colonies consisting of pleomorphic forms were regularly observed within colonies aged 2–3 weeks. The two phases were not easy to separate, but on occasion subcultures from the papillae on MacConkey agar apparently consisted entirely of gram-negative coccal forms. On further incubation of these subcultures for 3–4 days gram-negative rods reappeared. When subcultures were made on MacConkey agar or tryptose blood-agar base, coccal forms were present after 12–24 h and later rod-forms appeared. This was observed many times during a period of 6 months.

Phase-contrast microscopy

On incubation at 37°C for 12 h, subcultures from any stages in the developmental cycle gave rise to small colonies of coccal forms, usually 2–3 μ m but occasionally as large as 6.0 μ m in diameter. These forms were subsequently identified as large spheroplast L-forms and will be referred to as such for the remainder of this paper. At this stage the small colonies resembled classical L-form colonies in that the organisms of which they consisted were rounded and often vesiculated (fig. 1) but macroscopically they did not have a 'fried-egg' appearance. On further incubation for 12–48 h, smaller coccal forms 0.5–1.0 μ m in diameter, some of which were shown by electron microscopy to be small spheroplasts and some to be small bacterial forms (fig. 2) appeared. The latter forms seemed to represent a stage in the reversion process to the rod-form (see below). Because it was not possible to separate the small coccal forms from the large spheroplast L-forms by plating out, this two-phase stage, where appropriate, will be referred to as the large spheroplast L-form/small coccal-form (SLCF) stage.

At room temperature, the reversion process appeared to be different, in that the reverting forms were mostly tadpole-shaped, occurring at the edges of

the large spheroplast colonies after incubation for 12–24 h (figs. 3 and 4). At this temperature the tadpole-shaped and the coccal forms were actively motile; the latter were non-motile at 37°C.

After 48–72 h at 37°C and at room temperature, rod-forms resembling *Y. enterocolitica* strain NCTC10460 biochemically, serologically and, to some extent, morphologically, appeared at the edges of the colonies (fig. 5). Subcultures from this or any other part of the colonies produced large spheroplast L-forms and the cycle was repeated. Subculture into broth yielded mainly large spheroplast L-forms and small coccal forms (fig. 6) which tended to stay in this form, although occasional organisms of either type were seen to give rise to rod-forms (fig. 7). Again, motility was demonstrable at room temperature but not at 37°C.

After 3 months of subculturing, another type of growth was observed on subculture on solid medium. As already observed in fluid medium, the large spheroplast L-forms were capable of producing rod-forms directly (fig. 4) and this process sometimes produced growth consisting of organisms resembling *Streptobacillus moniliformis*; these were composed of filaments and swollen elements (fig. 8). This type of growth maintained the same morphology during many weeks of daily subculture.

As already emphasised, subcultures of any stage of the cycle including apparently revertant rod-forms usually gave rise to large spheroplast L-forms. Repeated subculture from the edges of colonies where there was profuse growth of such rod-forms eventually resulted in successful isolation of the rod-forms. However, careful observation always revealed a few large spheroplast L-forms among the rods (fig. 9). Revertant forms and parental forms were never entirely free from large spheroplast L-forms and close examination of apparently normal colonies of the other strains (NCTC nos. 10461 and 10462) of *Y. enterocolitica* revealed very occasional spheroplast forms.

Electron microscopy

Cultures were grown under the same conditions as those used for phase-contrast microscopy. Sections of parent bacteria, large spheroplast L-forms, small coccal forms and revertant bacteria were examined. Figs. 10–12 are low-magnification electron micrographs of normal bacteria, large spheroplast L-forms and reverting bacteria, respectively. It is apparent that the large spheroplast L-forms are swollen and that the revertant bacteria have irregular contours compared with the parent bacteria. Characteristic appearances of the cell wall of the parent bacteria are shown in figs. 13 and 14. In fig. 13 the cell wall is shown to be adhering to the cell membrane, whereas in fig. 14 it has split away. The latter appearance was characteristic of the large spheroplast L-forms except for a minority of organisms that possessed only a single membrane (fig. 15) and could therefore be designated as protoplast L-forms. Thus the majority of L-forms retained cell-wall material and this, with their behaviour and further structural evidence to be discussed below, justifies their identification as spheroplast L-forms.

Apart from the difference in contour regularity already noted, no structural differences were detected between the cell-walls of parent and revertant rod-shaped bacteria. In the case of some of the small coccal forms a thickened cell wall, adherent to the cytoplasmic membrane, was observed (fig. 16). At the same time many of the small coccal forms had cell-wall appearances indistinguishable from those of the large spheroplast L-forms. A feature common to large and to small forms was the presence of membranes separating small cells, often containing compressed cytoplasm, from the main cell (fig. 17). It was not established whether some of these small cells were homologous with the tails of the tadpole-shaped organisms, representing a stage towards the development of the rod form but this seemed probable, particularly because the culture from which these preparations were taken was incubated at room temperature. Occasionally, it seemed that these cells were budded off and behaving like mini-cells (fig. 18).

The large spheroplast L-forms showed other interesting features. Some cells had granular structures, ranging from 0.06 to 0.2 μm in size, between the cytoplasmic membrane and the cell wall (fig. 19), and membranous structures probably situated in the vesicular regions seen in phase-contrast preparations (fig. 1) could be seen in some cells (fig. 20). Cells containing membranous structures and particulate matter (fig. 21) were particularly spectacular and probably represent mother cells, the particles being elementary bodies which, when shed, may have a reproductive potential. In some cells the membranous material was arranged in more organised form. Figs 22 and 23 show membranes of different thicknesses within different spheroplast L-cells and fig. 24 shows such membranes arranged in concentric circles within the same cell. Higher magnifications of some of these membranous structures with a range of thicknesses from 8.0 to 30.0 nm are shown in figs 25 and 26. The structure shown in fig. 27 appears to be tubular.

Another point of interest was the presence of fibrillar structures within the large spheroplast L-cells. These most often occurred singly (fig. 28) but sometimes two or three were observed in one cell (fig. 29). They ranged from 200 nm to 1.2 μm in length. Fig. 30 shows a bundle of fibrils and fig. 31 shows an enlargement of an area similar to the structures in fig. 29 with particulate material and fibrils. Fig. 32 is an enlargement of part of fig. 28 showing undulated fibrils with a wavelength of *c.* 30 nm.

Biochemical reactions

These were determined at 37°C and at room temperature on 48-h cultures of *Y. enterocolitica* strains NCTC10460, 10461 and 10462, on 24-h cultures of SLCFs, on 24-h cultures of the streptobacillus phase and on 24-h cultures of reverted rod-forms containing very few large spheroplast forms. All the tests listed by Cowan and Steel (1974) for the identification of *Y. enterocolitica* were done on several occasions and all the forms tested showed the reactions characteristic of *Y. enterocolitica*. No marked difference was seen between tests done at 37°C and at room temperature, except in respect of the Voges-

Proskauer (VP) reaction; this is usually positive at room temperature and negative at 37°C for *Y. enterocolitica* (Cowan and Steel, 1974). The parent strain (NCTC10460) was VP negative at both temperatures, whereas the large spheroplast L-form/small coccal-form cultures were positive at room temperature and negative at 37°C. The revertant rods had a tendency when inoculated into fluid medium to give rise to large spheroplast L-forms and small coccal forms which gave positive VP reactions at room temperature; on two occasions when the rod-form persisted, the reaction was negative at both temperatures, as in the parent form.

Agglutination tests

Antisera were prepared to the parent *Y. enterocolitica* strain NCTC10460 incubated at 37°C and at room temperature (RT), to its SLCF stage grown at 37°C and to *Y. enterocolitica* strain NCTC10462 grown at 37°C. These were tested against their homologous antigens grown at 37°C and room temperature and also against revertant bacteria. Slide-agglutination tests were also performed with organisms from cultures on solid medium. The results of the quantitative agglutination tests are shown in table I and it can be seen that the SLCF cross-reacted with its parent strain, its own revertants and *Y. enterocolitica* strain NCTC10462. The reason for the differences in titre with organisms grown at different temperatures was not investigated. The organisms also cross-reacted in slide tests. By means of phase-contrast microscopy it was possible to observe the agglutination of the individual large spheroplast L-forms as well as of the small coccal forms with antiserum to the parent bacterial form, thus showing that both forms, which were inseparable by conventional bacteriological methods for the purposes of antigen preparation, were related serologically to one another as well as to the parent bacteria.

Control tests with pre-immunisation sera and with *Staphylococcus aureus* and *Escherichia coli* antigens gave no agglutination.

Gel-diffusion tests

These tests, by the occurrence of multiple lines of identity (fig. 33), also showed that the SLCF cross-reacts with its parent and revertant forms and with *Y. enterocolitica* strain NCTC10462. Again, no attempt was made to investigate differences in degrees of reaction with organisms incubated at different temperatures. Control tests with pre-immunisation sera and with dilutions of medium from 1 in 10 to 1 in 10 000 000 against antisera were negative. *Streptobacillus moniliformis* antigen also gave negative results with the test antisera.

Fluorescent-antibody tests

These were particularly useful in providing a means of simultaneously demonstrating morphological and serological relationships. Observations

TABLE I
Agglutination titres of Yersinia enterocolitica large spheroplast L-form/small coccal forms (SLCF), and revertant bacteria

Antisera to <i>Y. enterocolitica</i> , strains (and incubation temperature)	Agglutination titres of sera tested against indicated strains (and incubation temperature) of <i>Y. enterocolitica</i>							
	NCTC 10460 (RT)	NCTC 10460 (37°C)	SLCF (RT)	SLCF (37°C)	RB (RT)	RB (37°C)	NCTC 10462 (RT)	NCTC 10462 (37°C)
NCTC10460 (RT)	3840	3840	480	960	7680	960	3840	960
NCTC10460 (37°C)	480	480	120	120	240	240	1920	960
SLCF (37°C)	960	1920	240	960	240	960	960	7680
NCTC10462 (37°C)	480	960	240	480	1960	240	3840	7680

RT = Room temperature.

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FIG. 1.—Large spheroplast L-forms in microcolony, 12 h at 37°C. Phase contrast. $\times 3000$.

FIG. 2.—Large spheroplast L-form and small coccal forms, 12 h at 37°C. Phase contrast. $\times 3000$.

FIGS. 3 and 4.—Tadpole-shaped forms incubated 12 h at room temperature. Phase contrast. $\times 3000$.

FIG. 5.—Revertant rod forms at colony edge, 48 h at 37°C. Phase contrast. $\times 3000$.

FIGS. 6 and 7.—Large spheroplast L-forms, incubated in fluid medium for 12 h at 37°C. Phase contrast. $\times 3000$.

FIG. 8.—Streptobacillus form incubated 12 h at 37°C. Phase contrast. $\times 3000$.

FIG. 9.—Reverted rod forms, incubated for 12 h at 37°C. Phase contrast. $\times 3000$.

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FIG. 10.—Parent rod form incubated for 12 h at 37°C. Electron micrograph (EM) section. $\times 10\,200$.

FIG. 11.—Large spheroplast L-forms, incubated for 12 h at 37°C. EM section. $\times 10\,200$.

FIG. 12.—Reverted rod forms incubated for 12 h at 37°C. EM section. $\times 10\,200$.

FIGS. 13 and 14.—Parent form incubated for 12 h at 37°C. EM section. $\times 100\,000$.

FIG. 15.—Large protoplast L-form incubated for 12 h at room temperature. EM section. $\times 100\,000$ and $30\,000$.

FIG. 16.—Small coccal form incubated for 24 h at 37°C. EM section. $\times 50\,000$.

FIG. 17.—Small coccal form incubated for 48 h at room temperature in fluid medium. EM section. $\times 54\,000$.

FIG. 18.—Large coccal form incubated for 48 h at room temperature in fluid medium. EM section. $\times 63\,000$.

FIG. 19.—Large spheroplast L-form incubated for 48 h at room temperature in fluid medium. EM section. $\times 32\ 000$.

FIG. 20.—Large spheroplast L-forms incubated for 48 h at room temperature in fluid medium. EM section. $\times 26\ 000$.

FIG. 21.—Large spheroplast L-forms incubated for 21 h at room temperature. EM section. $\times 20\ 000$.

FIGS. 22–24.—Large spheroplast L-form incubated for 48 h at room temperature in fluid medium. EM sections. $\times 16\ 000$, $\times 32\ 000$, $\times 32\ 000$.

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FIGS. 25 and 26.—Enlargements from figs. 22 and 24. $\times 200\ 000$.

FIG. 27.—Portion of tubular structure in large spheroplast L-form incubated for 12 h at room temperature. EM section. $\times 200\ 000$.

FIGS. 28 and 29.—Large spheroplast L-form incubated for 24 h at 37°C . EM sections. $\times 45\ 000$, $\times 38\ 000$.

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FIGS. 30-32.—Portion of fibrillar material in large spheroplast L-form incubated at 37°C for 24 h. EM sections. $\times 50\,000$, $\times 100\,000$, $\times 300\,000$.

FIG. 34.—Impression preparation of large spheroplast L-form colony incubated at 37°C for 12 h. Stained with fluorescent antibody and antibody to strain NCTC10460. $\times 3000$.

FIG. 35.—Impression preparations of small coccal forms incubated at 37°C for 24 h. Stained as in fig. 34. $\times 3000$.

FIG. 36.—Impression preparation of large spheroplast L-forms, small coccal forms and tadpole-shaped forms incubated at room temperature for 12 h. Stained as in fig. 34. $\times 3000$.

FIG. 37.—Large spheroplast L-form producing rod-form, taken from culture incubated at 37°C for 12 h. Stained as in fig. 34. $\times 3000$.

FIGS. 38 and 39.—Impression preparations of streptobacillus stage showing coccal and rod forms, incubated at 37°C for 12 h. Stained as in fig. 34. $\times 3000$.

FIG. 33.—Gel-diffusion precipitin reactions. Antigens (Ag) of *Y. enterocolitica* strain NCTC10460 (Y.e.1), spheroplast L-form/small coccal forms (SLCF), revertant bacteria (RB) and *Y. enterocolitica* strain NCTC10462 (Y.e.2) against antiserum (As) to *Y. enterocolitica* strain NCTC10460.

TABLE II

Fluorescent antibody reactions of Yersinia enterocolitica, large spheroplast L-form/small coccal forms (SLCF) and revertant bacteria (RB)

Antisera to <i>Y. enterocolitica</i> strains (and incubation temperature)	Reaction of sera tested against indicated strains (and incubation temperature) of <i>Y.</i> <i>enterocolitica</i>			
	NCTC 10460	NCTC 10460 SLCF	NCTC 10460 SBI	NCTC 10460 RB
NCTC10460 37°C	—	—	++	—
NCTC10460 RT	+++	+++	+++	+++
SLCF 37°C	—	+++	+++	—
NCTC10462 37°C	—	—	++	—

— = No reaction; ++ = moderately positive reaction; +++ = strong positive reaction.

were made on impression preparations of all the stages in the developmental cycle, and the serological relationships between these stages were confirmed. The results of some cross reactions are shown in table II, in which it can be seen that the SLCFs cross-reacted with the parent and revertant strains, and figs. 34–39 show the serological relationships between the morphological types already described under *Phase-contrast microscopy*. The antiserum to *Y. enterocolitica* strain NCTC10460 was used in all the preparations from which the illustrations were taken. Tests with pre-immunisation sera were negative. Fig. 34 shows a microcolony containing stained large spheroplast L-forms and fig. 35 shows stained small coccal forms, indicating that, as in the slide-agglutination tests, these two forms, which cannot be separated, are nevertheless each serologically related to the parent form. That the large spheroplast L-forms

stain evenly while the coccal forms stain only at the periphery may be because the latter possess a thicker cell wall as has been demonstrated by electron microscopy. Tadpole-shaped forms can be seen stained in fig. 36, and fig. 37 shows a large spheroplast L-form giving rise to a rod, indicating that these two are alike serologically; the same applies to the streptobacillus types of colonies showing filaments and spheroplast forms (figs. 38 and 39).

DISCUSSION

The evidence presented in this paper demonstrates that a strain of *Y. enterocolitica* can pass through a developmental cycle interpretable as an L-cycle.

In this instance the L-forms described occurred without any special inducing factors and their relationship with parent and revertant bacteria is established by the evidence. Appearances suggesting the presence of L-forms within colonies examined *in situ* were observed, but not separate classical 'fried egg' colonies. Thus, it appears that whereas these strains of *Y. enterocolitica* are capable of producing L-forms, the rod-form is normally the dominant form under these conditions of culture.

The effect of temperature upon the reactions of *Y. enterocolitica* is well-documented and it has now also been shown to affect the L-form. The reversion process and especially the production of tadpole-shaped forms occurred much more readily at room temperature than at 37°C although in fluid medium the organisms tended to remain in the SLCF regardless of temperature. They were, like the conventional rod forms, motile at room temperature but not at 37°C, and resembled classical *Y. enterocolitica* in their Voges-Proskauer reaction, being positive at room temperature and negative at 37°C, whereas the parent rod form was atypical, being negative at both temperatures. In all other biochemical tests the SLCF and rod-forms were identical, thereby supporting their relatedness.

Further confirmation of the relationship between the various forms described is provided by the serological tests. Agglutination, gel-diffusion and fluorescent-antibody results show relationships between the parent forms, the SLCFs, the revertant rods and another strain of *Y. enterocolitica*. Because it was not found possible to obtain small coccal forms free from large spheroplast L-forms, no conclusions could be drawn concerning differences in the antigenic structure of the different stages from small differences in cross reactions. The microscopic observations of the separate agglutination of large spheroplast L-forms and small coccal forms by the bacterial antisera and the individual staining by fluorescent antibody of the two forms (figs. 34 and 35) provided evidence that they were, in fact, each related serologically to the parent forms. The fluorescent antibody staining also demonstrated the serological relatedness of the large spheroplast L-forms to the rod forms to which they were giving rise (fig. 37).

Although the revertant forms seemed to be identical to the parent bacteria

in most respects, the irregular contours of the revertant bacteria (fig. 12) as compared with those of the parent organisms (fig. 10)—a phenomenon that also occurs in the *Proteus* L-cycle (Panchas and Schor, 1976)—may indicate a permanent ultrastructural change.

The ultrastructural studies taken in conjunction with observations on their behaviour, showed the large coccal forms to be spheroplast L-forms, rather than protoplast L-forms (Hofschneider and Martin, 1968), because they retained cell-wall material, even though they had lost rigidity. Although spheroplast L-form was the term selected, B-type L-form (Dienes and Bullivant, 1968) would have served equally well.

Further studies revealed more similarities between the large spheroplast L-forms of *Y. enterocolitica* and other L-forms studied by other workers. For example, in the forms described in this paper and in B-type L-forms of *Proteus* (Dienes and Bullivant, 1968), granular structures occur between the cell-wall and cytoplasmic membrane (fig. 19). Other similarities to the internal structure of the large spheroplast L-forms are apparent in the work of Green, Heidger and Domingue (1974) on group-D streptococcal L-forms. These workers published electron micrographs of sections of L-cells of the protoplast type, showing varying degrees of vesiculation, which they believed to be stages in the development of highly vesicular mother cells. They postulated that elements, several times smaller than mature mother cells, were stem cells capable of development into mother cells, of direct reversion to parental bacteria or of division by budding. Similarly, it seems that the large spheroplast L-forms of *Y. enterocolitica* can develop in the following ways: produce mother-cells (fig. 21), that is to say, cells containing not only membranous structures but also smaller elements which may function as elementary bodies; divide by budding (fig. 1); develop into revertant rod-forms through tadpole-shaped forms (figs. 3 and 4) or small coccal forms (fig. 2); give rise to cells resembling the mini-cells occurring in some strains of *E. coli* (Dennis and Rogerson, 1975), the fates of which were not determined, although some probably developed into tadpole-shaped forms, either directly (fig. 18) or through the small coccal-form stage (fig. 17); or give rise to a *Streptobacillus moniliformis* type of growth (fig. 8).

Membranous (figs. 20–27) and fibrillar (figs. 28–32) structures have been described in this paper and some of the former have similarities to the lamellate or mesosome-like structures seen in the L-forms of group-A streptococci (Cole, 1968; Kats *et al.*, 1977), group-D streptococci (Green *et al.*, 1974), *E. coli* (Eda, Kanda and Kimura, 1977) and *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Salmonella typhi*, *Proteus vulgaris*, *Vibrio* NAG and *Brucella abortus* (Kats *et al.*, 1977). Eda and Kats and their co-workers believe such structures are, indeed, L-form mesosomes and it seems likely that at least some of the structures described in this paper behave as mesosomes especially because the large spheroplast forms in which they occur seem to be in an active form from which they can develop in several directions. Fig. 25, in particular, resembles the electron micrographs of *Vibrio* NAG and *S. typhi* published by Kats *et al.* (1977). Possibly the structure shown in fig. 22 represents a primitive type of spore. The fibrillar structures (figs. 28–32) bear superficial resemblance to the

fibrillar and lamellate structures described by Corfield and Smith (1970) in group-A streptococcal L-forms but differ in that the fibrils are rigid, apparently solid (fig. 32) and not attached to the cytoplasmic membrane. At present, no interpretation of their composition or function can be made and it is apparent that further research is needed on L-form intracytoplasmic systems before their nature can be properly understood.

Whether the occurrence of L-forms such as those described in this paper is a widespread phenomenon in *Y. enterocolitica* and whether they have a role in the organism's sometimes obscure role in disease processes remains to be seen.

SUMMARY

Phase-contrast and electron microscopic, serological and biochemical evidence is advanced to show that a strain of *Yersinia enterocolitica* can pass through a developmental cycle interpretable as an L-cycle.

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