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Non-motile *Escherichia coli* O55, B5 Strains

By JOYCE WRIGHT*

St Ann's General Hospital, London, N. 15

SUMMARY: A description is given of twelve non-motile *Escherichia coli* O55, B5 strains isolated during an outbreak of diarrhoea. Possible uses of such strains in serological practice and in epidemiological investigations are indicated.

Knowledge of the *Escherichia coli* group has been considerably extended by the studies of Kauffmann (see review, 1947), Vahlne (1945) and Knipschildt (1945, 1946) upon their antigenic structure. They used the term 'K antigens' to comprise the A (capsular) and L and B (thermolabile, surface) antigens; strains possessing K antigen were not agglutinated in the living state by homologous O antiserum and were described as O-inagglutinable (Kauffmann, 1944). B antigens differed from L antigens in that their agglutinin-binding power was not destroyed by heating; treatment of an OB antiserum with an O suspension of the homologous strain resulted therefore in the absorption of both the O and B agglutinins (Knipschildt, 1945). The O (somatic) antigens were shown to be thermostable and to resist heating at 100°.

Giles, Sangster & Smith (1949) reported the isolation from infants with diarrhoea of a specific serological type of *Escherichia coli* which they termed the β variety. Subsequently, Kauffmann & Dupont (1950) found that such strains possessed the antigens O 55 and B 5; their H antigens have been reported as types 2 (Laurell, 1952), 4 (Grönroos, 1954), 6 (Kauffmann & Dupont, 1950), 7 (Krepler & Zischka, 1952), 11 and 21 (Le Minor, Le Minor, Nicolle & Buttiaux, 1954).

The present paper gives an account of twelve non-motile strains of *Escherichia coli* O55, B5. They were isolated by Dr K. B. Rogers, in 1952, during an outbreak of infantile diarrhoea in a residential nursery in Birmingham. Nine of the strains were derived from young children, one from a nurse and two from dust. Three strains were selected for more detailed investigation and have been designated *E. coli* 886 (from a child), 899 (from a nurse) and 916 (from dust).

METHODS

Serological and biochemical tests. O and OB antisera were prepared with a representative *Escherichia coli* O55, B5, H6 strain, designated β 80; an OB antiserum with one of the test strains, *E. coli* 886; and antisera, for H agglutinin testing, with *E. coli* strains devoid of O55, B5 antigens, namely, Bi 7455/41 (O43, K?, H2), 5680 (O111, B4, H4), A20a (O2, K1, H6), and U5/41 (O1, K1, H7). In addition, two antisera were kindly provided by Dr J. Smith

* Member of the Medical Research Council's Scientific Staff.

for use in testing for H reactions; they were prepared with *E. coli* Su 4321/41 (O13, K11, H11) and U11a/44 (O8, K?, H21).

Particulars of the preparation of *Escherichia coli* antisera, of their examination for certain non-specific agglutinins, and of the serological and biochemical methods used in testing *E. coli* strains have already been reported (Wright & Villanueva, 1953a, b).

Tests for motility. Each of the twelve strains under investigation was subcultured into two Craigie tubes (Craigie, 1931) containing semi-solid nutrient agar (0.3 % agar content), one tube being incubated at 37° and the other at room temperature. The progress of the growth was observed daily. Examination of the outer growth for motility was made on the day when it was first observed on the surface of the medium and was repeated on each of the two subsequent days. Each Craigie tube culture was transplanted serially through a second and then a third Craigie tube; these were incubated under the same conditions as the first and were examined for motility in the same way.

In addition, strains 886, 899 and 916 were subcultured daily for 12 days in semi-solid nutrient agar stabs, one series with each strain being incubated at room temperature and the other at 37°. Each culture was examined daily for motility.

Tests for motility were carried out by the microscopic examination of hanging-drop suspensions in normal saline.

Staining for flagella. Stained preparations of strains 886, 899 and 916 were examined for the possible presence of flagella. Two 18 hr. MacConkey agar stab cultures and two 6–8 hr. nutrient broth cultures of each strain were tested, one of each duplicate set having been incubated at room temperature and the other at 37°. O55, B5 strains having H antigens 2, 6 or 7 were cultured in parallel, and on each slide prepared for the staining of a test strain one or other of these motile cultures was included as a control.

Mordant was freshly prepared by mixing 20 ml. 10 % (w/v) aqueous tannic acid with 20 ml. saturated aqueous potassium alum and then adding 2 ml. 10 % (w/v) ferric chloride; after standing for 30 min., it was filtered through a Whatman no. 1 filter-paper. A little growth from each solid medium culture was transferred to 4 ml. distilled water and the suspension allowed to stand for 5 min.; each broth culture was centrifuged and the deposit also treated in this way. A loopful of each suspension was spread on a microscope slide and left to dry in air. Mordant was applied to the slide preparation for 3–5 min., washed off with distilled water and the slide gently blotted to remove excess water. Steaming Fontana's silver nitrate solution was flooded on to the slide and left for 3–5 min. The stain was then washed off with distilled water, the slide gently blotted, dried in air, mounted in Canada balsam and examined immediately under the oil-immersion lens.

RESULTS

Serological tests

O-inagglutinability and B antigen tests. β 80 O antiserum at dilutions higher than 1/80 did not agglutinate living suspensions of the twelve strains under investigation; it agglutinated to a titre of 1/3200 their steamed suspensions.

The strains were thereby shown to be 'O-inagglutinable', indicating that each possessed a thermolabile surface antigen (see Kauffmann, 1944). That this antigen was of the B kind was demonstrated by the results of the following test. β 80 OB antiserum 1/25 was absorbed with O suspension of each of the strains 886, 899 and 916, and the absorbed antiserum was tested with saline suspensions of β 80 and of the three strains used for the absorption. In each instance, the absorption of both the O and B agglutinins was complete.

Tube-agglutination tests. 886 OB antiserum agglutinated OB suspensions of β 80 and of the twelve strains under test to a titre of 1/800. It agglutinated O suspensions of all these strains to a titre of 1/6400. It gave no agglutinative reactions with strains having α -antigen of Stamp & Stone (1944), β -antigen of Mushin (1949), a non-specific O antigen (strain 'Straughan') or Vi antigen.

β 80 OB antiserum agglutinated OB suspensions of β 80 and of the twelve strains to a titre of 1/400.

β 80 O antiserum agglutinated O suspensions of β 80 and of the twelve strains to a titre of 1/3200.

Slide-agglutination tests. Both 886 and β 80 OB antisera gave prompt and complete slide agglutination of saline suspensions of β 80 and of the twelve strains.

Agglutinin-absorption tests. 886 OB antiserum 1/25, using OB antigens, (a) after absorption with β 80, was tested with this strain and with each of the strains 886, 899 and 916, and (b) after absorption with each of these three strains, was tested with β 80 and with the strain used for the absorption. Absorptions were carried out in the same way with (a) 886 OB antiserum 1/50, using O antigens, (b) β 80 OB antiserum 1/25, using OB antigens and (c) β 80 O antiserum 1/50, using O antigens. In each instance, absorption of the O or OB agglutinins, respectively, was complete.

Possible H antigens in test strains. Investigations were made for the possible possession of H antigens by the strains 886, 899 and 916. Accordingly, 8 and 18 hr. nutrient broth cultures and formol-saline suspensions from the surface growth of (a) Craigie tube cultures in semi-solid nutrient agar, and (b) MacConkey agar stab cultures were prepared. Each type of culture was incubated in duplicate at room temperature and at 37°. Tube-agglutination tests were carried out with these suspensions, using antisera prepared with *Escherichia coli* strains possessing H antigens of type 2, 4, 6, 7, 11 or 21 but devoid of O55, B5 antigens. No H agglutination reactions were observed at antiserum dilutions 1/50–1/12800. Conversely, 886 OB antiserum was tested for the possible presence of H agglutinins of types 2, 4, 6, 7, 11 or 21. *E. coli* strains possessing H antigen of one or other of these types, but devoid of O55, B5 antigens, were cultured in MacConkey agar stabs at room temperature for 18 hr. and formol-saline suspensions prepared from them. H agglutination reactions were not observed at antiserum dilutions 1/50–1/12800. In every series of tests, appropriate control tests of the H antisera and suspensions were included and they yielded results which demonstrated the activity of the reagents employed.

Tests for motility

The period taken for the growth of the twelve strains to progress through the Craigie tubes to reach the outer surface of the medium varied from 2 to 15 days. Inspection showed a characteristically non-motile type of *Escherichia coli* growth which extended up the outer side of the inner tube and across the surface of the medium but did not diffuse through it. Motile organisms were not observed in the microscopic examinations of the cultures.

The growth of strains 886, 899 and 916 throughout twelve daily passages in semi-solid nutrient agar stab cultures remained of the non-diffusive type and motile organisms were not observed by microscopic examination.

Flagella staining

Flagella were not observed in the stained preparations of strains 886, 899 and 916. The control preparations of motile O55, B5 strains possessing H antigens of types 2, 6 or 7 all showed well-marked flagella development.

Biochemical reactions

The twelve strains under test produced acid and gas within 24 hr. at 37° in arabinose, glucose, lactose, maltose, mannitol, and xylose, and in MacConkey broth at 44°; produced acid or acid and gas between 24 and 48 hr. at 37° in sucrose and between 48 and 72 hr. in rhamnose; failed to ferment dulcitol, salicin, inositol, or inulin or to liquefy gelatin. They were methyl red-positive and Voges-Proskauer-negative; they produced indole, reduced nitrates to nitrites, did not utilize citrate, and did not produce urease.

DISCUSSION

Motile strains of *Escherichia coli* O55, B5 have been isolated from infants with diarrhoea by a number of workers during recent years (Giles *et al.* 1949; Kauffmann & Dupont, 1950). In 1953, however, Le Minor described three non-motile *E. coli* O55, B5 strains received by her from Copenhagen and particulars of twelve non-motile *E. coli* O55, B5 strains isolated in England are reported here. The latter strains differed in their fermentative reactions from the non-motile strains of Le Minor (1953) and from motile *E. coli* O55, B5 strains possessing H antigens of types 2, 4, 6, 7, 11 or 21 (Kauffmann & Dupont, 1950; Wright & Villanueva, 1953*a*; Le Minor *et al.*, 1954; Grönroos, 1954); they formed, nevertheless, a biochemically homogeneous group. The twelve non-motile strains had been isolated from different individuals, or from dust, during an outbreak of diarrhoea in a nursery, and the uniformity of their biochemical activities suggests that such reactions may be of use in defining stable subtypes applicable to epidemiological investigations.

Two further points of interest in connexion with non-motile strains of *Escherichia coli* O55, B5 are their possible use in the preparation of OB antisera free from H agglutinin (Wright & Villanueva, 1954) and in H antigen transduction experiments such as those undertaken with salmonella strains by Stocker, Zinder & Lederberg (1953).

Finally the importance of method in the examination of *Escherichia coli* strains for their possible motility may be stressed. It is evident from the work of Stuart & Carpenter (1949) that the majority of *E. coli* strains are motile; careful investigation of strains under conditions optimal for the development of motility (Wright & Villanueva, 1953*b*) is therefore required before this characteristic can be excluded and a report of non-motility be made.

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