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BIOCHEMICAL PROPERTIES OF HAEMOLYTIC E. COLI STRAINS BELONGING TO O-GROUPS 138, 139 AND 141 [1])

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A previous paper, *Månsson* (1962), described a material of 279 haemolytic E. coli strains, 209 of which could be serologically grouped with 5 O-sera. Serotype O 138:K 81(B):(H 14) was represented by 79 strains, O 139:K 82(B):H 1 by 6 strains, and O 141:K 85(B):(H 4) by 86 strains. From the available writings on the subject it appears that the biochemical properties of these serotypes have been studied to only a limited extent.

Gitter & Lloyd (1955) examined 4 strains which were reported to resemble typical E. coli biochemically. Of the 28 strains of haemolytic E. coli studied by *Quinchon, M. Henry & Mme G. Henry* (1959), 16 were biochemically identical and the other strains represented 5 different fermentation patterns. The 16 strains were also urease positive. No serological formulae were given for their strains. *Rees* (1959) has described results for strains belonging to the serotypes O 138:K 81(B) and O 139:K 82(B) as well as RVC 2909 (later shown to be O 141:K 85(B)). All 3 serotypes were indole positive, methyl-red positive, and Voges-Proskauer negative and none grew in sodium citrate. All 3 serotypes fermented glucose, lactose, maltose, mannitol, arabinose, sorbitol, rhamnose, trehalose, and xylose with gas formation. Inositol was not fermented. Serotypes O 138:K 81(B) and O 139:K 82(B) fermented saccharose and dulcitol promptly while O 141:K 85(B) required some days for this reaction. Both O 138:K 81(B) and O 139:K 82(B) were negative in salicin; O 141:K 85(B) was positive but only after several days. This serotype was negative in sorbose but O 138:K 81(B) and O 139:K 82(B) were positive, the latter after

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several days. No tests for urease activity were reported. The strains referred to by *Hess & Suter* (1959) as S_1 (O 139:K 82(B):H 1) gave varying results in dulcitol, sorbitol, and salicin. Half the strains were positive in sorbitol, the majority were positive in sorbose and the majority of the strains were negative in salicin. The strains required 48 hours to ferment saccharose, had no urease activity, and did not grow in KCN medium. *Ørskov et al.* (1960) have reported the biochemical reactions of a strain with the serological formula O 141: K 85(B):H 4. What was particularly interesting was the negative reaction in saccharose, even after incubation for 14 days, and the prompt fermentation of dulcitol. The strain was positive in salicin after 2 days and negative in sorbose as well as in ammonium citrate, urea, and KCN.

This paper is an account of the biochemical reactions of 171 strains representing the serotypes O 138:K 81(B):(H 14), O 139:K 82 (B):H 1, and O 141:K 85(B):H 4.

These strains form part of the material described in a previous paper (*Månsson,* 1962).

METHODS

Conventional methods were used throughout and will be mentioned only briefly here. Fermentation tests were carried out in meat-extract broth to which one per cent of the respective sugars had been added together with brom-cresol-purple as an indicator. Durham tubes were used. The strains were tested in adonitol, dulcitol, sorbitol, sorbose, arabinose, xylose, rhamnose, maltose, salicin, inositol, lactose, saccharose, mannitol, and glucose. The observation time was 14 days.

The methyl-red and Voges-Proskauer reactions were carried out in buffered peptone-glucose broth. Test reagents were a 0.25 per cent alcoholic solution of methyl red (MR-reaction) and a potassium hydroxide and creatinine solution (VP-reaction), see *Ewing,* 1960. The formation of indole was examined in casein broth by the method of *Kristensen et al.* (1925). Ehrlich-Böhme's reagent was used. The nitrate reaction was carried out in peptone water with the addition of 0.02 per cent nitrite-free KNO_3 and with sulphanilic acid and alphanaphthylamine as indicator.

The hydrogen-sulphide test was made in Bacto lead acetate agar (Difco). Tests for the production of urease were made in *Christensen's* (1946) urea agar. Gelatin liquefaction was followed by the manner described by *Ewing* (1960). Koser's salt solution was used for testing the ability of the organisms to grow in citrate. The KCN test was carried out by *Møller's* (1954) method. Tests

for the decarboxylation of lysine were made in the manner described by *Falkow* (1958). After inoculation, however, a layer of sterile paraffin oil was added to each tube. Arginine breakdown was tested in the same way.

RESULTS

For the sake of clarity the results have been summarised in Table 1. Qualitative differences have not been included in the table. Except for those which were urease positive, the strains

Table 1.

Biochemical behaviour of the haemolytic E. coli strains.

O-group	138		139		141	
No. of strains	79		6		86	
	+	—	+	—	+	—
Motility (semi-solid medium)	6 (8)	73 (92)	6		78 (91)	8 (9)
Adonite	4 (4)	75 (96)		6		86
Dulcitol	78 (99)	1 (1)	5	1	79 (92)	7 (8)
Sorbitol	79		6		86	
Sorbose	62 (78)	17 (22)	4	2	19 (22)	67 (78)
Arabinose	79		6		86	
Xylose	79		6		86	
Rhamnose	79		6		86	
Maltose	79		6		86	
Salicin	9 (11)	70 (89)		6	41 (58)	45 (52)
Inositol		79		6		86
Lactose	79		6		86	
Saccharose	73 (92)	6 (8)	2	4	5 (6)	81 (94)
Mannitol	79		6		86	
Glucose	79		6		86	
Indole	75 (96)	4 (4)	6		79 (92)	7 (8)
H_2S		79		6		86
Gelatin		79		6		86
MR	79		6		86	
VP		79		6		86
KNO_3	79		6		86	
Urease activity	52 (66)	27 (34)	5	1	48 (56)	38 (44)
Na-citrate	2 (3)	77 (97)		6	7 (8)	79 (92)
KCN	4 (5)	75 (95)		6	11 (13)	75 (87)
L-lysine	46 (58)	33 (42)	3	3	34 (40)	52 (60)
L-arginine	8 (10)	71 (90)		6	44 (51)	42 (49)

+ = reaction
— = no reaction
The figures in brackets refer to relative numbers in per cent.

have given typical reactions for E. coli. O-group 138 included 4 strains which were adonitol positive, 4 which were indole negative, and 2 which were citrate positive. Since these properties resemble those of the Klebsiella group it can be pointed out that none of the strains examined here had all 3 of these characteristics. The 4 adonitol-positive strains, for example, were also indole positive. It was only in O-group 138 that adonitol-positive strains were encountered and the reaction was weak. Most of the strains were also dulcitol positive with the exception of 7 strains in O-group 141 and one strain in each of the 138 and 139 groups. All the strains were sorbitol positive. A positive reaction in sorbose was given by 78 per cent of the O 138 strains. There were both sorbose-negative and sorbose-positive biotypes among the O 139 strains. The majority (78 per cent) of the O 141 strains, however, were sorbose negative. All strains gave positive reactions in arabinose, xylose, rhamnose, and maltose. In salicin, 89 per cent of the O 138 strains but only 52 per cent of the O 141 strains were negative. The 6 strains in O-group 139 were negative. All strains were inositol and lactose positive. In saccharose, 92 per cent of the O 138 strains were positive but 94 per cent of the O 141 strains were negative. O-group 139 contained both saccharose-positive and saccharose-negative strains. All the strains were positive in mannitol and glucose. Gas as well as acid was formed. A few of the O 141 strains (8 per cent) and of the O 138 strains (4 per cent) were indole negative. None of the strains formed H_2S or liquefied gelatin. All the strains were MR positive and VP negative and all reduced nitrate. Many of the strains were urease positive. In O-group 138 66 per cent of the strains were considered to be positive, in O-group 141 56 per cent, and in O-group 139 5 of 6 strains. It can also be seen from the table that some strains were positive in sodium citrate, 3 per cent in O-group 138 and 8 per cent in O-group 141. Thirteen per cent of the O 141 strains and 5 per cent of the O 138 strains were judged to be positive in KCN medium. Lysine decarboxylation was also observed for 46 per cent of the O 138 strains, 3 of 6 O 139 strains, and 40 per cent of the O 141 strains. A few strains in O-group 138 (10 per cent) and relatively more strains in O-group 141 (51 per cent) could break down arginine.

Certain qualitative differences could be discerned between the strains of different O-groups. Strains belonging to O-group 138 usually fermented sugars either promptly (24 hours) or not

at all. The 4 adonitol-positive strains, however, showed a positive but weak reaction only after 3 to 5 days. In dulcitol 12 of the strains developed a positive reaction after 2 to 3 days, in salicin 6 of 9 strains after 3 to 6 days, and in saccharose 10 strains only after 2 to 3 days. No biochemical differences were observed between motile and non-motile strains. The 6 strains in O-group 139 also usually fermented sugars promptly, in one day. Two strains, however, showed a positive reaction in saccharose after 2 days and one strain a positive reaction in dulcitol after 6 days. Of the 79 dulcitol-positive strains in O-group 141, 31 (or 36 per cent) developed a positive reaction after 24 hours and 48 (or 56 per cent) became positive after 2 to 4 days. In salicin a positive reaction was observed after 2 to 4 days for 37 of the strains (43 per cent) giving this reaction. Five sorbitol-positive strains (6 per cent) gave this reaction after 48 hours, the others (94 per cent) after 24 hours. Xylose was fermented in 24 hours by 83 strains (97 per cent) and in 2 to 3 days by 3 strains.

The O 141 strains had K antigen 85(B) in one of two forms. Of 30 strains examined, 12 had K antigen 85ab(B) and 18 strains had K antigen 85ac(B). The biochemical differences between these strains are listed below.

	K 85ab(B) (12 strains)		K 85ac(B) (18 strains)	
Salicin	11 $+^{1-4}$	1 —	3 $+^{1-4}$	15 —
L-lysine	7 +	5 —	7 +	11 —
L-arginine	8 +	4 —	8 +	10 —
KCN	6 +	6 —	3 +	15 —
Indol	8 +	4 —	18 +	

Among the 11 salicin-positive strains in the group K 85ab(B) there was one which fermented salicin promptly. The others developed a positive reaction after 2 to 4 days. Unlike the other 11 motile strains (H 4), the salicin-negative strain was non-motile. One strain in the group K 85ac(B) fermented salicin promptly and the rest in 2 to 4 days. One strain in this group was non-motile but did not show any biochemical differences. The group K 85ab(B) was distinguished not only by the number of salicin-positive strains but also by having a majority of strains which could decarboxylate lysine, break down arginine, and grow in KCN medium. Four of the strains were in addition indole negative.

DISCUSSION

It is customary to regard as E. coli, cultures which form indole, lack urease activity, and which are VP negative and MR positive. They usually ferment lactose. Some strains can grow in ammonium medium containing sodium citrate but this property is variable. Several biotypes can be identified according to the sugars fermented. This definition for E. coli (*Kauffmann,* 1954) agrees closely with that given by the Enterobacteriacae Subcommittee (see *Edwards & Ewing,* 1955). One difference is that indole is "usually produced" according to the latter definition. Indole-negative cultures can be encountered and as an example can be cited the first O 111:B 4 strain isolated in Denmark (*Kauffmann & Dupont,* 1950).

According to these definitions the strains in the present material can be considered as typical E. coli with the exception of those which are urease positive (Table 1). This property of haemolytic E. coli has been mentioned by *Quinchon et al.* who found 16 of 27 strains to be urease positive but did not report the serological formulae for the strains they investigated and by *Ørskov et al.* (1961) who found some O 141 strains to be urease positive. This observation is somewhat disconcerting. The reaction has been carried out both in Christensen's urea agar and in a fluid medium according to M. Kristensen's modification (*Kauffmann,* 1954). The results have often been difficult to evaluate. Colour changes in the substrate were not as intensive or as rapid in comparison with those produced by Proteus strains. To attain a definitely positive reaction has generally required incubation for 2 days. Nor has further incubation resulted in an as intensive colour change of the medium as was obtained with Proteus strains. Furthermore, the results in fluid medium were less distinct and clear-cut than in solid medium. On the other hand, most of the strains examined gave a purple tinge which after 2 or 3 days extended through most of the solid medium. The results of this reaction should apparently be accepted with reservations for the present.

About 9 per cent (15 of 171) of the strains have grown in KCN medium within 2 days. All the strains examined by *Quinchon et al.* behaved typically in this respect and were negative.

Falkow's (1958) method for demonstrating decarboxylation of lysine did not give unequivocal results. The addition of paraffin to each tube according to *Møller* (1955), proved effective.

E. coli strains usually decarboxylate lysine and break down arginine (*Møller, Falkow*). About half the strains examined here did not decarboxylate lysine but the relative proportion varied in the different O-groups (Table 1). Arginine was broken down by only 10 per cent of the O 138 strains and by 44 per cent of the O 141 strains.

A number of strains have shown properties which are fairly uncommon for E. coli, mainly in urease activity but also in decarboxylation of lysine, arginine breakdown, and growth in KCN medium. There were also clear biochemical differences between the O-groups. The general validity of the observations on O-group 139 is severely limited, of course, by the small number of strains available for examination. On the other hand, there were significant differences between the O-groups 138 and 141. The O 138 strains fermented sugars either rapidly or not at all. The O 141 strains often required more than 24 hours to give positive fermentation results. The O 138 strains, unlike the O 141 strains, were on the whole sorbose positive, saccharose positive, and salicin negative (Table 1). Furthermore, they were usually nonmotile in semi-solid medium. The biochemical reactions, of course, can give no more than a hint of the particular serotype to which a particular strain is likely to belong. Study of a larger material is desirable in order to extend the observations recorded here.

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SUMMARY

The biochemical reactions of 171 strains of haemolytic E. coli were examined. Of these strains, 79 had the serological formula O 138:K 81(B) : (H 14), 6 belonged to the serotype O 139:K 82(B) : H 1, and 86 to the serotype O 141:K 85(B) : (H 4). The O 138 strains, unlike the O 141 strains, were usually sorbose positive, saccharose positive, and salicin negative (see Table 1). As a rule they fermented sugars either promptly or not at all. Several strains (105 of 171) were considered to show urease activity. This reaction is discussed. About 50 per cent of the strains decarboxylated lysine and about 25 per cent could break down arginine although the relative numbers of the strains with these properties were different for the various O-groups. Twelve indole-negative and 15 KCN-positive strains were encountered.

ZUSAMMENFASSUNG

Biochemische Untersuchungen von hämolytischen E. coli Stämmen den O-Gruppen 138, 139 und 141 zugehörig.

171 hämolytische E. Coli Stämme sind biochemisch untersucht worden. 79 Stämme hatten die serologische Formel O 138:K 81/B/: /H 14/, 6 Stämme O 139:K 82/B/:H 1 und 86 Stämme O 141:K 85/B/:

/H 4/. Die O 138-Stämme waren im Gegensatz zu den O 141-Stämmen in der Regel sorbosepositiv, saccharosepositiv und salicinnegativ (siehe Tabelle 1). Die Vergärungen finden meistens prompt nach einem Tage oder garnicht statt. Mehrere Stämme (105 von 171) wurden ureaspositiv bezeichnet. Die Reaktion wird diskutiert. C:a 50 % der Stämme dekarboxylierten Lysin und c:a 25 % spalteten Arginin. Die Prozentziffern waren verschieden bei verschiedenen O-Gruppen. 12 indolnegative und 15 KCN-positive Stämme kamen vor.

SAMMANFATTNING

Biokemiska undersökningar av hämolyserande E. coli stammar tillhörande O-grupperna 138, 139 och 141.

171 hämolyserande E. coli stammar undersöktes biokemiskt. Av dessa hade 79 st den serologiska formeln O 138:K 81(B):(H 14), 6 st formeln O 139:K 82(B):H 1 och 86 st formeln O 141:K 85(B):(H 4). O 138 stammarna voro i motsats till O 141 stammarna i regel sorbospositiva, sackarospositiva och salicinnegativa (se tabell 1). Som regel skedde förjäsningarna snabbt eller ej alls. Ett flertal stammar — 105 av 171 — betecknades som ureaspositiva. Reaktionen diskuteras. Ca 50 % av stammarna dekarboxylerade lysin och ca 25 % nedbröt arginin. Procentsiffrorna varierade i de olika O-grupperna. 12 indolnegativa och 15 KCN positiva stammar påvisades.

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