COLIFORM BACTERIA IN THE INTESTINE OF MICE*

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A recent study of the factors affecting the experimental colonization of mice with *Escherichia coli* 026:K60 (1) prompted a survey of the coliform bacteria normally present in the gastrointestinal tract of normal mice. The fact that the indigenous coliforms were not eliminated, but only temporarily suppressed by the introduced test strain of *E. coli* 026:K60, made it essential to identify the serotypes prevalent at any given time during the course of the experimental colonization. It proved of interest, furthermore, to differentiate between the resident and transient coliform strains in the normal intestinal flora in the mice colonies and to record the presence of other Gram-negative facultative aerobes.

Two colonies of mice, NCS and NCS-D, were investigated. Both are maintained at the Rockefeller University and are characterized by a fecal flora poor in Gram-negative facultative aerobic bacteria; they are somewhat more sensitive to certain experimental bacterial infections than are ordinary mice (2). Previous studies reported the presence of slow lactose-fermenting *E. coli* strains in these colonies (1, 3, 4). In addition, the NCS mice used in the present study harbored typical *E. coli*, which they have acquired following an accident in the breeding room; *Enterobacter*, *Klebsiella* were also occasionally cultured from them. The term coliform as used in this paper refers to bacteria belonging to the above mentioned groups.

Materials and Methods

Bacteriological studies were carried out with Tergitol-7 agar medium containing tetrazolium (for the recovery of coliforms) (4) and with a nonselective nutrient agar. The organisms were tested by standard biochemical methods (5).

It soon became apparent in the course of this work that some of the strains isolated from the mouse colonies were probably endemic, and diagnostic antisera were therefore prepared in rabbits to identify them. Four representative strains, slow and typical lactose-fermenting *E. coli*, were chosen for this purpose.

Broth cultures boiled for 2½ hr were used as somatic antigens for the preparation of the sera. These reagents made it possible to achieve a preliminary grouping of the organisms. Analysis of the antigenic structure of the isolated *E. coli* and *Klebsiella* strains was carried out at the Communicable Disease Center, Atlanta, with reagents made available through the courtesy of Dr. P. R. Edwards and Dr. W. H. Ewing. As there was uncertainty with label-

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ing the somatic antigens of the two most frequently recovered strains, a typical and slow lactose-fermenting *E. coli*, these cultures were further examined by Dr. F. Orskov and Dr. I. Orskov, at the International Escherichia Centre, Copenhagen.

RESULTS

It was found convenient to make a preliminary classification of the organisms isolated from the NCS and NCS-D mouse colonies into slow lactose-fermenting (SLF) and typical lactose-fermenting (LF) coliforms. These two groups were separated according to their appearance on Tergitol-7 agar (T7T) isolation plates after 24 hr at 37°C.

Slow Lactose-Fermenting Coliforms (SLF).—As seen in Table I, 96% of the slow lactose-fermenting coliform cultures isolated from NCS and NCS-D mice belonged to serotype *E. coli* 081±: :H21. This strain was recovered from

TABLE I

Slow Lactose-Fermenting Coliforms (SLF) in the Intestine of Mice*

Total No. of isolations	*E. coli* 081±: :H21			Miscellaneous serotypes		
	Isolations			Isolations		
	No.	Per cent	Source	No.	Per cent	Source
171	165	96	64 litters	6‡	4	6 litters

* SLF were encountered in NCS and NCS-D colonies.

‡ 4 of these strains were a homogeneous Enterobacterlike group.

feces of adult mice, and on autopsy from the intestine of young mice. As it constituted the predominant serotype among the coliform bacteria under investigation, the main characteristics of this organism will be described here in some detail.

On T7T plates the lactose nonfermenting colonies gave rise to lactose-fermenting daughter colonies after 48 hr at 37°C. The organisms isolated from these daughter colonies were identified as *E. coli* on the basis of the usual criteria (5).

Acid and gas (25% by volume) were produced in lactose media after 48 hr, and in salicin media after 5 days' incubation. Fermentation occurred within 24 hr in media containing the following sugars: glucose, mannitol, sucrose, maltose, raffinose, sorbitol, dulcitol, and rhamnose. No fermentation was observed with inositol, adonitol, and cellobiose. Lysine and ornithine gave a positive, and arginine a negative decarboxylase reaction.

Table II shows the results of O agglutinin-absorption tests, carried out by Dr. F. Orskov and Dr. I. Orskov, with a representative isolate R8 and type culture *E. coli* 081. On the basis of these tests, the somatic antigen of R8 is 081±. The symbol "+" denotes that this strain possesses an additional 0 antigenic factor to the test strain, and "—" means that it is not capable of depleting the test 0 serum of 0 antibodies. K factor has not been elucidated. The flagellar antigen was identified as H21 after serial passaging of the culture through semisolid agar.

E. coli 081±: :H21 was repeatedly recovered from mice during the 9 months of this survey and therefore is endemic in the two colonies under consideration. Further, the study of stock cultures maintained at Rockefeller University revealed that a strain which was recovered from NCS mice colony about 3 yr ago was biochemically and antigenically identical with the current isolates.

Of the six slow lactose-fermenting strains not corresponding to the above description, four Enterobacterlike organisms formed a biochemically homogeneous group (Table I). They produced a yellowish water nonsoluble pigment, fermented lactose without gas production after 5 days incubation, and liquefied gelatin. The two remaining strains were SLF *E. coli*, untypable with the available somatic 0 sera.

TABLE II

Agglutinin-Absorption Tests with E. coli 081

Antigen	Antisera	
	081	081 absorbed with R8
Test strain 081	≧5120	1280
R8	320	0
	OR8	OR8 absorbed with 081
Test strain 081	2560	0
R8	≧5120	80

Lactose-Fermenting Coliforms (LF).—Escherichia, Enterobacter, Klebsiella. As shown in Table III, *E. coli* was the predominant strain in the group of typical lactose-fermenting coliforms.

Amongst *E. coli* isolates, type 0109±:K48:H14 was most often encountered, especially in stools from adult mice. This strain exhibited a characteristic colony pattern on T7T plates. Prompt fermentation with the production of acid and gas was recorded in lactose, glucose, mannitol, maltose, raffinose, sorbitol, and rhamnose. Dulcitol revealed acid and gas production after 48 hr of incubation; sucrose was acidified in 24 hr but no gas was produced from this sugar during the 30 days observation period. Inositol, adonitol, and cellobiose were not fermented. The decarboxylase test was positive in lysine and ornithine, and negative in arginine medium.

Table IV shows the results of 0 agglutinin-absorption tests, carried out by Dr. F. Orskov and Dr. I. Orskov, with a representative isolate R10 and type culture *E. coli* 0109. The somatic antigen of R10 was found to be 0109±; the meaning of symbol "±" was previously explained.

E. coli 0109± :K48:H14 described above was endemic in the NCS colony, as

indicated by repeated isolations from several litters during the 9 month period of investigation. This strain was identical with a stock culture which was recovered from the colony about 3 yr ago.

The remaining *E. coli* strains were transient in occurrence (Table III). Of those tested, 21 were antigenically rough, and 1 was untypable with the available sera. Bacteria of group 068 appeared with some frequency. A few *Enterobacter-Klebsiella* strains of a very mucoid character were isolated and, amongst them, *Klebsiella* capsular type 63 was cultured on a few occasions.

TABLE III

A. Lactose-Fermenting Coliforms (LF) in the Intestine of Mice*

Total No. of isolations	*E. coli* 0109±:K48:H14			Miscellaneous serotypes		
	Isolations			Isolations		
	No.	Per cent	Source	No.	Per cent	Source
123	77	63	27 litters	46	37	30 litters

*B. Classification of 46 Miscellaneous Serotypes (LF)**

E. coli			*Klebsiella*			*Enterobacter*
No. of isolations	0 serotype	No. of isolation of each serotypes	No. of isolations	Capsular serotype	No. of isolations of each serotype	No. of isolations
35	68	12	9	63	7	2
	22	1		55	1	
	Rough	21		16	1	
	Untypable	1				

* LF were encountered in NCS colonies only.

Nonenterobacteriaceae Strains.—Pseudomonas aeruginosa was represented by 11 isolates which were recovered from 8 litters. The pyocyanin pigment was extracted from all these cultures.

A group of 21 isolates showed weak fermentative reactions and consisted mostly of strains which failed to grow on T7T medium, produced a yellowish water nonsoluble pigment, and lost viability on nutrient agar within a week after isolation.

Coliforms in other Litters of Mice.—An attempt was made to determine whether the *E. coli* serotypes predominant in NCS and NCS-D mice colonies at the Rockefeller University exist also in other mouse colonies.

Animals from from two sources, "Souris" and CFW, were tested. (*a*) The "Souris" colony was established by Dr. Robert Fauve at the Pasteur Institute (Garches, France) with ani-

mals of the NCS mouse colony obtained from the Rockefeller University 4 yr. ago (6). Some of Dr. Fauve's mice were brought back to New York in August 1964 and a new colony ("Souris") was developed from them at the Rockefeller University. These mice are still free of *E. coli*, as was the case for the original NCS colony. Of twelve SLF *E. coli* isolates obtained from the "Souris" colony, none was antigenically related to the type at present endemic in the NCS colony and described in this paper. These isolates were untypable with the available diagnostic 0 sera.

(*b*) CFW mice were obtained from a commercial producer (Carworth Farms, New City, New York). Of ten *E. coli* cultures from the CFW colony, eight proved to be typable; four of them were identified as 07, two as 06, and one each as 015 and 022. Of ten cultures of SLF *E. coli* which were tested, only one was typable (085).

In other words, all the cultures isolated from CFW were antigenically unrelated to the strains endemic in the NCS colony.

TABLE IV

Agglutinin-Absorption Tests with E. coli 0109

Antigen	Antisera	
	0109	0109 absorbed with R10
Test strain 0109	≧5120	2560
R10	1280	0
	OR10	OR10 absorbed with 0109
Test strain 0109	≧5120	0
R10	≧5120	320

DISCUSSION

Several reports indicate that particular strains of *E. coli* can persist in the intestine of human adults and infants for periods of a few weeks or months (7–9). The fact that the slow lactose-fermenting *E. coli* 081±: :H21 and the typical lactose-fermenting *E. coli* 0109±:K48:H14, have been continually present in mice of the NCS colony for the past 3 yr is consistent with the findings in man. The persistence of these strains is probably accounted for by the inbred nature of the colony which constitutes a "closed" type of community, and by the fact that animals are fed a pasteurized diet. Nevertheless, it is surprising that human handling, and exposure to ordinary environmental conditions such as unfiltered air and contaminated bedding, did not result in exogenous contamination.

Amongst the strains of SLF *E. coli* which were tested, 96% were found to belong to one serotype, namely *E. coli* 081±: :H21, indicating a well established host-parasite relationship. Amongst the *Escherichia-Enterobacter-Klebsiella* strains tested, *E. coli* 0109±:K48:H14 was predominant but

occurred less frequently than the serotype mentioned above. Other coliform strains proved to be transient in occurrence.

Many attempts have been made to explain the mechanism by which some *E. coli* strains become established in the intestine of man and animal despite competition by other strains. A study of the comparative antagonistic activity of various *E. coli* strains did not reveal any significant difference between enteropathogenic and nonenteropathogenic types in this regard (7). As shown earlier (1), colonization of the intestine of mice with *E. coli* 026:K60 suppressed the resident *E. coli* strains for only a limited period of time.

Experiments on the role of colicines in the intestinal tract have indicated that animals with the highest percentage of colicine-producing resident strains tend to have the most stable flora (10). On the other hand, other observations indicate that the antagonistic activity of resident strains is not greater than that of transient strains (6). Colicine production by the resident strains was not assessed in our study.

According to information from Dr. F. Orskov and Dr. I. Orskov, both *E. coli* 081 and 0109 strains were originally isolated from human sources by Knipschildt and Ewertsen, 081 from urine, and 0109 from feces. These strains are uncommon amongst cultures received for serotyping by the International Escherichia Centre, but only very few strains come from mice. The fact that the resident *E. coli* strains isolated in the course of this survey possess somatic 0 antigens apparently not of common occurrence in man or animal may be an indication of mouse host specificity.

SUMMARY

Mice of the NCS and NCS-D colonies, bred at the Rockefeller University, harbored in their intestine an endemic strain of slow lactose-fermenting *Escherichia coli* 081±: :H21 serotype. In addition, NCS mice have recently acquired *E. coli* 0109±:K48:H14. Both strains persisted during the period of observation, whereas they were not encountered in the feces of mice from two other colonies. Other coliform strains encountered were more transient in their occurrence.

Since strains of *E. coli* 081±: :H21 and 0109±:K48:H14 are extremely uncommon in human beings, it seems probable that they possess specificity for the mouse host.

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BIBLIOGRAPHY

1. Mushin, R., and Dubos, R., Colonization of the intestine of mice with *Escherichia coli*, *J. Exp. Med.*, 1965, **122**, 745.
2. Dubos, R. J., and Schaedler, R. W., The effect of the intestinal flora on the growth

rate of mice, and on their susceptibility to experimental infections, *J. Exp. Med.*, 1960, **111,** 407.

3. Dubos, R., Schaedler, R. W., and Costello, R. L., The effect of antibacterial drugs on the weight of mice, *J. Exp. Med.*, 1963, **117,** 245.
4. Schaedler, R. W., and Dubos, R. J., The fecal flora of various strains of mice. Its bearing on the susceptibility to endotoxin, *J. Exp. Med.*, 1962, **115,** 1149.
5. Edwards, P. R., and Ewing, W. H., Identification of Enterobacteriaceae. Minneapolis, Burgess Publishing Co., 1962, 2nd edition.
6. Fauve, R. M., L'emploi, enlpathologie expérimentale, de souris bactériologiquement propres La souche NCS, *Med. et Hyg.*, 1963, **21,** 178.
7. Sears, H. J., Brownlee, I., and Uchiyama, J. K., Persistence of individual strains of *Escherichia coli* in the intestinal tract of man, *J. Bact.*, 1950, **59,** 293.
8. Spaulding, E. H., Gunther, C. B., and Deutsch, E. T. Competitive antagonisms among *E. coli* strains, *Bact. Proc.*, 1961, 117.
9. Gage, P., Gunther, C. B., and Spaulding, E. H., Persistence of *E. coli* serotypes in the stools of infants, *Bact. Proc.*, 1961, 117.
10. Branche, W. C., Young, V. M., Gillem, H. C., and Massey, E. D., Observations on *Escherichia coli* as a component of the normal intestinal flora; effect of colicin production, *Bact. Proc.*, 1960, 134.