ASPECTS OF COLONISATION OF THE MOUSE INTESTINE BY COLIFORM BACTERIA

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MUSHIN AND DUBOS (1965) have described the successful colonisation of the intestine of suckling mice of the NCS colony by orally administered enteropathogenic *Escherichia coli* O26:B6. The important features associated with the experimental colonisation were: susceptibility to infection at the suckling stage, a high mortality with large doses of the bacteria and the clearing of the implanted organism starting at approximately the weaning stage. It was suggested that the composition of the indigenous intestinal flora of NCS mice may play a role in their susceptibility to infection.

The reported studies prompted further investigations with the following main aims in view: (a) to determine whether the suckling NCS mouse could be used as a biological model for intestinal colonisation with various enteropathogenic *E. coli* serotypes; (b) to establish whether enteropathogenic *E. coli* serotypes could be differentiated from non-pathogenic coliforms on the basis of pathogenicity to NCS mice; and (c) to study the dynamics of the initial implantation of these organisms in the gut of the mouse and to investigate factors affecting the colonisation and the subsequent clearing of the infection.

In the present paper consideration was given to the possibility of some antagonistic factors in the gut being able to influence the implantation and the clearance of the coliforms. The possible existence of an immune mechanism is the subject of another study.

MATERIALS AND METHODS

NCS mice. The albino NCS mouse colony, established early in 1966 at the Microbiology Department of the University of Melbourne, was derived from the NCS colony maintained at the Rockefeller University under "specific pathogen-free" conditions (Dubos and Schaedler, 1960). Our maintenance of the mouse colony was also under highly sanitary conditions. The cages and sawdust bedding were autoclaved at 121°C for 20 min. to kill the vegetative bacterial cells. The food and drinking water were also autoclaved and water was acidified to pH 4.3 to suppress bacterial growth. The food was subsequently supplemented with vitamins and calcium tablets.

The NCS colony was kept in isolation in an air-conditioned breeding room. For experimental infections and subsequent lodging the animals were transferred to an infectious unit which housed conventional mice, but the maintenance regimen continued to be similar to that provided in the breeding room.

The important characteristic of the NCS mice was the remarkably uniform and numerically restricted intestinal coliform flora, lacking typical *Escherichia coli* strains. During the course of the present investigation the animals usually carried only one coliform serotype, namely the slow-lactose-fermenting *E. coli* O81±:H21. This strain had been endemic in the original NCS colony at the Rockefeller University (Mushin and Dubos, 1966).

Received 18 Feb. 1970; accepted 6 May 1970.

Conventional mice. These albino animals, used for a limited series of experiments, possessed a more abundant and more varied intestinal coliform flora than that found in the NCS mice.

Colonisation experiments

Coliform cultures. The cultures used in the experiments comprised five enteropathogenic and two non-pathogenic *E. coli* serotypes and one *Enterobacter aerogenes* strain. The enteropathogenic *E. coli* serotypes were: O26:K60 (B6), O55:K59 (B5), O111:K58 (B4), O127:K63 (B8) and O128:K67 (B12). The enteropathogenic strains and non-pathogenic serotype *E. coli* O117 were obtained by the courtesy of Dr F. Ørskov and Dr I. Ørskov of Copenhagen, and the late Dr P. R. Edwards and Dr W. H. Ewing of Atlanta. The strains from the above collections were designated "Or" and "E" respectively. One additional *E. coli* O55:B5 strain, labelled "QVH", was a recent isolate from a case of gastro-enteritis. The second non-pathogenic *E. coli* serotype used was the slow-lactose-fermenting O81±:H21, endemic in the NCS mouse colony. *Enterobacter aerogenes*, also a slow-lactose-fermenting strain, was included in this study for its apparent ability to colonise the intestine of babies. This organism appeared in faeces of many infants after chloramphenicol chemotherapy administered to control an outbreak of salmonellosis in a children's hospital.

Experimental infections with coliforms. The mice used were 9–12 days old. The infective dose was administered by mouth, except in one set of experiments in which the intranasal route was chosen.

Oral route. The cultures were prepared by inoculating a 10-ml nutrient broth medium with a standardised loopful of an 8-hr broth culture at 37°C and incubating the freshly inoculated medium for 16 hr at 37°C. Depending on the strain, this procedure gave viable counts usually in the range of $(5-10) \times 10^8$ organisms per ml, as estimated from surface platings of tenfold dilutions of the culture. A drop of broth culture, undiluted, diluted or occasionally concentrated, was placed in the mouth of the mouse by means of a 1-ml syringe with the tip of the needle covered by fine polyethylene tubing. On calibration the tubing was found to deliver 0.01-ml drops. The infective doses ranged from 10^3 to (200×10^6) bacteria.

Intranasal route. Mice were infected with *E. coli* O26:B6 by two methods: (i) inhalation of a drop of a nutrient broth culture placed on the nostrils of the animal, previously anaesthetised with a chloroform-ether mixture; (ii) inhalation of an atomised suspension of approximately 2×10^5 bacteria per ml of air by animals exposed to the spray for 50 min. in a closed container.

Bacterial sampling before and after infection. The coliform flora of mice was examined prior to infection by killing one animal in each litter, and after infection at selected time-intervals. The material was sampled from the following organs: lungs; heart, liver and spleen combined; stomach; duodenum, and the remainder of the gut. The tissues were homogenised in 4 ml charcoal-water in McCartney bottles. Standard loops delivering 0.02-ml drops of suspension were used for plating on isolation media and the plates were incubated overnight aerobically at 37°C.

Media and identification of coliforms. Tergitol-7-agar, a Difco product, with the addition of tetrazolium chloride (T₇T medium) (Schaedler and Dubos, 1962) was used for the isolation and visual differentiation of coliforms. The smooth type of colonies appearing on this medium proved to be suitable for direct slide agglutination tests. The diagnostic sera were prepared with OB-, and occasionally with O-antigens of the coliform bacteria.

Histological examination. Sections from the intestine of mice infected at 9 days of age with *E. coli* serotypes O26:B6 or O111:B4 were examined microscopically for evidence of pathological changes such as inflammation and penetration into the gut wall. The sections were obtained from the upper and lower intestine and caecum and were stained by the haematoxylin-eosin method. The sampling was carried out 2, 5, 7 and 9 days after the oral administration of 10^6 bacteria. Control sections were prepared from the intestine of normal mice of the same age.

Investigation of possible antimicrobial factors in the gut

Coliform strains. The eight strains used in the colonisation experiments were examined for their ability to produce colicines and for their possible role as indicators. The technique

was similar to the one applied for testing *Shigella sonnei* strains for the production of colicines (Gillies and Brown, 1966). The potential colicine producer strain was inoculated on a tryptone soya blood agar plate in a diametric streak and incubated overnight at 35°C. The growth was removed, the indicator strains were applied at a right-angle to the original streak and duplicate plates were incubated both aerobically and anaerobically overnight at 37°C.

Bacterial groups. Lactobacilli, coliforms, enterococci and anaerobic bacteria were isolated from the intestine of normal adult NCS mice and examined for inhibitory activity against the range of eight coliform strains. The selective isolation media and the conditions of growth were as described by Schaedler and Dubos, except for anaerobic bacteria which were also cultivated on RCM medium (Barnes, Impey and Goldberg, 1966) with and without neomycin. The composite growth from each plate was examined for the production of antagonistic factors. The harvested growth was inoculated in a diametric streak on the appropriate medium, or a suspension was made in phosphate buffer at pH 7.0 and subjected to ultrasonic disintegration before it was streaked on a plate. The subsequent procedure was similar to that described for testing the colicine production.

Intestinal material. NCS mice of five age-groups were used: 9, 16, 21, 23 and 28 days old. Either the whole intestine was removed and homogenised in sterile water, or a homogenate was made from a mixture of faeces and scrapings from the intestinal wall. The homogenates were spun in a Spinco ultracentrifuge and the sterile supernatants were streaked on plates; the subsequent procedure was similar to that applied for testing the colicine production. In addition, filter disks dipped in the liquid were placed on plates previously swabbed with 2-hr broth cultures of the coliform bacteria, and the plates were incubated overnight at 37°C.

RESULTS

Comparison of experimental infections of NCS and conventional mice

The NCS mouse was compared with the conventional mouse for suitability as an experimental model for intestinal coliform infections. The susceptibility to infection was assessed on the basis of the duration of colonisation and mortality in the two groups of mice.

Table I presents the data on the duration of infection after oral administration of $(5-10) \times 10^6$ organisms to 9-12-day-old mice. Three *E. coli* serotypes were used: enteropathogenic O26:B6 "Or" strain for both groups of animals, non-pathogenic O117 for NCS mice and O81, endemic in NCS, for conventional mice.

It can be seen that the oral administration to NCS mice of either enteropathogenic or non-pathogenic *E. coli*, both foreign to their gut, produced colonisation lasting from 1 to 3 wk. The clearance of the introduced organism from the intestine started at the weaning stage, at about 19-21 days of age, and was usually complete by 30 days of age. In contrast, the conventional mice rejected the implanted coliforms more rapidly, but less uniformly; clearance was observed after as little as 1 day, and the duration of colonisation exceeded 10-11 days in only one of the mice.

Under similar environmental conditions in the "infectious unit", the experimental NCS mice regained the endemic *E. coli* O81±:H21 strain, and seldom acquired a different coliform, whilst the conventional mice were found to harbour a more abundant and varied flora.

Table I gives data on mortality in NCS and conventional mice. The challenge dose of $(5-10) \times 10^6$ non-pathogenic *E. coli* strains caused no deaths,

whilst the enteropathogenic serotypes were associated with mortality, and the percentage of deaths was higher in NCS mice. Thus the NCS mouse proved to be a more sensitive and a more consistent model for intestinal colonisation than the conventional mouse. Consequently, in further experiments only NCS mice were used.

Mortality in NCS mice after oral administration of various coliform strains

From the results in table I it seemed that mortality results in suckling mice could provide a means of differentiation between enteropathogenic and non-pathogenic coliform strains. To investigate this possibility, groups of 9–12-day-old mice were given orally various coliforms—five enteropathogenic and two

TABLE I

*Duration of infection and mortality after oral administration of $(5-10) \times 10^6$ of various strains of *E. coli* to 9–12-day-old NCS and conventional mice*

Type of mouse	<i>E. coli</i> serotype used	Enteropathogenic (+) or non-pathogenic (—)	Number of mice challenged	Duration of infection (days)	Mortality	
					Number	Per cent.
NCS	O26:B6, "Or"	+	41	7–21	19	46
Conventional	O26:B6, "Or"	+	39	1–10 (20*)	9	23
NCS	O117	—	43	7–21	0	0
Conventional	O81 ± :: H21	—	35	1–11	0	0

* Result in a single mouse.

non-pathogenic strains. The challenge doses of the organisms varied from 10^3 to (200×10^6) bacteria.

Table II illustrates the results of these experiments. Data obtained by Mushin and Dubos (1965) at the Rockefeller University with a strain of *E. coli* O26:B6 different from that used in the present study were also included to supplement the results.

Several significant features are apparent. (a) The mortality in the suckling NCS mice reflected the virulence of the coliform strains administered. The percentage of deaths following oral challenge with $(5-10) \times 10^6$ enteropathogenic *E. coli* was highest with serotypes O26:B6 and O55:B5, and lower with O111:B4 and O128:B12; the stock culture O127:B8 may have lost its virulence. The two non-pathogenic strains *E. coli* O117 and *Ent. aerogenes* were avirulent. (b) Oral challenge with large doses such as 200×10^6 bacteria greatly increased the mortality, causing deaths even with the non-pathogenic coliform strain, *Ent. aerogenes*. On the other hand, small doses of 10^3 enteropathogenic *E. coli* O26:B6 had no lethal effect.

(c) *E. coli* serotypes, causing mortality in mice, apparently exerted a toxic effect, as deaths were usually recorded within 24 hr after an oral challenge with

200×10^6 bacteria, within 48 hr with $(5-10) \times 10^6$ bacteria, and within 4 days with smaller doses. No signs of diarrhoea were observed.

TABLE II

Mortality in NCS mice challenged when 9-12 days old with graded doses of coliform strains

Oral challenge dose	Number of mice dying/number challenged (and percentage mortality) in challenge tests with							
	<i>E. coli</i> strain						<i>Ent. aerogenes</i>	
	O26:B6	O55:B5 “QVH”	O111:B4 “Or”	O128:B12 “E”	O127:B8 “E”	O117 “Or”		
200×10 ⁶	62/72 (86)*	7/7 (...)	10/11 (91)				14/19 (74)	
20×10 ⁶	109/300 (36)*	55/76 (72)	5/25 (20)	10/35 (29)			0/25 (0)	
(5–10)×10 ⁶	43/80 (54)†	68/104 (65)	11/62 (18)	3/16 (19)	1/53 (2)	0/43 (0)	0/19 (0)	
3×10 ⁶	30/72 (42)†							
1×10 ⁶	14/109 (13)†	10/76 (13)	1/19 (5)			1/12 (8)		
0.5×10 ⁶	4/51 (8)†							
10 ³	0/41 (0)†					0/16 (0)	0/17 (0)	

* Figures derived from experiments at the Rockefeller University (Mushin and Dubos, 1965), with a different *E. coli* O26:B6 strain. † *E. coli* O26:B6 strain "Or".

Effect of strain selection on mortality. Experiments with two strains of *E. coli* O26:B6 indicated (table II) different degrees of virulence. Thus an oral dose of 20×10^6 bacteria of the strain used in the experiments at the Rockefeller University caused 36 per cent. mortality, and a lower dose of

TABLE III

Mortality in NCS mice challenged when 9-12 days old with three different E. coli O55:B5 strains

Oral challenge dose	Number of mice dying/number challenged (and percentage mortality) in challenge tests with <i>E. coli</i> strain		
	"Or"	"E"	"QVH"
10×10^6	6/30 (20)	12/20 (60)	68/104 (65)

$(5-10) \times 10^6$ bacteria of "Or" strain given in the present study was responsible for 54 per cent. mortality. Similarly, as seen in table III, three strains of *E. coli* O55:B5 obtained from various sources and administered orally in doses of 10×10^6 bacteria showed differences in virulence, the lowest mortality being caused by the "Or" stock strain.

Examination of mice after oral administration of coliform bacteria

Location of the introduced organisms. Post-mortem examinations were carried out at selected times after the oral challenge. For several hours before

succumbing to infection the animals appeared to be unwell. Necropsies carried out at this stage invariably revealed bacteraemia, and the introduced strain was recovered in large numbers from the lungs, the heart, liver and spleen, and from every part of the intestine. Mice challenged with doses of $(5-10) \times 10^6$ bacteria either died within 48 hr, or survived without obvious signs of infection. However, bacteraemia was often found in the survivors within 24 hr after the challenge. Two days later the introduced strain was only occasionally recovered from the lungs, which in these cases were markedly haemorrhagic, and even less frequently from the heart, liver or spleen, but the organism was consistently found in the various sites of the intestine. Challenge with 10^3 bacteria established an intestinal infection but no bacteraemia. Thus the extent of infection

FIG. 1.—Duration of infection in NCS mice challenged orally when 9–12 days old with $(5-10) \times 10^6$ coliform bacteria. The clearance of the implanted *E. coli* strains commenced at approximately 21 days of age and the elimination was usually completed by the age of 25–30 days. The clearance of *Ent. aerogenes* was not as marked.

varied according to the number of bacteria in the administered dose. The colonising strain persisted in the lower gut for the longest period of time. It was observed that several mice with *E. coli* O55:B5 infection developed lung abscesses, which were noticed at necropsy 4–5 wk after the oral challenge, and yet the animals appeared to be physically normal.

Duration of infection. Fig. 1 demonstrates the duration of infection after oral challenge of 9–12-day-old mice. Three enteropathogenic and two non-pathogenic coliform strains were given in doses of $(5-10) \times 10^6$ bacteria and approximately 30 mice per serotype were examined. The results refer to four age-groups: 10–13 days, 14–17 days, 18–24 days, 25–30 days and over. Mice were considered to be infected regardless of whether they carried the implanted organism in the proportion of 1 per cent. or 100 per cent. of their coliform flora.

The general trend in these experiments was for the clearance of the introduced bacteria to commence at the weaning period in mice, at approximately 21 days of age, and for the elimination to be usually complete by the age of 25–30 days. However, the clearance of *Ent. aerogenes* was not as marked as

that recorded for other strains. This observation calls for further investigation as the possibility exists that this strain produced a benign type of infection and thus was able to establish itself in the gut for a prolonged period of time.

The over-all pattern of duration of infection initiated by the various coliform strains, pathogenic and non-pathogenic, was similar to that reported by Mushin and Dubos (1965) in their study of *E. coli* O26:B6.

Degree of colonisation. The degree of colonisation of the gut by the implanted coliforms was estimated on post-mortem examination, by the identification of the growth on T₇T isolation media on the basis of colony appearance and slide agglutination tests. This enabled the numerical ratio of the introduced strain to be expressed as a percentage of the total coliform flora.

FIG. 2.—Degree of intestinal colonisation in NCS mice challenged orally when 9–12 days old with $(5-10) \times 10^6$ coliform bacteria. The introduction of a coliform strain was followed by the suppression of the resident *E. coli* O81 serotype. In time, the percentage of the introduced coliforms decreased and *E. coli* O81 increased. At the age of 25–30 days the mice that remained carriers retained only a small percentage of the implanted bacteria in the lower colon.

Fig. 2 shows the degree of colonisation of the gut of mice of various age-groups as indicated for fig. 1; the percentage colonisation in each age-group has been averaged. As expected, the over-all pattern was similar to that in fig. 1. Within 24 hr of the introduction of a coliform strain, the new organism totally or partly suppressed the resident slow-lactose-fermenting *E. coli* O81. However, the percentage of the introduced coliforms decreased in time, whilst the percentage of *E. coli* O81 increased. At the age of 25–30 days most mice had completely cleared the infecting strain from their gut, but some remained carriers, retaining only a small number of the introduced bacteria in the lower part of the colon.

Histological examination. At necropsy of mice infected with oral doses of 10^6 *E. coli* O26:B6 or O111:B4 strains, no abnormalities were evident on macroscopic examination. Stained sections from the various sites of the colonised intestine showed no pathological changes compared with corresponding sections from normal mice.

Intranasal infections

Experiments were carried out to investigate whether mortality in mice after oral challenge with enteropathogenic *E. coli* was due to invasion of the lungs, as infection of this organ was evident not only in mice with bacteraemia but also in animals in which coliforms were not detected in the heart, liver and spleen. It was thought that lung infection may have occurred accidentally during oral administration of coliforms.

Direct intranasal challenge with doses of 10×10^6 *E. coli* O26:B6 was carried out on 25 mice 9–12 days old. No mortality was recorded. The lungs were infected, but appeared to be normal and were cleared of infection within 48 hr, whilst the intestine harboured the bacteria up to 1 wk after the challenge. Thus it seems unlikely that the mortality following oral administration of enteropathogenic *E. coli* could primarily be due to lung infection.

No deaths were recorded in seven suckling mice exposed for 50 min. to an atomised suspension of *E. coli* O26:B6. At necropsies carried out 24 hr later, the introduced coliforms were detected in the intestinal tract, but not in the lungs, heart or spleen.

Tests for antibacterial factors in the gut

These experiments were designed to determine whether the introduction of a new coliform strain into the gut of suckling NCS mice might be met with antagonistic activity of the indigenous microflora, resulting in the elimination or suppression of the implanted strains. The mouse intestine was considered to be an ecological niche in which the antagonistic factors, if present, could be studied.

Under the conditions of the tests, the coliforms examined, namely five enteropathogenic *E. coli*, two non-pathogenic *E. coli* and *Ent. aerogenes*, did not show colicine activity when the above strains were used as potential producers and indicators. As the experiments were designed to investigate exclusively the resident and the experimentally introduced microflora in the gut of NCS mice, this has narrowed the range of coliforms used as potential producers and indicators. The limitation became apparent in another investigation, in which the indigenous serotype O81 was shown to be capable of colicine production, as it inhibited the growth of two coliforms and one *Shigella sonnei* strain.

No antagonistic activity was displayed when whole cells or sonicated suspensions of bacteria belonging to various groups such as lactobacilli, coliforms, enterococci and anaerobes, isolated from normal adult NCS mice, were examined. Nor were inhibition effects demonstrated when the homogenised material from the gut was used. However, it should be noted that our isolates of strictly anaerobic bacteria comprised bacteroides and strains of enterococci and lactobacilli, whilst fusiform rods, which represent a large and important component of the flora of an adult NCS mouse, were not included because of the difficulty of culturing these organisms (Savage, Dubos and Schaedler, 1968).

DISCUSSION

The data presented in this paper on colonisation of the intestine of NCS mice with several enteropathogenic *Escherichia coli* serotypes confirmed the observations of Mushin and Dubos (1965) on infections with *E. coli* O26:B6. The age of the experimental animals has been proved to be a critical factor and mice 9–12 days old were considered to be suitable for colonisation. Kétyi (1964) used conventional newborn mice for the implantation of *E. coli* O101 and indicated the analogy between those infections and the corresponding disease in premature and newborn human babies.

The comparison of NCS with conventional mice indicated the suitability of NCS mice as a model for colonisation, as they proved to be more sensitive to infection and more uniform with regard to the duration and degree of colonisation of the intestine.

An oral challenge of NCS mice with $(5-10) \times 10^6$ enteropathogenic or non-pathogenic coliforms was followed by their implantation in the gut. In all cases the clearance of the introduced organisms started at about the weaning time and was usually completed by the age of 25–30 days. The suckling NCS mouse is a suitable model for the differentiation of enteropathogenic from non-pathogenic coliforms on the basis of mortality. Thus a dose of $(5-10) \times 10^6$ of some enteropathogenic *E. coli* was responsible for a high mortality, whilst an equal dose of non-pathogenic coliforms was not lethal to mice. Mortality rates after comparable doses of various supposedly enteropathogenic strains ranged from 54 per cent. to 2 per cent.; death appeared to be due to toxæmia. Doses of both enteropathogenic and non-enteropathogenic strains exceeding $c. 200 \times 10^6$ caused a very high mortality.

Death of mice was associated with bacteraemia and the involvement of the heart, lungs, liver, spleen and the whole length of the intestine. Mice that survived the oral administration of high doses, e.g., $(5-10) \times 10^6$ bacteria, occasionally harboured them in the lungs, but cleared them from the heart, liver and spleen. Infections of mice with *E. coli* O26:B6 via the intranasal route produced infection of the lungs, but no deaths, thus showing that lung infection was not the critical factor associated with mortality. Polotsky and Arbuzova (1967) and Novgorodskaya (1968) used the intranasal route of infection in mice and described an inflammatory process in the lungs followed by a rapid recovery when enteropathogenic *E. coli* O111:B4 and O55:B5 were introduced. On the other hand, several other coliform serotypes, e.g., O143 and O124, caused progressive infections and death.

Histological examination of stained sections from mice infected with a dose of 1×10^6 *E. coli* O111:B4 or O26:B6, which caused intestinal colonisation but no deaths, showed no pathological changes and no accumulation of bacteria in the epithelium of the intestinal wall. The observations of Savage *et al.* (1968), based on examination of frozen sections from the gut of normal NCS mice, pointed to a rich microflora embedded in the mucous layer of the epithelium. This discrepancy in observations could be due to the differences in the techniques of preparing the sections.

An interesting feature of the intestinal colonisation was the clearance of the gut, commencing approximately at the weaning stage. It has been suggested (Rauss and Kétyi, 1960; Mushin and Dubos, 1965) that the intestinal flora may exert an antagonistic effect on the proliferation of the implanted coliforms. Experiments were designed to determine whether colicine-producing strains or some other antagonistic factors present in the tissues and the lumen of the gut could play a role in the process of clearing.

Our results were negative. However, it should be noted that in the tests for colicine production the range of strains examined for their possible role as producers or indicators was limited to the resident and experimentally introduced coliforms. Thus, under different environmental conditions colicine production may be of significance. In the search for antagonistic factors involving a variety of resident bacteria in the gut, our range of anaerobes was restricted to bacteroides, and some strains of streptococci and lactobacilli; fusiforms were not included because of the difficulty of cultivation. Savage *et al.* suggested that fusiform bacteria, which are embedded in great numbers in the mucous layer in the intestinal epithelium of the caecum and colon, may exert some influence on the composition of the flora in the gut of NCS mice. Implantation of lactobacilli, anaerobic streptococci and bacteroides into the intestine of germ-free mice (Schaedler, Dubos and Costello, 1965) did not prevent the subsequent colonisation with coliform bacilli, which multiplied to a very high level and remained numerous during the 4-mth period of observation. On the other hand, the coliform population decreased precipitously when the germ-free mice were fed with intestinal contents from healthy NCS mice. Thus apparently no interference was evident during the association of various bacterial groups with coliform bacteria, and some other factors present in the gut of NCS mice seemed to be responsible for the inhibitory effect exerted on the coliform flora.

Kétyi pointed out that the decreased susceptibility to infection in growing mice may be related to the biological maturing of the gut epithelium. Several other mechanisms operating in the alimentary canal, such as competition for nutritional factors or the inhibitory effect attributed to certain short-chain fatty acids, were investigated by various workers and were discussed previously (Mushin and Dubos, 1965).

Our additional studies, which included aspects of immunity, will be presented in another paper.

SUMMARY

The suckling NCS mouse proved to be a more sensitive and a more consistent subject for intestinal colonisation than the conventional suckling mouse.

An oral challenge dose with $(5-10) \times 10^6$ coliforms, enteropathogenic or non-pathogenic, was followed by intestinal colonisation. The clearance of the introduced strains from the gut started at the weaning period in mice and was usually complete at 25-30 days of age.

However, the NCS mouse proved to be a suitable model for the differentiation of enteropathogenic from non-pathogenic coliforms on the basis of

mortality, as only the enteropathogenic virulent *E. coli* serotypes caused deaths, apparently due to toxic effects.

Sections from the intestine of mice infected orally with doses of 10^6 *E. coli* O26:B6 or O111:B4 showed no pathological changes.

Lung infections, following the oral challenge of mice with coliform strains, did not seem to be the sole cause of deaths as infections of the lungs via the intranasal route were not associated with mortality.

The dynamics of the implantation and of the clearance of intestinal coliforms were considered. Antagonistic activities involving the introduced coliforms and the resident bacterial groups such as coliforms, enterococci, lactobacilli and bacteroides were not observed.

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