Pathology of experimental neonatal diarrhoea from *Escherichia coli*

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RABBITS 8 to 12 days old develop diarrhoea with distinctive symptoms when various human enteropathogenic bacteria are injected into the duodenum by laparotomey (Ghosh, 1969). Only the pathology of cholera in baby rabbits has been studied in detail, and it closely resembles the natural infection in man. Evidence is now presented that **E. coli** enteritis in baby rabbits has a close similarity to infantile diarrhoea in children and also to experimental cholera. Professor Scott Thompson (1955) and others have already emphasised the clinicopathological similarities between natural cholera and infantile diarrhoea.

MATERIAL AND METHODS

Cultures: The enteropathogenic **E. coli** (EEC) 65/56 of serotype 0-26:B-6:NM was received from Dr. Joan Taylor, Central Public Health Laboratory, London and maintained without subculture for about three years on an egg-saline slope at room temperature (ca. 26° C). EEC 0-127a:B-8:H-6 was isolated in our laboratory from the liquid stool of a child. It is peculiar for its serotype in being motile (Edwards and Ewing, 1967). Controls were inoculated with a K **E. coli** isolated in this laboratory from urine and inagglutinable with polyvalent EEC antiserums (Burroughs Wellcome Ltd.).

Animals: Three strains of rabbits were used. Strain I was a family (i.e. closed colony started with a single litter) of blackpointed Californians which have a relatively simple genotype and are highly susceptible to cholera vibrios (Cruickshank et al. 1966). Strain II came from a long-established closed colony started with mongrels. Strain III was derived from Strain II a few generations ago, but is kept under different conditions.

Methods: The inoculum was 0.5 ml of a four-hour culture at 37 C in Difco brain-heart infusion broth and contained ca. 10 viable cells. Rectal contents collected with flexible nylon microswabs and diarrhoeic stools were plated on MacConkey's medium and grown overnight at room temperature. At this temperature, most indigenous coliforms of the rabbits formed rosy pink colonies while the human strains, together with a few rabbit bacteria, formed red colonies, which allowed some discrimination in selecting suspicious colonies for transfer to agar slopes. The subcultures were suspended in saline, steamed for one hour and tested by slide agglutination with specific 0 serums prepared in rabbits in the laboratory and diluted 4 – 6 fold with saline to agglutinin titres of 1:150 (Edwards and Ewing, 1967).

Results

E. coli of urine: Of four rabbits from different litters given the **E. coli** from urine, one did not excrete it in detectable numbers. In the rest, about 5 – 30% of

coliforms in rectal swabs, from 24 hours after inoculation till the termination of the experiment seven days later, were of the administered type. At necropsy, it constituted about one-third of all bowel coliforms which were limited to the distal ileum (total count of coliform colonies ca. 10) and colon (count ca. 10).

EEC 65/56 & rabbit strains: All rabbits inoculated with EEC either became healthy carriers, or developed transient or fatal diarrhoea. Litters varied considerably in proneness to develop diarrhoea. Thus seven out of eight rabbits from two litters of Strain II infected with EEC/65/56 had fatal diarrhoea, while all four from another litter of the same strain became merely carriers. Of eight rabbits from three litters of Strain III, three became carriers, three had transient diarrhoea and two died with diarrhoea. Four rabbits from two litters of Strain I were inoculated with only ca. 10 viable cells: three had fatal diarrhoea and one became a carrier.

Carriers: In carriers of all rabbit strains, EEC 65/56 was first detectable in rectal swabs 24 – 26 hours after inoculation, forming 8 – 20% of all coliform colonies. Within the next 12 hours, it formed 50 – 90% of coliforms. Only one rabbit showed appreciable reduction in the proportion of EEC from the sixth day after inoculation; others continued to excrete almost pure EEC with a few streptococci and other noncoliform bacteria as long as the ninth day, when they were killed. At necropsy blood, spleen, bile, stomach and duodenum were sterile. A loopfull of the contents of the mid-ileum yielded moderate pure growth of EEC, while the terminal ileum and colon gave heavy growths. Haematoxylin-eosin stained paraffin sections of formalin-fixed tissues from various levels of gut showed no abnormality.

Fatal diarrhoea: Began on the second or third day in rabbit Strains I and II, lasting 2 – 4 days. One rabbit of Strain III had diarrhoea on the fifth day and died two days later, while the other developed diarrhoea only on the seventh day and died next morning. The liquid discharge was greenish and faecal (not rice-watery) with a strong offensive odour. In rectal swabs EEC 65/56 appeared in small numbers 24 – 36 hours after inoculation, irrespective of the clinical incubation period, and rapidly reached a concentration of 50 – 80% of the coliforms. In diarrhoea stool, they grew in almost pure culture. Wet and gramstained films showed dense coliforms and a few epithelial cells with absence of pus cells, red cells and parasites.

The general condition deteriorated markedly with onset of diarrhoea. Culture of stomach juice during diarrhoea gave light to moderate growth of EEC in four out of six rabbits. In these four, the acidity was lowered to about pH 3 from the normal pH 1 – 2. At necropsy, the viscera looked normal, apart from signs of moderate dehydration, a little opalescent mucoid fluid in the distal half of the ileum, and 5 – 15 ml of faecal liquid (pH 7 – 8) in the colon. On gentle centrifuging, about half the volume of colonic fluid sedimented, leaving a cloudy watery fluid. This was filtered through membrane filters of 0.8u APD. 0.1 ml of the filtrate injected into the skin of adult rabbits did not elicit any local reaction, unlike the increased vascular permeability with cholera stool. Cultures of blood and spleen were negative in three out of eight rabbits. Bile culture was negative in all, and the stomach in five of six rabbits. There was moderate to heavy growth of EEC 65/56 from the duodenum of all eight, with heavy growth from their ileum and colon. Culture from the small intestine gave pure EEC on aerobic plates, and from the colon an almost pure growth.

Histology revealed nothing striking. The columnar epithelium of the lower ileum showed slightly increased basophilia, loss of surface mucin layer, hyperactivity of goblet cells, and groups of bacilli adhering to the intact brush border, especially near the base of the villi and in crypts without invasion of the epithelial cells or deeper tissues. A variable proportion of the villi showed mild subepithelial oedema at the tips and some congestion. There was no necrosis or inflammatory cell infiltration. The colon was normal.

Transient diarrhoea: In four rabbits, diarrhoea resolved in 1 – 4 days. EEC was cultivable from stomach juice in two during diarrhoea only. These continued to excrete almost pure EEC till killed 3 – 4 days after the cessation of diarrhoea, by which time the general condition had returned to normal. The cultural and histological findings at necropsy were the same as those in healthy carriers.

Serum agglutinin: The serum of carriers and diarrhoeic animals collected 6 – 8 days after inoculation had titres of less than 1:20 in tube agglutination and passive haemagglutination tests (Neter et al., 1952) using the inoculated strain as antigen.

EEC 0:127: Apart from the above rabbits, seven rabbits of Strain III infected with EEC 0-127a all became carriers; as did one of Strain II, the other one having transient diarrhoea only. Their pathological and bacteriological features were similar to those already described.

Discussion

In spite of the small number of observations, the results suggest that environmental factors like housing, apart from the inherent variations in **E. coli** and rabbit strains, contributed to the differences in susceptibility, e.g., in incidence of diarrhoea in rabbit Strains II and III. This may have some bearing on the observation that EEC strains may spread and persist in children's institutes without causing symptoms (Payne and Cook, 1950).

The symptomatology in baby rabbits, in both carriers and sick ones, showed a close resemblance to natural infantile enteritis. It is notable that a normal human **E. coli** strain could establish itself in the rabbits in the face of competition from a large excess of the indigenous bacteria, reaching a concentration of up to a third of the total aerobic bacteria. However, Cooke et al. (1969) have shown that the stools of normal men contain waves of different **E. coli** strains, each lasting from a few days to months although animal **E. coli** do not seem to establish easily in human bowel (Smith, 1969). It is plausible that the urinary strain gained a foothold in the distal ileum of rabbits where the normal flora comprised few bacteria. This strain failed to reach the high concentration that EEC attained and did not involve the proximal ileum. It would be interesting to see whether EEC produces colicines to inhibit the normal flora.

Thomson (1955) recovered by intubation EEC from the stomach of many children with diarrhoea and from the duodenum of nearly all. It has been speculated from such findings together with necropsies on scours of calves and piglings (Smith and Hall, 1968) that the occurrence of diarrhoea requires the invasion of the proximal ileum. Although similar findings were made in baby rabbits, it is by no means certain that the organisms did not invade the oral end of the ileum after derangement of the normal cleansing mechanism of the gut associated with diarrhoea, as is probably the case in experimental cholera. The failure of EEC to infect the proximal ileum in healthy carriers and its disappearance from this region in convalescents indicate that such localisation is conditioned largely by the host.

In milk acidified with conc. HCl, all three **E. coli** strains survived at 37° C for about three hours at pH 3, but only a few minutes at pH 2. This explains the recovery of the bacteria from the stomach of sick animals with high pH.

The absence of rise in serum agglutinin titre to EEC accords with recent experience in children (McNaught, 1958). Undoubtedly, EEC entered the circulation in many rabbits with advanced disease, but any belated rise in agglutinin due to this in rabbits would have been missed in the series.

Little is known about the internal pathology in human infantile diarrhoea, mainly because death is infrequent with treatment and postmortem changes rapid in the intestines. The findings in baby rabbits are essentially similar to those in experimental cholera (Cruickshank et al., 1966), although milder.

In cholera, the absence of inflammatory cells and other features suggested exotoxin-provoked lesions, and led to the discovery of an enterotoxin (Cruickshank et al., 1966). Smith and Halls (1968) have shown that EEC of piglings produce exotoxin under the control of a plasmid. The same remains to be confirmed in human strains.

Summary

Three strains of baby rabbits, infected with two EEC strains, became carriers or developed diarrhoea, the EEC almost replacing the indigenous coliforms. The EEC were located on the cell surface in the distal ileum and colon, and in sick animals also in the proximal ileum and sometimes in the stomach. Systemic invasion was terminal and irregular. Histologically, there was minimal congestion and oedema of villi of the distal ileum only. A nonvirulent strain was able to establish itself in the terminal ileum only and remained a minority in the coliform population. Similarity of this experimental system with infantile diarrhoea in man and with natural and experimental cholera is emphasised. A suitable strain of baby rabbits could provide a useful model for investigating the pathogenesis and management of human **E. coli** diarrhoea.

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