PERSISTENCE OF INDIVIDUAL STRAINS OF *ESCHERICHIA COLI* IN MAN AND DOG UNDER VARYING CONDITIONS

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It has been shown in 2 previous papers (Sears *et al.*, 1949, 1951) that the *Escherichia coli* strain occurrence in the feces of normal individuals tends to follow a regular pattern. That is, each person carries 2 types of strains with respect to the length of time during which they persist in his bowel—residents, which establish themselves and multiply for months or years, and transients, which have a tenure of a few days or weeks only. The resident strains eventually disappear and are replaced by others. Some interest attaches to the question of what brings about a change in the resident strain. In our previous studies some inconclusive evidence was produced that diarrheic attacks might precipitate these changes. Recently we have studied 2 people, a middle-aged man and wife, both of whom had for years been victims of a bowel condition that subjected them at intervals to bouts of diarrhea. Fecal specimens were collected weekly from each by means of anal swabs and plated within an hour on plates of eosin methylene blue agar from each of which, after 18 to 20 hr of incubation, 10 *E. coli* colonies were picked and their serologic O group determined. During a period of 451 days, 34 specimens were taken from each subject and 340 *E. coli* colonies studied. The wife showed a complete constancy of her resident strain. A strain of O group 10 was found in 30 of the specimens and accounted for 260 or 76.5 per cent of all cultures isolated. Of the 80 other cultures, 39 belonged to 8 other O groups and 41 could not be grouped because of spontaneous agglutination. The husband yielded only O group 8 from his first 3 specimens, then O83 became dominant and persisted for 140 days, giving way gradually to O4 which had first appeared on the ninetieth day of our observations and continued throughout the rest of the period. From this subject 31 cultures belonging to 6 different O groups were isolated and 107 others did not fall into any of the 112 O groups for which we had antiserums. There is a possibility that these unclassified cultures were all or nearly all of 1 strain in which case they would have constituted a second resident almost exactly paralleling the O4 strain. From the results on these 2 subjects it seems evident that their frequent diarrheic attacks did not cause their *E. coli* strain pattern to differ in any striking way from persons with entirely normal bowel motility. There was no indication that these 2 persons ever exchanged their *E. coli* strains.

In our previous papers we reported that strains ingested by human subjects occasionally appeared transiently in the feces but did not displace the current resident strain. Since ingestion experiments could be performed more freely with lower animals, we undertook to study the *E. coli* strain composition of dogs. Two male mongrels of about 40 to 50 lb were used. They were kept in adjacent cages which were cleaned daily by hosing. They were fed on horsemeat and prepared dog foods and fresh water was kept available. Fecal specimens were collected from the animals at intervals of approximately a week by means of anal swabs. About 10 cultures were made from each specimen.

At the beginning of the study of dog R he appeared to have 2 resident strains. One, an O1 strain, persisted for 56 days, the other, of O group 4, continued dominant for 146 days longer when an O83 strain, which had first appeared 2 weeks earlier, became dominant and persisted as the only resident. Since it was evident after a 394-day study of dog R that this O83 strain had become firmly established as his resident strain, he was used for certain experiments to be described below.

Dog D's resident strain from the beginning was of O group 83. It was impossible to determine whether this was the same strain as dog R's O83 strain, and it is of no consequence so far as our study is concerned. During the 397 days of the preliminary study of dog D, his O83 resident was absent from only 1 of the 43 specimens

examined and accounted for 373 of the 410 cultures isolated.

It was obvious that both dogs were showing the same general pattern of strain occurrence as had been observed with humans.

To determine what effect the massive feeding of a foreign strain might have on the resident strain, dog R was given, over a period of 24 days, 13 feedings of a whole agar slant culture of another strain emulsified in milk. The strain fed was an O28 of human origin. Fecal specimens were examined during the period of feeding and for 19 days afterward. From 11 specimens, 106 *E. coli* cultures were examined. All of them were O83. The fed strain O28 was not recognized once. A similar experiment with dog D yielded similar results. Fourteen feedings of a whole agar slant culture of the same O28 strain were given over a period of 32 days. The ingested strain was not isolated at any time during the 57 days following the first feeding. All of the 125 cultures isolated from 14 fecal specimens were of his resident strain, O83, except 3 which were unidentifiable as to O group.

To rule out the possibility that the ingested strains in the above experiments were being largely killed off in the stomach, both dogs were subjected to a similar test except that the fed cultures, this time an O2 strain isolated from another dog, were placed in enteric-coated capsules. These were placed in meat balls which the dogs swallowed quickly without chewing. Dog R received 9 such capsules, each containing the entire growth from a 24 agar slant culture, over a period of 24 days. Thirteen specimens were examined during a 50-day period. On the twenty-first day of this experiment 5 of 9 cultures proved to be O2. All of the rest of the 129 cultures were of the resident strain O83. Dog D, in an exactly similar experiment, failed to yield any cultures of the fed strain. All 127 cultures, isolated during and after the period of feeding, belonged to O83, this dog's resident strain.

Dog R was used for an experiment in which the *E. coli* content of the bowel was greatly reduced by chemical means, after which a foreign strain of *E. coli* was fed to the animal. A total amount of 4½ g of sulfaguanidine was administered, by mouth, over a period of 9 days. Fecal cultures made during this period showed a gradual decline in the number of *E. coli* to a stage where a very heavy inoculum on the plates was necessary to yield the 10 colonies picked. On the tenth, eleventh, twelfth, thirteenth and fourteenth days respectively, the dog was fed 1 enteric-coated capsule containing the growth from 1 agar slant culture of the O2 strain. None of the 237 cultures examined during the observation period of 88 days belonged to the ingested O group. The resident strain, O83, remained dominant.

Clearing of the bowel by an enema consisting of a quart of water containing 1 teaspoonful of sodium bicarbonate was attempted with dog R. The animal had an immediate profuse discharge of feces and shortly afterward was given an enteric-coated capsule containing the growth from a whole agar slant culture of the O2 strain. The day after this feeding, a fecal specimen yielded 5 O2 cultures of the 7 examined and on the third and fourth days 3 of 10 and 4 of 10 cultures, respectively, were of this O group. No O2 were recognized in later specimens. All of the rest of the 87 cultures isolated were O83. The resident strain was not displaced by the treatment.

In order to determine whether *E. coli* might enter through the rectum and more readily establish itself as a resident in the colon, dog D was given an injection per rectum of 1.0 ml of salt solution in which were suspended 6 loopfuls from an agar slant culture of the O2 strain. The amount was insufficient to produce any ejection of intestinal contents. Specimens taken on the first and second days after the injection yielded 7 and 2 colonies of O2, respectively, of the 10 picked. On the third day all 10 colonies belonged to his resident strain, as were all of the 10 colonies picked from each of 3 subsequent weekly specimens.

We continued to culture specimens from both dogs at varying intervals for about 6 months following these experiments. Dog D at the end of this time showed very little change in his *E. coli* flora. Cultures of O83 continued to comprise the great majority isolated. From dog R, however, most specimens yielded an O4 strain and numerous transients together with his O83 resident which became irregular in appearance and constituted a smaller proportion of the total colonies isolated. Only 3 of the 40 cultures picked during the last 4 weeks were of this group. Twenty-one were O4 and 14 belonged to at least 5 different O groups. Four days after the last rectal culture, dog R was killed with nembutal and the intes-

tinal tract tied off in sections and the contents cultured. Fifteen colonies each were selected from the cultures from the upper ileum, the lower ileum, the cecum, the ascending colon and the descending colon. All of the 75 cultures except 3 proved to belong to O group 16 which had hitherto been unrecognized in this dog. The other 3 cultures were O4 and were isolated from the cecum and ascending colon. Considering the antemortem as well as postmorten findings on this dog it seems likely that he was, at the time of death, in a stage of change of resident strain. We have frequently observed with humans that the loss of a resident strain is followed by the appearance of 1 or more strains having short tenure before a new resident strain becomes fully established.

SUMMARY

The fecal *Escherichia coli* strain composition of 2 people subject to frequent diarrheic attacks were studied for more than a year and found to differ in no important respect from that of normal individuals. They showed neither more frequent changes of resident strain nor more numerous transients than normal subjects. One must conclude that frequent clearing of the bowel plays little or no role in bringing about these changes.

Caged dogs, in spite of the fact that their eating habits undoubtedly cause them to ingest large numbers of *E. coli* of many strains daily, show the same general strain composition pattern as human beings. Their resident strains may persist for many months. Perhaps they tend to show a few more transients than humans, but the difference is not striking. All attempts artificially to establish new resident strains in the animals failed. Feeding of massive doses of foreign strains of *E. coli*, even in enteric-coated capsules and after greatly reducing the bacterial population of the bowel by chemical means or by enema, led neither to the loss of the current resident nor to the establishment of the ingested strains for longer than a few days. Injection of foreign strains per rectum was no more successful.

The finding, at death, that the dominant strain of *E. coli* is the same in all parts of the intestinal tract gives assurance that the fecal strains really represent the ones multiplying in the bowel and not merely those that survive to be excreted.

From these studies one cannot escape the conclusion that the establishment of new strains of *E. coli* in the bowel of man and animals is not easily accomplished and that the conditions which lead to its natural occurrence from time to time are complex and probably highly individual.

REFERENCES

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