PERSISTENCE OF INDIVIDUAL STRAINS OF ESCHERICHIA COLI IN THE INTESTINAL TRACT OF MAN[1]

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There is ample evidence that man invariably acquires *Escherichia coli* during the first day or two of life, if not, indeed, even before birth, and that he is never thereafter without it. The constancy of this occurrence makes it a safe presumption that every day throughout life new strains of this organism are introduced into the intestinal tract. If all strains established themselves there and multiplied with equal facility, one would expect that the strain composition of the *E. coli* of the feces of any individual over a period of time would present an exceedingly complicated and constantly changing picture. There are so many unknown factors involved in this picture, however, that inferences are of little value unless supported by observed facts. For most organisms there would be no way to study the strain composition of material containing them, but the great antigenic diversity of *E. coli* offers an opportunity to determine at least whether the number of strains present at any one time is small or large and whether individual strains tend to persist over long periods or whether their tenure is very brief.

Two studies have been made in recent years that throw some light on this subject. Kauffmann and Perch (1943) studied two persons over a period of approximately 4 months, plating out stool specimens from each at irregular intervals, selecting 2 to 4 colonies from each plate and classifying them with respect to their O groups. The results from one of their subjects revealed one to three different antigenic groups in each specimen. In only one instance was the same group found in two specimens, and in this case the interval between its first and last appearance was so long (90 days) as to suggest that it more likely represented the appearance of a new strain of the group than the persistence in the bowel of the original one. Their other subject, however, yielded the same antigenic group in each of 10 successive specimens collected over a period of 42 days, together with occasional more transient groups, and after its disappearance another group appeared in 3 successive specimens collected during a period of 58 days. Wallick and Stuart (1943) made a study on a single subject, collecting specimens at more frequent and more regular intervals and picking 10 colonies from each specimen. Their study continued over a period of 15 months and revealed a more or less definite pattern in the *E. coli* strain composition of the feces of the individual studied. Three of their antigenic groups were almost continuously present for several months each, but not concurrently, though there was some overlapping. Along with these, in most specimens, there appeared from one to three others that were either not found at all in later specimens or in only a few successive

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ones. Their results suggest that at any particular time the *E. coli* of the bowel consists of a single dominant antigenic type that persists over a long period and several others that are definitely transient in their occurrence. Eventually the dominant type disappears, however, and its place is taken by another. It seems likely that at least one of the subjects of Kauffmann and Perch would have shown this same kind of type succession also if the study had been continued over a longer period.

It is the purpose of the present paper to present further evidence that the *E. coli* strain composition of the human bowel is generally not quantitatively complex, but consists, at any time, of not more than three or four strains, and that, over an extended period of time, it tends to conform to a fairly regular pattern, qualitatively. The method of arriving at these conclusions is derived from the great diversity of antigenic types of *E. coli* strains and is based on what we believe to be a quite reasonable assumption, viz., that when a stool specimen is plated and a number of colonies on the plates prove to belong to the same antigenic type, they represent the progeny of the same strain; and, further, that when from specimens examined at frequent intervals over a period of time the same antigenic type is regularly isolated, it means the continuous multiplication in the bowel of the same strain rather than the development of several strains belonging to the same antigenic group.

Our method therefore was simply to plate out fecal specimens from the persons selected for study at frequent intervals, pick a number of colonies from each plate, and classify them into antigenic groups. For the most part we used eosin methylene blue agar as our plating medium and confined our attention to the colonies typical on that medium of *E. coli*. It should be recorded, however, that *Aerobacter* species were not encountered at all and paracolon type organisms only two or three times. We chose as probably most practicable the classification of our cultures on the basis of their O antigens. For the preparation of serums we injected rabbits with 20-hour broth cultures that had been heated 2½ hours at 100 C and preserved with 0.25 per cent formalin. The antigens for agglutination were prepared in a similar manner except that they were heated only 1 hour in flowing steam. Most of our serums gave a titer with the homologous antigen of 1:5,120 or higher. Whenever a culture gave a titer with a particular serum within one dilution of the titer of the serum with its homologous antigen, it was presumed for the purpose of our study to belong to the same O group. The validity of this presumption was tested in a few instances by cross absorptions. It was unusual for a serum to agglutinate a heterologous O antigen in a dilution higher than 1:160.

During the greater part of our study the Kauffmann O group antigens were not available to us. Most of our serums were prepared with cultures isolated from the subjects themselves. Later we received these antigens from Dr. Kauffmann and prepared serums from most of them. We were able to identify some of our serum strains with the Kauffman O groups. We have indicated these in the tables setting forth our data by the symbols K-1, K-2, etc. Four persons have been studied who are referred to below as subjects A, B, C, and D.

From subject A the isolation of cultures from stool specimens at frequent intervals continued, with a few interruptions, over a period of more than 2½ years. The voluminous data accumulated are condensed in table 1. In this table, as in succeeding ones, the vertical column on the left represents a continuous period of time divided by the horizontal lines into shorter periods. The lengths of the shorter periods were chosen arbitrarily to show the duration of the principal antigenic groups. The numbers A-17, A-18, heading the vertical columns to the right, designate specific antigenic groups, and the numbers in these columns express the number of cultures, among the total examined during the period, which belong to the designated groups. It is seen that group A-17 was continuously present throughout the period of January 12, 1947, to October 8, 1947,

TABLE 1

Data on 574 cultures of E. coli isolated from subject A over a period of 2½ years

PERIODS OF COLLECTION OF SPECIMENS	DAYS IN PERIOD	NUMBER OF SPECIMENS	NUMBER OF CULTURES	NUMBER OF CULTURES IN SPECIFIED O GROUP							
				A-17	A-18	A-1	A-3	A-4 (K-1)	B-1	Various groups	Ungrouped
1/12–1/16/47	5	5	19	17							2
1/17–2/7	22	6	16	1			11*	1		1	2
2/8–3/7	29	19	87	45				8		4	30
3/8–3/31	24	17	143	94		26*	1	5		9	8
4/1–10/8	191	15	59	28	1					5	25
10/9–12/1	54	No specimens collected during this period (subject ill)									
12/2–2/18/48	79	23	112		105			3		1	3
2/19–3/2	13	5	50		39				10*		1
3/3–3/17/49	380	20	40		130			1			9
3/28–4/29	48	5	48		28			19			1
Totals.....	845	115	574	185	303	26	12	37	10	20	81

* Approximately three days before the appearance of these cultures the subject had purposely swallowed several millions of the corresponding strain in water.

a total of 271 days. There then followed a period of 2 months when the subject was ill and no specimens were examined. When study of the subject was resumed after this interlude, this group was not found again. Another O group, A-18, which had been found as a single culture prior to the interruption in the study, became the dominant group and persisted throughout the rest of the period covered by the table, 574 days. A few days after the collection of the last specimen represented in the table, subject A was absent from the city for about a month on an extended journey. On his return specimens were again examined at intervals of about 10 days for 2 months. Group A-18 was not isolated from any of these specimens. The tenure of this strain appears to have ended during the subject's absence. Each of these two strains, during the period of its presence, not only was present in nearly all stool specimens examined during the period but represented a far greater proportion than any other strain of the total cultures taken. Together the two strains, A-17 and A-18, accounted for approximately

73 per cent of all cultures examined during the entire period. During the tenure of each of these two strains a number of others were found for short periods. Those marked with an asterisk, A-1, A-3, and B-1, represent deliberately ingested strains. More will be said of them later. A-4 cultures appeared in the feces of this subject on a number of occasions during the period of study, but such long intervals occurred when this group was not found that it is probable that A-4 cultures represent several rather than a single strain. It may be a significant ob-

TABLE 2

Data on 1,219 cultures of E. coli isolated from subject B over a period of 2½ years

PERIODS OF COLLECTION OF SPECIMENS	DAYS IN PERIOD	NUMBER OF SPECIMENS	NUMBER OF CULTURES	NUMBER OF CULTURES IN SPECIFIED O GROUP									
				B-1 (K-2)	B-2	B-8	K-7	B-9	B-3	B-4	B-5	B-6	Ungrouped
1948													
2/11–3/26	44	11	41	23	18								
3/27–3/31	5	1	10		4				6				
4/1–4/7	7	1	10	1	9								
4/8–4/13	6	1	10	1									
4/14–4/15	2	1	10		1					9			
4/16–4/21	6	1	10							10			
4/22–4/29	8	3	25	13	5					7			
4/30–7/16	78	22	220	145	75								
7/17–7/21	5	1	10	5	4						1		
7/22–7/23	2	1	10	4	4						1	1	
7/24–8/11	19	3	121	24	97								
8/12–8/19	8	1	10	1	7						1	1	
8/20–8/26	7	1	10		5							5	
8/27–9/30	35	7	55	3	20							25	7
10/1/47–6/9/48	253	32	352	159	193								
6/10–7/27	58	No specimens during this period (subject on motor trip to east coast)											
7/28–8/31	35	6	75			63							12
9/1–12/14	105	6	61			27	30						4
12/15/48–5/27/49	235	8	120				32	88					
5/28–7/29	63	4	49				48						1
Totals.....	981	111	1,219	379	442	90	110	88	6	35	3	32	24

servation that our A-4 serum strain proved to belong to the Kauffmann O group 1 and that this O group has been isolated from several persons in this laboratory. A more detailed study of the cultural and antigenic characters of these cultures would probably throw light on the question of whether they represent the same or several different strains. Such a study has not been made. The column headed "Various groups" in table 1 represents a number of different antigenic groups, none of which were found in more than one fecal specimen. A large number of cultures from this subject had to remain ungrouped because of their tendency to agglutinate spontaneously. Cultural studies indicated the likelihood that a number of antigenic groups were represented among them.

Subject B was studied also over a period of more than 2½ years, the study involving a total of 111 stool specimens from which 1,219 *E. coli* cultures were isolated. In table 2 it is seen that for a period of 16 months at the beginning of the study this person carried two strains concurrently, B-1 and B-2. Almost every specimen examined showed these two strains in varying proportions, and they were nearly always in preponderance. Of 904 cultures examined during their period of tenure, 821 were one or the other of these two strains. Of the remainder, 66 cultures belonged to four other groups and 7 remained ungrouped. Group B-4 became prominent in specimens early in the study of this subject, and for the brief period of 22 days predominated among the cultures studied. However, it disappeared without displacing completely either B-1 or B-2 and was never subsequently encountered. Later B-6 became prominent and persisted for more than 2 months but also disappeared leaving again B-1 and B-2 undisturbed. In early

TABLE 3

Data on 102 cultures isolated from subject C over a period of thirteen weeks

PERIOD OF COLLECTION OF SPECIMENS	LENGTH OF PERIOD IN DAYS	NUMBER OF STOOL SPECIMENS EXAMINED	NUMBER OF CULTURES EXAMINED	NUMBER OF CULTURES IN SPECIFIED O GROUP			
				C-1 (K-X)	C-2	C-3	Ungrouped
1946–1947							
12/30–1/5	7	1	5	5			
1/6–1/8	3	1	4		4		
1/9–2/9	32	7	33	24	9		
2/10–2/16	7	2	10		10		
2/17–2/19	3	1	5			4	1
2/20–3/28	37	9	45		34		11
Totals	89	21	102	29	57	4	12

June, 1948, this subject was absent from the laboratory on a motor trip to the east coast. On his return approximately 6 weeks later, stool specimens failed to yield either of groups B-1 and B-2. A new group, B-8, was present and with the exception of 12 ungrouped cultures, all from one fecal specimen, was the only organism found for a period of 35 days, when it was joined by group K-7. Groups K-7 and B-8 were present together for a period of 105 days, when B-8 disappeared and a new group, B-9, took its place. For 235 days, during which only 8 specimens were examined, K-7 and B-9 were constantly present and accounted for all of the 120 cultures examined. B-9 appeared last in a specimen taken May 27, 1949, in which it was represented by a single culture, the 12 others from the same specimen proving to be K-7. The next four specimens from subject B taken over a period of 63 days failed to yield B-9. All but one of the 49 cultures examined from these specimens proved to be K-7.

Subject C, table 3, could be studied for only 13 weeks but shows for this period a pattern of occurrence of the different antigenic groups that is essentially similar to the above. Group C-2 was present throughout the study and was accompanied

for shorter intervals by groups C-1 and C-3 and the ungrouped cultures, which probably represent several groups as they varied considerably in their biochemical characters.

Subject D, studied for only 2 months, gives too few data to be strictly interpreted, but it is seen from table 4 that group D-3 was present in all of the 10 specimens but one, and that of the total of 38 cultures this group accounts for 29. It was clearly the dominant group during the short period of study.

From the data on these four persons, as well as from the somewhat similar studies of Wallick and Stuart and of Kauffmann and Perch, cited above, one cannot escape the conclusion that the *E. coli* flora of the human bowel is made up of two kinds of strains, those which establish themselves firmly and continue to multiply over extended periods of time and those which are found only in a single or a few successive specimens, their total tenure being a few weeks at most. It is convenient to speak of these two kinds of strains as *residents* and *transients*, respectively. Even the residents, however, maintain a limited tenure, each being

TABLE 4

Data on 38 cultures isolated from subject D over a period of 56 days

PERIOD OF COLLECTION OF SPECIMENS	LENGTH OF PERIOD IN DAYS	NUMBER OF STOOL SPECIMENS EXAMINED	NUMBER OF CULTURES EXAMINED	NUMBER OF CULTURES IN SPECIFIED O GROUP			
				D-1	D-2	D-3	Ungrouped
1946–1947							
12/20–12/25	6	1	4	2	1		1
12/26–1/9	15	3	10			9	1
1/10–2/13	35	6	24	3		20	1
Totals.....	56	10	38	5	1	29	3

destined eventually to yield its position of dominance to new resident strains. That two strains may be resident at the same time is shown by the data on subject B. It is a curious fact, which may or may not be a matter of coincidence, that joint tenure of two resident strains was demonstrated in this individual for a total of 856 days out of the 923 days he was under study.

If the above is the correct interpretation of the data here presented, then it becomes a matter of some interest to learn why, among the undoubtedly numerous strains of *E. coli* that reach the intestinal tract of an individual, some become residents, others mere transients, and still others presumably do not survive at all in numbers sufficient to be detected by the methods used. It is of particular interest to know why strains that have been resident in the bowel continuously for months are suddenly displaced by other strains. We have made some attempts to throw light on these problems, but without reaching any clear conclusions. Wallick and Stuart thought it possible that a bacteriophage might be instrumental in bringing about the disappearance of a resident strain, but their single attempt to demonstrate such a phage was unsuccessful. We have also failed to find in the stool a bacteriophage active against a recently lost resident strain. More

recently, Stuart (1949) has expressed the opinion that the change of resident strains is associated with diarrheic attacks or other intestinal upsets. Some of our observations lend support to this theory, but others seem opposed to it. In our subject B the change of resident strain from B-1 and B-2 to B-8 occurred some time during a 6 weeks' vacation trip. The subject reported that during this period he suffered from a diarrheic attack of 1 day's duration. However, later, during the tenure in this subject of strain K-7, he again suffered a moderately severe diarrhea, but without loss of his resident strain. The experience of subject A indicates that considerable disturbance of normal intestinal physiology may take place without interfering with the tenure of the resident *E. coli* strain. In this subject, during the entire period of residency of strain A-17 he was suffering from a chronic urinary tract infection due to an *E. coli* strain belonging to the Kauffmann O group 75. Incidentally, this organism was never at any time found in the stool of this subject. During the illness, however, the patient received much medication. Sulfadiazine and sulfacetimide were taken orally for a week at a time on four different occasions. At another time during the period the patient took 3 million units of penicillin intramuscularly for a corneal ulcer. Also, a rather stubborn constipation was treated on several occasions with saline purges. None of these experiences disturbed the tenure of A-17. The change came during a period of hospitalization during which the patient suffered with a continuous moderate fever for 10 days and received mandelic acid orally and streptomycin intramuscularly. As the new resident strain, A-18, recognized after this experience, had appeared together with A-17 prior to the hospital experience, the association may have been only a coincidence. Recently, during the tenure of A-18, subject A suffered a severe diarrheic attack, having six liquid stools in one day, without loss of the resident strain.

We have made several attempts to establish new resident strains in two of our subjects, A and B, by ingestion of fairly large numbers of organisms of known antigenic type. Pure agar slant cultures were suspended in water and drunk either between meals or gradually with meals. In some instances the stomach acidity was first neutralized with sodium bicarbonate. The number of organisms swallowed at each time was of the order of from 2×10^6 to 40×10^6. In 4 of 10 trials the ingested organisms were not recovered in the feces at all. In 7 other attempts they were recovered, usually after a period of 3 days, for short periods of time only. They could be established as transients but not as residents. In a recent experiment subject A ingested over a period of 10 days at 2-day intervals a total of approximately 225 million organisms of strain A-18. As seen from table 1, this strain had been the dominant strain in this subject for more than a year. It had disappeared, however, and had not been encountered in stool specimens for about 3 months prior to this ingestion experiment. Five days after the first dose was swallowed the organism appeared in the stool, along with the current resident strain, and continued to accompany the latter until the sixth day after the last dose was taken. It was not found in later specimens. The resident strain was not displaced.

Gratia and Fredericq (1946, 1947) have shown that some *E. coli* strains pro-

duce antibiotic substances of considerable potency against other *E. coli* strains. It occurred to us that this phenomenon might be a factor in the alternation of resident strains in the intestinal tract. It seemed possible that the resident strains might prove to be organisms possessing a wide range of antibiotic activity against other *E. coli* strains. This hypothesis was tested in the following manner. Using the strains that had been long-term residents in our subjects as possible antagonistic strains and a large number of strains that had either been transients in our subjects or had come from various other sources as possible susceptible cultures, we set up a series of experiments using a method similar to that recently employed by Halbert (1948) in his study of the antagonisms of *E. coli* strains toward dysentery organisms. Pour plates were made of the organisms to be tested for susceptibility, the plates being heavily seeded in order to obtain small colonies very thickly distributed. The plates after hardening were dried in the incubator with the covers off for 1 to 2 hours; then cultures to be tested for antagonistic properties were inoculated onto the surfaces of the plates as spots, with about eight cultures to each plate. After 24 hours' incubation antagonistic cultures were shown by clear areas of widths varying from 2 to 6 mm surrounding the masses of growth developing from the inoculated spots. Occasionally one of the spotted cultures would show little or no growth, a circumstance indicating antagonism of the background culture for it. Using several hundred *E. coli* strains in this way, we found that two of our resident strains possessed a wide range of antagonistic activity against other *E. coli* strains but that the others had little or no activity, more often appearing in the susceptible class. On the other hand, the two most active antagonists we discovered in this way had both been isolated from subject A as transients, each appearing actually in only one stool specimen. There appeared, therefore, to be no correlation between the range of antagonistic activity of *E. coli* strains isolated from our subjects against other *E. coli* strains and the duration of their residence in the bowel.

SUMMARY AND CONCLUSIONS

In the study reported in this paper *Escherichia coli* cultures have been isolated from stool specimens taken at frequent intervals from four different persons over periods of time amounting to 2½ years in the case of two subjects, less than 3 months for two others. The very large number of cultures so obtained have been classified into groups on the basis of their O antigens. On the assumption that the continuous isolation from the stools of a single individual of the same O group indicates the persistence in the bowel of that individual of the same *E. coli* strain and that, conversely, the disappearance of a strain will be indicated by the disappearance of that O group, our results seem to justify the following conclusions:

(1) At any particular time the *E. coli* flora of the human intestinal tract consists of strains that persist over relatively long periods of time, accompanied at times by not more than three or four other strains that maintain a tenure of a few days or a few weeks only. We have called these two types of strains *residents* and *transients*, respectively.

(2) Though the resident strains may persist for many months, they always eventually disappear to be replaced by other resident strains.

(3) The change of resident strain is sometimes accompanied by a diarrheic attack, but our experience does not indicate that this is always a factor in bringing about the change. A resident strain may persist in spite of somewhat drastic alterations of intestinal physiology.

(4) Strains of known antigenic type, deliberately swallowed in large numbers, have sometimes not been recovered in the stool; at other times they have been recovered for limited periods only. They have not been established as residents.

(5) The antagonistic activity of resident strains against other *E. coli* strains has not been found greater than that of transient strains or strains picked up from other than intestinal sources.

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