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Effect of Colicine Production on *Escherichia coli* in the Normal Human Intestine. (28624)

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Microorganisms which cause disease in the human intestine and the host-parasite mechanisms involved have long been under investigation, but less attention has been given to the normal intestine and the factors which influence its flora. The influence of one of these factors, the antibiotic "colicine"(1) which is produced by *Escherichia coli,* has been investigated, but results were inconclusive. The present study was undertaken to reevaluate existing information by utilizing more extensive and definitive methods to demonstrate production and activity of colicines of *E. coli* strains recovered from 5 normal individuals for a 6-month period.

Materials and methods. Fecal samples were collected monthly from June through November from 5 persons for a period of 6 months. Each sample was streaked immediately onto eosin methylene blue and blood agar plates, and after incubation for 18 hours at 37°C, a total of 10 typical *E. coli* colonies was picked from the 3 plates when possible. Biochemical characterization of each isolate was based on lactose, sucrose, and mannitol fermentations, indol, methyl red, Voges-Proskauer and citrate reactions. To assure that typical *E. coli* were selected for study, only those isolates which displayed the IMViC formula ++ −− were included. Somatic (O) serotypes were determined according to the methods of Edwards and Ewing(2) using antisera for *E. coli* serotypes 01 through 025 and for enteropathogenic *E. coli* types 026, 055, 086, 0111, 0112ab, 0112ac, 0125ab, 0125ac, 0126, 0127, 0128ab, 0128ac. Isolates not belonging to any of these serotypes were used for production of antiserum prepared according to the technique of Ewing(3) for the purpose of identifying all similar serotypes. Cross absorptions were done when necessary to determine the identity of some isolates.

All 10 *E. coli* isolated from each of the 5 individuals during a one-month period were tested for colicine activity against *E. coli* isolated during the previous month. In parallel all isolates were assayed for colicine activity against the colicine indicator strain of Gratia(4), *E. coli* phi.

Two methods were employed for demonstration of colicine activity. The first is essentially the simultaneous pour plate method of Blackford *et al.*(5). A thin layer of Proteose no. 3 agar (Difco) was poured into a Petri dish, allowed to harden and covered with a second layer of agar which had been seeded with approximately 4×10^7/ml of the substrate organism(6). Twenty-four hour cultures of the isolates to be tested for colicine activity (producer strains) were then spot inoculated on the surface and the plates incubated for 24 hours at 37°C.

The second method is a modification of the chloroform plate diffusion technique of Gratia and Fredericq(4). Twenty-four hour agar slants of *E. coli* isolates to be tested for colicine production were spot inoculated with a sterile cotton swab onto thick Proteose no. 3 agar plates. The plates were then incubated for 48 hours at 37°C, flooded with chloroform and allowed to stand for one hour to assure death of the organisms. The chloroform was decanted and the plates allowed to remain with the lids ajar for one hour for the residual chloroform to evaporate. Growth from 24-hour agar slants of the substrate

TABLE I. Summary of Colicine Production of *E. coli* Strains Isolated from 5 Subjects During 6 Months (June through Nov.).

	Residents				Transients			
Subject	*E. coli* designation	No. of months isolated	No. colicine positive strains	No. colicine negative strains	Strain designation	No. of months isolated	No. colicine positive strains	No. colicine negative strains
A	x(d)	5	48	0	02(d)	1	10	0
					z	1	0	2
B	b(d)	5	36	1	a	1	4	1
C	022(d)	3	21	0	e(d)	1	0	6
					c	1	1	0
					06(d)	1	2	7
					018	1	0	3
					y	1	3	0
D	02(d)	3	13	1	j(d)	1	1	5
	f	3	5	3	g(d)	1	4	6
					k	1	2	2
					h	2	0	9
					i	1	1	0
E	01(d)	4	2	16	n(d)	1	0	7
					m(d)	2	0	12
					0112ab(d)	1	0	8
					s	1	0	3
					t	1	1	1
					p(d)	2	5	6
					014	1	0	1

(d) dominant strain.

E. coli was washed off and suspended in 0.85% saline and then further diluted with saline to give 6×10^8 organisms/ml by nephelometric method. Two-tenths ml of this suspension was thoroughly mixed with 8 ml of melted Proteose no. 3 agar and poured over the plates containing the killed colicine producer strains. These plates were incubated for 24 hours. Since the size of the zone of inhibition is not indicative of amount of antibiotic produced, only the presence or absence of inhibition was recorded.

Sears and associates(7) have divided the *E. coli* serotypes of the human intestine into two groups, resident and transients. However, for the purpose of this study resident isolates are further defined as those which appeared for at least 3 of the 6 months and transients as those which appeared for shorter periods. Moreover, strains were considered dominant when they comprised at least 6 of the 10 isolates for one month.

Results. Although a sixth individual was studied the data are not presented because the *E. coli* recovered were aberrant and isolated only on 3 occasions during the 6-month period(8). Data showing colicine production of the *E. coli* of the other 5 individuals isolated over a period of 6 months are presented in Tables I and II.

Subject A had the most stable flora (Table I); only 3 different *E. coli* serotypes were recovered during the 6-month investigation. Ninety-seven per cent of the isolates produced colicine (Table II). The x strain, a dominant resident during the last 5 months of the study, was a consistent colicine producer when tested against the indicator strain, *E. coli* phi. However, colicine produced by strain x was not active against *E. coli* serotype 02 which was dominant and present only during June, the first month of the study, therefore colicine apparently played no direct role in the disappearance of *E. coli* 02 from subject A's intestinal flora.

Results obtained from individual B also

TABLE II. Percentage of Total *E. Coli* Strains Isolated Which Produced Colicine.

Subject	Total serotypes	Total strains	Total No. colicine positive	% colicine production
A	3	60	58	96.6
B	2	42	40	95.2
C	6	43	27	62.8
D	7	52	26	50.0
E	8	62	8	12.9

show an association between stability of the fecal flora and consistent colicine production (95%). *E. coli* strain b, a resident which replaced the June flora, was the sole organism recovered during the last 5 months of the study and was a consistent colicine producer, with the exception of a single b isolate recovered in November.

In subject C the *E. coli* flora of June was partially rough and none of these isolates produced antibiotics. This flora was supplanted in July by *E. coli* serotype 022, a consistent colicine producer. Serotype 022 was dominant in July, appeared in September as a single isolate during a change-over in flora and then reestablished itself as a dominant resident in November. In contrast to the 2 previous individuals subject C had 5 transient strains as well as the resident *E. coli* 022 and only 62.7% were colicine producers.

Colicine activity of the *E. coli* of subject D was also more variable and was associated with isolation of a greater number of serotypes during the 6 months. *E. coli* 02, a resident serotype and colicine producer in 13 of the 14 strains isolated, appeared as occasional isolates in June and August and emerged as a dominant organism in September. The resident strain f appeared in June as colicine producers although this serotype was a less consistent producer in July. It was found as a single non-colicine producing isolate in August and was not recovered in the following 3 months. This was the only resident *E. coli* in the study which never appeared as a dominant strain. Though 50% of the *E. coli* isolated produced colicine, only *E. coli* 02 was a consistent producer.

From individual E a total of 8 serotypes was isolated, the greatest number from any of the subjects in this study. Only 13% of these 8 serotypes were colicine producers, whereas the 3 and 2 strains respectively, which were isolated from subjects A and B, were consistent in colicine production. The single resident, *E. coli* serotype 01, achieved dominance without the production of colicine, although colicine was produced by this serotype in the month of September. Of the 7 transients only 2 produced colicine, *i.e.*, *E. coli* t and p.

A total of 20 transient *E. coli* serotypes and 6 resident serotypes was isolated from these 5 individuals. Nine of the transients were not colicine producers. However, 4 did show consistent colicine activity. Of these 4 serotypes, 2 were isolated only on one occasion, *i.e.*, strain c from subject C and strain i from subject D. A third transient colicine producer was represented by 3 isolates, *i.e.*, strain y from subject C recovered in September. The 4th colicine producer, strain 02 from subject A isolated in June, is classified among the transients but may have been present prior to the beginning of this study. The remaining 7 transient *E. coli* serotypes were irregular in colicine producing ability. Some of these did produce colicine, but in no instance was colicine production detected in over half of the isolates representing a single serotype.

Four of the resident *E. coli* serotypes, x of subject A, b of subject B, 022 of subject C, and 02 of subject D, were totally or predominantly producers of colicine and were recovered during 3 to 5 of the 6 months. *E. coli* 01 of subject E was the sole serotype to be recovered during the 4 months that it was dominant, but it rarely produced colicine.

The indicator strain, *E. coli* phi, made possible the detection of colicine production by resident *E. coli* which were dominant over a period of months. On some occasions the *E. coli* proved sensitive to colicine produced by flora isolated the subsequent month, but in all instances this activity was also shown by the indicator organism.

Discussion. Although little is known concerning the factors which bring about the alteration of normal intestinal flora, our data suggest an apparent association between the presence of a multiplicity of *E. coli* serotypes and little or no colicine production in contrast to the presence of a few serotypes and consistent colicine production. Moreover, the production of colicine by an *E. coli* seems clearly associated with its survival in the intestine as a dominant resident. However, it cannot be concluded from the evidence here that colicine production enables an organism to establish itself as a resident.

Several other factors which might have an

effect on *E. coli* of the intestine have been investigated. For example, it has been suggested that diarrhea might be associated with alteration of the *E. coli* flora of the intestine. Although occasional bouts of mild diarrhea were noted in 3 individuals of this study, there were no apparent changes in the resident *E. coli* serotypes which could be correlated with these episodes. However, in none of these cases were pathogenic enteric organisms recovered. Robinet(9) investigated the role of serum antibody and found no relationship between antibody levels and behavior of *E. coli* in the alimentary tract. Wallick and Stuart(10) and Sears *et al*(11) investigated the influence of bacteriophage on changing intestinal flora and found none. This possibility is being further investigated using material from the same subjects as those studied in the present report.

The chloroform and simultaneous colicine assay methods used in this study were nearly equal in sensitivity, but several *E. coli* possessed antibiotic activity which was detected by one method and not by the other. Hentges and Freter(12) also observed that the various *in vitro* colicine assay methods lack complete correlation. However, through the use of a colicine indicator strain, *E. coli* phi, it is felt that most of the colicine production was detected. Blackford *et al*(5) employed the simultaneous pour plate method and used 11 indicator strains in their survey of enteric bacteria for antagonistic properties. They demonstrated that 51.1% possessed some activity, whereas in the present study 61% of the *E. coli* produced colicine as determined by the techniques described. Sears *et al*(7, 11) also employed the simultaneous method but did not utilize a known colicine sensitive strain as an indicator. In the latter experiments, although omission of an indicator strain reduced the number of colicine producers detected, the lack of activity of residents against the transients was demonstrated. This lack would not seem surprising if the production of colicine by resident flora prevents the multiplication of susceptible *E. coli* strains in detectable numbers. This supposition is strongly supported by our data wherein an association has been demonstrated between production of colicine by an *E. coli* strain and its survival in the intestine as a dominant resident.

Summary. To re-evaluate the effect of colicine production on *Escherichia coli* in the normal human intestine, the *E. coli* flora of 5 healthy individuals was studied for 6 months. Each isolate was identified as to serotype or hyperimmune antisera were prepared to check similar but less readily identifiable strains. The *E. coli* isolates were assayed for colicine production using organisms isolated from one month against organisms isolated the preceding month and against the colicine indicator strain, *E. coli* phi, by the chloroform and simultaneous pour plate methods. Results indicate that there was an association between multiplicity of serotypes and little or no colicine production as compared with few serotypes and high colicine production. Also, resident serotypes produced colicine more consistently than did transient serotypes. It is suggested that the ability of some *E. coli* strains to maintain themselves in the human intestine as dominant residents is associated with the production of colicines.

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