TENURE OF INDIVIDUAL STRAINS OF ESCHERICHIA COLI IN THE INTESTINAL TRACTS OF TWO CASES OF CHRONIC IRRITABLE BOWEL

by

Helen Irene Janes, B.S.

A THESIS

Presented to the Department of Bacteriology
and the Graduate Division
of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

May 1953

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

TABLE OF CONTENTS

TENURE OF INDIVIDUAL STRAINS OF E. COLI
IN THE INTESTINAL TRACTS OF TWO CASES OF CHRONIC IRRITABLE BOWEL

Originally Breslau in 1866(1), Billroth in 1874(2), and confirmed by Nothnagel in 1881(3), demonstrated the sterility of the meconium of new-born babies and the appearance of micro-organisms with the first yellow stools. However, after the fundamental studies of Escherich in 1885(4) and in 1886(5) in which he established the colon bacillus as the main organism in the fecal mass, more careful cultural observations have been made on the bacteriology of the digestive tract. Through the original observations of Escherich, confirmed by Schild in 1895(6), Popoff in 1892(7), and Szego in 1897(8), and others since that period, it was learned that the intestinal discharge of babies remained free of bacteria for several hours after birth. However, through the use of improved methods and enriched media there is now evidence that the meconium of babies contain bacteria at birth(9). The occurence of Escherichia coli in the meconium at birth and within minutes after, is reported by Hall & O'Toole in 1934(10) and in 1935(11), and by Snyder in 1936(12). From birth, and in instances perhaps even before delivery as discussed by Snyder in 1936, until death man harbors E. coli in the intestinal tract. Since coliform organisms occur in the new-born, usually cared for under relatively sanitary conditions, it is evident that adults during the normal course of every day encounter and swallow many E. coli from various sources, such as from the food we eat, the water we drink and use otherwise, and from the general surrounding, deposited there by other people and by animals.

In 1899 Levin(13) presented valuable evidence supporting the hypothesis that man acquires his intestinal micro-organisms from the

surroundings. Levin studied the intestinal contents of animals in the arctic regions, such as white bears, eiderducks, penquins, reindeer and seals born in surroundings of such intense cold that few bacteria live. He found the digestive tracts of these animals in most cases, entirely sterile. In the arctic zone the great rarity of micro-organisms in the air and the small number found in the water ingested by these animals probably accounts for the absence of an intestinal bacterial flora. Cushing and Livingood in 1900(14) carried out experiments which they interpreted as indicating that the number of micro-organisms in the intestinal canal depends largely upon the number introduced by the mouth and not upon the multiplication of pre-existing bacteria in the medium of the intestinal contents.

In addition to the voluminous literature on the presence of coliform bacteria in milk and dairy products, and water supplies, there are some reports on its presence in various foods ingested by humans such as in shellfish, in olives, (Tracy 1934(15)), and in various canned foods.

The data condensed in table 1, on the incidence and isolation of coliform organisms from dairy products delivered for patient consumption to a large local hospital, from April 1948 thru April 1953, by various dairies, helps substantiate the hypothesis that we ingest many different strains of E. coli every day.

With respect to foods in general, the following statement was made by Jensen in 1945(16), "Reduced to its fundamentals, the manufacture and subsequent handling of food products may be considered as a race between man and microbes to see which will be the first to consume such material". Therefore it is interesting to speculate that the human

TABLE I

INCIDENCE and ISOLATION OF COLIFORM ORGANISMS
from PASTEURIZED DAIRY PRODUCTS
DELIVERED OVER A FIVE YEAR PERIOD TO
V. A. Hospital, Vancouver, Washington
For Patient Consumption

Year	Pasteurised Milk & Cream		Ice Cream	
	Number of Spec. Tested	% Coliform Positive	Number of Spec. Tested	% Coliform Positive
(Apr.)1948	153	14.9%	53	37.7%
1949	361	4.9	129	42.6
1950	299	3.0	127	4.0
1951	228	1.3	53	2.0
1952	387	20.7	82	8.5
(Apr.)1953	68	28.0	21	24.0
Totals	1496		465	

intestinal tract must be inoculated with many different strains of E. coli coming from diverse places because of rapid transportation of foods, as well as a result of travel.

The following main questions now suggest themselves about the strain composition of the E. coli of the intestinal tract of man:

1. Does one always retain the first strains acquired as a baby while other strains come and leave the intestinal tract?
2. Do all the strains ingested remain and multiply with equal facility?
3. Are there some ingested strains more adapted to the human bowel than other strains? Are there individual differences in people which make the bowel of some more suitable than others?
4. Is man at one age more susceptible to certain strains of E. coli than at another age?
5. Do we expect to find many different strains, or only a few strains present in the human bowel at any one time?

The question arises about how to differentiate one strain of E. coli from another. Kauffmann 1943[17], Knipschildt[18], and Vahlne 1945[19] established an antigenic schema making it possible to classify strains of E. coli according to their O, K and H antigens. At that time a total of 112 different "O" groups were demonstrated. Individual E. coli colonies can be taken from any culture and differentiated with respect to their "O" antigens (somatic or cell body antigens) employing this K.K.V. schema. With many bacterial species there is no way of demonstrating this, but the great antigenic diversity of E. coli offers us a method by which we can obtain reasonably reliable evidence either that

a given strain multiplies continuously in a given individual, or that a particular strain is displaced by another. Justification lies in the fact that if one isolates strains of E. coli from different persons or animals, or from many different sources in the environment, such strains will be found to fall into a large number of "O" antigenic groups. It may therefore be assumed that the strains ingested by a single individual would show a similar diversity of antigenic groups. It seems reasonable therefore, to assume that if one continues to isolate, over a period of time from a given individual, cultures belonging to the same "O" group, these cultures represent not the strains continuously ingested, but the progeny of a single strain which has been multiplying in that individual's intestinal tract. Furthermore, if at any time a new "O" group appears among the cultures from such an individual it seems certain that it represents a new and different strain.

Thru frequent sampling of the bowel contents over a period of time we are able to determine whether an individual harbors a large number of E. coli "O" groups, or whether only a few are present at any one time. It has been demonstrated by various workers, thru the use of the K.K.V. schema, that <u>normal</u> individuals carry a surprisingly small number of "O" groups at any one time. Sears, Brownlee, & Uchiyama in 1950[20] and Sears & Brownlee in 1952[21] found that at any one time the strain composition of the feces of normal individuals consists of two types of E. coli, with respect to their tenure. One type was found to be present consistently over long periods of time ranging from months to years. These they designated as <u>resident</u> strains. The second type included all those strains occuring only briefly for days, weeks, or months. These they designated as <u>transient</u> strains.

Resident strains are not permanent for the life of the subject, as studies indicate, but do disappear from the bowel. Sometimes they end their tenure rather suddenly with no clear relation to conditions of the subject. So far definite factors tending to bring about this change of tenure have not been reported in the literature. Various possibilities have been proposed, and others suggest themselves for this sudden disappearance of resident strains of E. coli from the human bowel:

(1) Antagonism of strains
(2) Bacteriophage
(3) Change in environment
(4) Sanitary living conditions
(5) Antibiotic therapy
(6) Dietary changes
(7) Increased intestinal motility

(1) Anatagonism of strains:

De Bary in 1879[22] was the first to emphasize the significance of the anatagonistic relations among micro-organisms. Waksman in 1937[23] was concerned with associations and antagonisms among micro-organisms in different habitats. In 1941[24] he presented the broad antagonistic relations between micro-organisms living in association, either in simple mixed cultures or in complex natural populations. He emphasized the fact that pathogenic bacteria would not survive long in water containing saprophytic bacteria. The pathogens were found to survive approximately three times as long in sterilized tap water. Certain other pathogens such as Mycobacterium tuberculosis and Brucella melitensis

survived for longer periods. There is also a marked difference in the length of survival of different strains of typhoid and paratyphoid bacilli. Ruchhoft in 1934[25] demonstrated that certain strains died off very rapidly in a few hours in sewage sludge and other strains survived for almost two weeks.

An antagonistic relation is often found to exist against certain bacteria in some soils, being traced to the presence of specific bacteria and fungi in the soil. E. coli is rapidly crowded out by other organisms in the soils as evidenced by Skinner and Murray in 1926[26].

Different strains of E. coli appear to repress the typhoid organism to a different extent. Freshly isolated strains of E. coli are more active than stock cultures[27]. Older cultures of E. typhosa are reported as being non-antagonistic. However, young actively growing cultures inhibit the growth of E. coli[28].

Sea water appears to contain an agent, other than its salts, which exerts a bactericidal effect, Waksman reported in 1937 from information by ZoBell in 1936[29]. The dysentery and typhoid organisms disappear rapidly in sea water, namely in 12 to 16 hours. The paratyphoid organisms survive for as long as 23 days in sea water.

Various types of antagonisms are re-emphasized by Waksmann in 1941[24]:

1) Antagonism in vivo vs. antagonism in vitro

2) Repressive, bactericidal, and lytic forms of antagonism

3) Direct, indirect and true antagonism

4) Hetero-antagonism - between strains of different species

5) Iso-antagonism - between strains of the same species.

We are particularly interested in iso-antagonism between different strains of E. coli as a possible explanation for the sudden loss of resident

strains from the human bowel. Sears, Brownlee & Uchiyama in 1950[20] based on studies of Gratia & Fredericq in 1947[30], suggested the possibility that the antibiotic substances, called colicines, produced by some E. coli strains might be antagonistic against other strains of E. coli and thereby affect the tenure of strains in the intestinal tract. A newly ingested strain could posses a colicine active against a resident strain, however, Sears, Brownlee & Uchiyama[20] were unable to obtain evidence in favor of this. They found no correlation between the antagonistic activity of E. coli strains and the length of tenure in the bowel. In fact they found that the two most active antagonists were isolated from one of their subjects as transient strains which appear actually in only one stool specimen.

Hoover in 1951[31] found no correlation between the production of colicine and the antigenic composition of E. coli.

2) Bacteriophage:

This is the second possibility proposed for the sudden disappearance of resident strains of E. coli from the human bowel.

The term "lysobacteria" was applied by Rosenthal[32] to those bacteria capable of dissolving both living and dead organisms. The following differences were recognized between the action of antagonists and that of phage:[24]

(1) The filtrate of the antagonist may be active against other bacterial species.

(2) Both living and dead bacteria of the antagonized species are dissolved by phage.

(3) The action of the antagonist is not as specific as that of phage.

(4) Races of E. coli resistant to phage are dissolved by the filtrate of the antagonist.

In 1945 Kauffmann and Vahlne[33] were able to demonstrate the effect of bacteriophage on capsulated and on non-capsulated strains of E. coli. It appeared that the capsule protected the bacterium from the action of the bacteriophages. They suggested also that bacteriophages with an effect against capsular strains of E. coli may exist in nature. However their limited experiments failed to reveal them.

Toft in 1946[34] succeeded in isolating these capsule-phages occuring in nature, from "waste water" obtained from various provincial towns of Zealand.

The single attempt by Wallick & Stuart in 1943[35] to isolate from the human bowel, bacteriophages active against a resident strain of E. coli which had suddenly disappeared from the bowel, failed to reveal such a strain-specific phage.

Sears, Brownlee & Uchiyama in 1950[20] likewise failed in a single attempt to demonstrate such a phage.

3) Change in environment:

Sears, Brownlee & Uchiyama in 1950[20], and Sears and Brownlee in 1952[21] present some evidence in favor of the possibility that change in environment of the subject can be correlated with a disappearance of the resident E. coli strains. However their studies indicate that such is not always the case for some of their subjects retained their resident "O" strains for several years in spite of several complete changes of environment.

4) Sanitary living conditions:

Those in unsanitary living conditions and who eat anything

anywhere, might be expected to harbor many different strains of E. coli. If the intestinal E. coli strain composition is a direct result of the numbers ingested, soon the strain composition would be most complex. Evidently sanitation has no effect on the tenure of E. coli in the intestinal tract for it has been shown that dogs have an E. coli strain pattern similar to that of man. Dogs also carry just one or two resident strains and a few transient strains at any one time(36).

5) Antibiotic therapy:

Sears, Brownlee, & Uchiyama in 1950(20) reported that one of their subjects receiving medication over a period of months, received sulfadiazine, sulfacetimide, and mandelic acid orally, and streptomycin and penicillin I.M. Their studies failed to show a change in the resident E. coli strain A-17 during this period. These authors reported a number of persons receiving no such medication who did show a change in resident strains. Further studies of the effect of these factors should be made.

6) Dietary changes:

Food we eat determines to some extent the pabulum in the intestinal tract upon which bacteria grow and multiply. One would expect a change in the diet to affect the composition of this mass in the intestinal tract and have some influence on the type of bacterial flora of the bowel. Since there is a chemical change in the food residues as they pass down the intestinal canal it is to be expected that the bacterial flora may be qualitatively and quantitatively different at different levels. Escherich showed in his early studies that breast fed infants harbor B. lactis aerogenes as the obligatory form of flora high in the

intestinal canal, while E. coli flourishes farther down.

Lembke in 1896[37] was among the first to demonstrate the variations in the intestinal flora of dogs fed for a series of days on different foods. He then compared the different bacterial forms found in the stools after a mixed diet, and after separate diets of bread, meat, or fat respectively. Numerous varieties of different micro-organisms were represented. In 81 human cases examined by Lembke, E. coli remained present in spite of changes in the diet. Some reduction could be noted in the relative number of organisms present under a restricted diet. However, little is found in the literature correlating the sudden disappearrance of resident E. coli strains with changes in the diet. Sears and Brownlee in 1952[21] reported that changes in the diet seemed to have no effect upon the E. coli strain composition of the bowel.

Cushing & Livingood in 1900[14] considered all the various species of bacteria in the intestinal tract to be only temporary, since it was possible to completely remove all bacteria through a sterile dietary regime followed by a fast long enough to allow the bowel to empty itself.

7) Increased intestinal motility:

Some workers are of the opinion that the sudden disappearance of resident strains of E. coli from the intestinal tract may be associated with diarrheic attacks or other intestinal upsets. Sears, Brownlee, & Uchiyama[20] reported some observations supporting this hypothesis, yet some of their studies indicated that considerable disturbance of the normal intestinal physiology may take place without interfering with the tenure of the resident E. coli strains.

Statement of Problem

It is the purpose of this paper to try to throw some light on the hypothesis that frequent clearing of the bowel could result in a loss of the resident and transient E. coli strains from the bowel. It seems a reasonable assumption that this frequent clearing of the bowel would result in a turnover of a large number of strains, and therefore the presence of many different coli "O" groups at any one time, and over a long period of time.

Therefore of the above discussed hypotheses, the one chosen for investigation in this study with its relationship to the disappearance of resident strains from the bowel is the last, namely <u>increased intestinal motility</u>.

EXPERIMENTAL STUDIES

It seemed useful therefore, to examine stool specimens from persons with an abnormal bowel condition in an effort to learn why strains of E. coli that have been resident in the bowel continuously for months to years suddenly disappear. It was desired to conduct the study on subjects normal in other respects, and able to lead a normal home, business and social life, except for the attacks of diarrhea.

We were fortunate in having the opportunity to conduct studies on two subjects with chronic irritable bowel. They are man and wife, both of whom have suffered from this condition for many years. In both subjects the beginning of their frequent diarrheic attacks antedated their marriage by many years. Subject B (the husband) has had an irritable bowel since he was about 6 years of age and is now in his late 60's. Subject A (the wife) has had the condition since before their

marriage 32 years ago at the time of this writing. These people own and operate a small manufacturing plant working with wood and metal, and are together during the working day as well as at home.

During the period of our observations from October 1951 to April 1953, their symptoms have been confined almost entirely to frequent diarrheic attacks with soft stools during the intervals between attacks. Such subjects should present a situation in which newly ingested coli strains are entering a relatively cleared intestinal tract. It would be a reasonable assumption that in these subjects the absence of enormous numbers of organisms already growing and multiplying in the bowel might offer an opportunity for numbers of incoming strains to multiply. One might expect therefore to isolate many different strains of E. coli from the bowel of subjects with frequent diarrheic attacks.

Methods:

The two subjects A and B included in this study were followed, except during the author's summer vacation, over a period of 19 months from October 1951 to April 1953. Fecal specimens were obtained from both subjects at weekly intervals for one year and for the remainder of the time once a month. These specimens were collected by means of sterilized rectal swabs inserted into the rectum for a distance of approximately 3 inches in an effort to eliminate strains of E. coli present around the anus. Each swab consists of a length of rubber tubing, approximately 3 1/2 inches long, cut on a bevel on one end, and large enough inside diameter to allow easy movement of the cotton tipped applicator. This device for securing fecal specimens is recommended by the Division of Infectious Disease, National Institute of Health, U. S. Public Health Service. It is in use in many factories, hospitals, and public health

laboratories conducting examinations for enteric pathogenic bacteria.

In this study each specimen was inoculated within an hour to an Eosin Methylene Blue Agar plate (Difco), and after 18 to 24 hours incubation at 37°C., ten E. coli colonies were picked at random from each of the two plates.

Each colony was inoculated into Brain Heart Infusion Broth (Difco) for antigen production, to a plain agar slant for storage, and when the size of the colony permitted, the indol, methyl-red, voges-proskauer, and citrate media (Difco) were all inoculated directly from the center of each selected colony. Otherwise the IMViCs were performed from the one week old agar stock cultures. These results were read as follows: after 24 hours incubation for indol, after 48 hours for V.P. & citrate, and after 4 days for M.R.; all incubated at room temperature. Each culture was also examined at a later time in the study for its ability to ferment each of the following carbohydrates: dulcitol, inositol, lactose, salicin, and sucrose.

"O" antigenic classification of each of the cultures was made on the 20-24 hour broth cultures heated in the Arnold steriliser to 100°C. for 1 hour. This destroys the "K" or capsule antigens of the bacillus which cause "O" inagglutinability. For the sake of preservation formalin was added to the antigen to attain a final concentration of 0.3% by diluting each of the antigens with 0.6% formalin in sterile saline. After the preliminary agglutinations had been performed, these antigens were stored in the refrigerator at + 4 to + 5°C. until time to do the titrations.

Classification of these cultures was facilitated by the possession in our laboratories of "O" serums made from most of the

112 coli "O" groups of the Kauffmann-Knipschildt-Vahlne (KKV) schema. Otherwise "O" serums were prepared by intravenous injection of rabbits with the desired culture previously heated to 100°C. in the Arnold for 2 1/2 hours to destroy the "H" antigens. Heating at higher temperatures must be avoided or the "O" antigen will be damaged(38).

Before preparing an antigen for use in inoculating a rabbit for "O" serum production, a test antigen was prepared from the desired culture, as follows:

(1) This 20-24 hour culture was heated for 1 hour in the Arnold at 100°C.

(2) The antigen was cooled and formalin added to a final concentration of 0.3%.

(3) The antigen was titrated with normal rabbit serum diluted 1:2 with normal saline, and serial dilutions of 1:4 through 1:64 with the antigen were employed.

(4) If agglutination occurred within this range, the antigen was considered rough and the culture was not used for animal inoculations, until converted from rough to smooth.

When the culture proved to be smooth or had been converted, then another antigen was prepared from it for use in inoculating the rabbit:

(1) This 20-24 hour culture was heated in the Arnold for 2 1/2 hours at 100°C.

(2) The antigen was cooled and formalin added to a final concentration of 0.5%. (Vahlne(39))

(3) The rabbit was given 5 I.V. injections of the antigen at four day intervals. The amount was respectively 0.25 cc., then 0.5, 1.0, 1.0, and 2.0 cc.

(4) Four days after the last injection a test bleeding was made and the rabbit serum titrated with the homologous strain. Generally the higher titer serums were found to occur 8 to 10 days after the last injection of antigen.

(5) The rabbits were bled to death from the heart by employing sterilized, evacuated bottles.

(6) After the serums were separated, merthiolate to a concentration of 1:10,000 was added for preservation.

(7) These "O" serums were titrated, labeled, and stored in the refrigerator at + 4 to + 5°C. and remained usable until quantity became exhausted.

The "O" group determination is performed in 3 stages(39),

(1) First, the 112 serums were mixed into 22 separate pools. Each individual serum was present in the mixture in a dilution of 1:160. Each pool contained from 5 to 6 of these known monovalent serums. Each pool was preserved with merthiolate 1:10,000. These when prepared aseptically may be stored in the refrigerator and are usable over a period of months.

Every antigen was then tested by means of a one tube dilution of 1:320 using equal quantities of the pooled serum and antigen. Ten to 20 antigens can conveniently be tested simultaneously with the 112 serums in this manner. All "O" antigen agglutinations were read after 20 to 24 hours incubation in a 50°C. water bath(39).

(2) In the second stage of this "O" group determination each monovalent serum comprising the pool in which agglutinations occurred was employed singly to ascertain to which

"O" group the strain being tested belonged.

(3) The 3rd stage of this determination was a titration with the monovalent serum in which agglutination occurred in stage two. Serial dilutions of 1:320 through 1:10,240 were used. When the serum titer with the unknown antigen was that obtained with the homologous strain, the culture was considered to belong to that specific "O" group.

In this manner 730 strains of E. coli from Subjects A and B were classified into "O" groups.

Kauffmann & Perch in 1948(40) demonstrated that stored E. coli cultures remained, with minor exceptions, biochemically constant after 5 to 6 years storage. Therefore the carbohydrate fermentation studies on cultures from Subjects A and B in this project were performed as a group toward the end of the study from the stock cultures on plain agar. These stock cultures had been rubber stoppered and stored in the refrigerator since the original 24 hour incubation period.

Each of the 730 strains in this study was examined for its ability to ferment each of the following carbohydrates: dulcitol, inositol, lactose, salicin, and sucrose. To insure freshness of media and uniformity of results each of these carbohydrates was carefully prepared by the author. First 10% solutions of each carbohydrate were made and sterilized at 15 pounds pressure for 6 minutes. Each carbohydrate was added aseptically to a final concentration of 1%, to sterile beef broth containing Andrade's Indicator 1%. Each lot was tubed aseptically and tested for sterility before being inoculated. These were incubated at 37°C. and results recorded after 24 hours, 4 days, 7 days, and 10 days(40). In this manner 3,650 fermentation studies were conducted on the 730 strains

TABLE II

"O" Group Classification of 730 E. coli Strains
Isolated from Two Cases of Chronic Irritable Bowel over a 19 month Period

	Date of Spec.	Number of Cultures	Number of Cultures in Specified "O" Groups								
			Subject A			Subject B					
			10	Other Groups	Un-grouped	8	83	4	Other	Un-grouped
1951	10-9	10 on each date			10	10				
	10-15		5		5	10				
	10-22			10=0=13		10				
	11-5		10				10			
	11-12		2	4=18 1=25 1=75	2		10			
	11-18		10				9		1=0=74	
	11-26		1	9=0=13			8		2=0=74	
	12-3			9=0=8 1=0=74					10=0=2	
	12-10		9	1=0=91			10			
	12-17		10				10			
1952	1-7		10				4	6		
	1-14		10				1	10		
	1-21		10						1=0=25	9
	1-28		9	1=0=96				10		
	2-4		10							10
	2-11		10					10		
	2-18		10				1	1		8
	2-25		10				1			10
	3-3		4		6			2		8
	3-10		10					7		3
	3-17		9		1	No E. coli from this spec.				
	3-24		10				3			7
	3-31		10					9		1
	4-7		10						1=25 7=73	2
	4-21		10					10		
	4-28		10					7		3
	5-5		10					1		9
	5-12		8		2					10
	5-19		10					1		9
	6-2		10					10		
		Author on Summer vacation								
	9-30		3	2=0=13	5	1				8
	10-27		10							10
	11-23		10							
	12-28					1	5	4		
					10 R.			1	4=36 5=68	
1953	2-2			10=0=14					10=0=1	
	2-23				10 R.				9=112 10=1	
	4-19				10 R.				10=0=1	
	Totals	A=370	160	49	61	32	71	89	61	107*
		B=360	90.3%	13.2%	16.5%	19	19.7	24.7%	16.9%	29.7%

R= rough

*66 of these were low titer 0-25.

of E. coli selected from Subjects A and B. Cultures which had not formed acid at the 10 day reading were recorded as negative.

Results:

The results from the investigation of intestinal E. coli strains from two individuals with increased intestinal motility, show that these subjects do not have any greater number of E. coli "O" groups than do persons with a normal bowel. Furthermore, in spite of their frequent diarrheic attacks they have the same kind of an E. coli strain pattern as is found in persons with a normal bowel, that is, each of the subjects carried a resident, and a few transient E. coli strains. In addition as may be seen in Table II, the frequent clearing of the bowel did not displace the resident strains. It is of interest however that we were no longer able to isolate the resident strain from either Subject A or B after November 1952. Reference to an accurate record kept on both subjects as to changes in their diets, medications, travel and illnesses, revealed none of these just prior to nor during that time. There was no correlation between conditions of the subjects and the apparent loss of the resident strains. In fact Table III from Subject B shows that no change in the "O" groups occurred when on three separate occasions B was severely ill with even more frequent diarrhea than usual. Table III reveals that changes in the diet in Subject B did not bring about changes in the "O" groups present. This was also true in Subject A. It is of interest also that a course of Terramycin therapy started on Subject B on 11-26-51 taken over 48 hours for sore throat caused no change in the "O" groups either.

Cultures of the coliform group cannot be satisfactorily classified on the basis of their biochemical reactions[39][42]. However, through

TABLE III

Effect of Diet in Case B, on Coli-O groups

	Date of Spec.	No. of Cults.	Diet Prior to Survey with Changes	No. Cults. in Specified O-Groups											
				8	83	4	74	2	25	73	36	68	112	1	U
			Split peas, carrots, squash, parsnips, meat (beef), white bread, milk (cooking), cooked fruit.												
1951	10- 9	10		10											
	10-15	each		10											
	10-22	date		10											
	11- 5				10										
	11-12				10										
	11-18		11-23: No milk, ice		9		1								
	11-26		cream or bread.		8		2								
	12- 3		12-3: Corn bread added					10							
	12-10				10										
	12-17				10										
1952	1- 7		(Subject had chill 7 PM)		4	6									
	1-14		Elim. cooked fruit, peas		1	10									
	1-21		Small quant wheat added						1						9
	1-28		starting 1-21			10									
	2- 4														10
	2-11					10									
	2-18		2-20: Elim. wheat, rye,		1	1									8
	2-25		soy added												10
	3- 3		Rich raisen-suet pudding			2									6
	3-10		ill 3-6 to 3-10.			7									3
	3-17				No	E.	coli	from	this	spec.					
	3-24				3										7
	3-31					9									1
	4- 7		4-13 & 4-14, B ill from						1	7					2
	4-21		"eating ham"			10									
	4-28					7									3
	5- 5					1									9
	5-12														10
	5-19					1									9
	6- 2					10									
			Author on summer vacation												
	9-30			1											8
	10-27		B has remained on same												10
	11-23		diet as of Jan. 1952.	1	5	4									
	12-28		All roughage foods &			1					4	5			
1953	2- 2		white flour products											10	
	2-23		eliminated. Still no										9	1	
	4-19		milk allowed.											10	

the use of numerous biochemical types, even though they are not all perfectly constant, it is possible to further subdivide the serological "O" groups[38][18] (Tables IV & V).

Biochemically, strains are generally considered to belong to the Escherichia genus if they are motile, aerogenic, form an IMViC pattern ++--, ferment lactose with gas, and ferment other carbohydrates like a typical E. coli. However, the failure of a strain to ferment lactose does not exclude it from the Escherichia genus if it behaves otherwise like a typical Escherichia[39][41].

For some years it was held that the inverse correlation MR + and VP - for E. coli, and MR - and VP + for Aerobacter aerogenes was constant. It is now known that coliform organisms occur which are positive to both and negative to both. Stuart, Griffin, & Baker[43] found that almost 10% of their coliform cultures did not correlate in this regard. Though 4.3% of the strains from Subject A, and 4.2% from Subject B were not IMViC regular, the irregularity did not occur in the MR-VP relation. In Subject A, 2.7% were citrate +, and 0.3% were VP +. For Subject B, 0.9% were citrate +, and 1.9% were VP +. The remaining few irregularities may be seen in Tables IV & V.

Subject A:

As is seen in Table II, we continued to isolate the single resident strain O-10 from Subject A, from October 15, 1951 through November 23, 1952. This represents a total of 70.3% of all the cultures isolated from A during the 19 months of this study. The transient strains isolated were all included in "O" groups (8, 13, 14, 18, 25, 74, 75, 91, & 96) and accounted for 13.2% of all the cultures found in Subject A. Only 16.5% of the cultures were ungrouped. Most of the serologically ungrouped cultures from A belong to the same biochemical group, our Type I, as seen in Table VI. Thus the bio-

For Determination of Biochemical Types of B. Coli in Cases A and B, for Tables IV and V.

Biochem Type	Dulcitol	Inositol	Indol	M.R.	V.P.	Citrate	Lactose	Salicin	Sucrose
X	-	-	+	+	-	-	+	-	-
XI	+	-	+	+	-	-	+	-	-
XII	-	-	+	+	-	-	+	-	+
XV	-	-	+	+	-	-	+	+	-
V	+	-	+	+	-	-	+	+	+
VI	-	-	+	+	-	-	+	+	+
VII	+	-	+	+	-	-	+	-	+
VIII	+	-	+	+	-	-	+	+	-
IX	-	-	+	+	+	-	+	-	+
X	-	-	-	+	-	-	+	-	+
XI	-	-	+	-	-	-	+	+	+
XII	+	-	+	+	-	+	+	-	-
XIII	-	-	+	+	-	+	+	+	-
XIV	-	-	+	+	-	+	+	-	+
XV	+	+	+	+	-	-	+	+	-
XVI	-	-	+	+	-	+	+	-	-
XVII	-	-	+	-	-	-	+	-	-
XVIII	-	-	+	-	-	-	+	-	+

TABLE IV

Further Subdivision of coli-O groups from Case A, into Fifteen Biochemical Types

	Date of Spec.	"O" Group	No. cultures in Biochemical Types															
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
1951	10-9	U	10														
	10-15	U	5														
		0-10	1	4													
	10-22	0-13			10												
	11-5	0-10	8			2											
	11-12	U					2										
		0-25						1									
		0-10	2														
		0-18	2		1	1											
		0-75			1												
	11-18	0-10	6			4											
	11-26	0-13	9														
		0-10		1													
	12-3	0-8	2		6*				1*								
		0-74							1								
	12-10	0-10		7	1					1							
		0-91			1												
	12-17	0-10		10													
1952	1-7	0-10	1	5	2*					1	1*						
	1-14	0-10	7	2		1											
	1-21	0-10	3	5						2							
	1-28	0-10		9													
		0-96										1					
	2-4	0-10		7							1		1*	1			
	2-11	0-10		9						1							
	2-18	0-10		10													
	2-25	0-10		3		2				3				1	1		
	3-3	U			6												
		0-10		3						1							
	3-10	0-10		10													
	3-17	U														1	
		0-10	2	5										1			1
	3-24	0-10		8					1*	1							
	3-31	0-10		10													
	4-7	0-10		9						1							
	4-21	0-10	2	8													
	4-28	0-10	1	8		1											
	5-5	0-10	2	8													
	5-12	U												1		1	
		0-10	1	½(L)										1			
	5-19	0-10	1	8					1								

TABLE IV .... continued

Further Subdivision of coli-O groups from Case A, into Fifteen Biochemical Types

	Date of Spec.	"O" Group	Biochemical Types															
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	
	6-2	O-10	4	2		2				2								
		Author on Summer Vacation																
	9-30	U										3				3		
		O-10		3														
		O-13		2														
	10-27	O-10	2	1	4****	2				1								
	11-23	O-10		10														
	12-28	Rough	4		1****	5												
1953	2-2	O-14	10															
	2-23	Rough			1*													
					5****			4****										
	4-19	Rough	7		1*													
					2													
	Totals		92	173	42	20	2	5	4	14	2	4	1	5	1	4	1	=370

U = Ungrouped serologically

* Each specimen represents 10 cultures.

* fermented sucrose late ... 7 days
** fermented salicin late ... 10 days
(L) fermented lactose late ... 5 days
*** fermented sucrose late ... 3 days
**** fermented sucrose late ... 10 days

TABLE V

Further Subdivision of coli O groups from Case B. into

Thirteen Biochemical Types

Date of Spec.*	"O" Group	No. cultures in Biochemical Types																	
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
1951 10-9	O-6	1	2		4				2					1					
10-15	O-6	2		8*															
10-22	O-6		XXXXO. 8 were ***-. 2 were ***-. Specimens lost.																
11-5	O-83			7				3											
11-12	O-83			10															
11-18	O-83 O-74	1		8															
11-26	O-83 O-74			8 2*															
12-3	O-2	4		1 4*													1		
12-10	O-83			10															
12-17	O-83			7							3								
1-7-52	O-4 O-83	5	1	4															
1-14	O-4 O-83	10		1															
1-21	U O-25			9 1															
1-28	O-4	5	5																
2-4	U			10															
2-11	O-4	10																	
2-18	O-4 O-83 U		1 1	1 6											1				
2-25	U			10															
3-3	O-4 U	2		8															
3-10	O-4 U	6 1		1														1	1
3-17	No E. coli from this specimen																		
3-24	O-83 U			3 7															
3-31	O-4 U	9		1															
4-7	O-73 O-25 U	6 1		1 1															
4-21	O-4	10																	
4-28	O-4 U	7		3															

TABLE V .... continued

Further Subdivision of coli O groups from Case B. into Thirteen Biochemical Types

Date of Spec.	"O" Group	Biochemical Types																						
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII
1952																								
5-5	O-4	1																						
	U			7				2																
5-12	U			4				6																
5-19	O-4	1																						
	U			3				6																
6-2	O-4			9			1(L)																	
	Author on Summer Vacation																							
9-30	O-8			1																				
	U			7				1																
10-27	U			9				1																
11-23	O-4			4																				
	O-83							5																
	O-8			1																				
12-28	O-4							1																
	O-36		4																					
	O-68			4			1																	
1953																								
2-2	O-1		5		1**				4**															
2-23	O-112	3		6***																				
	O-1			1***																				
4-19	O-1	2	1	1																				
				6*																				
Totals		87	20	196	5		3	25	6		3			1	1			1	1	1				

U= Ungrouped serologically
* Each specimen represents 10 cultures

* fermented sucrose late ... 7 days
** fermented salicin late ... 3 days
*** fermented sucrose late ... 3 days
(L) fermented lactose late ... 5 days

chemical reactions of the ungrouped cultures are in accord with the presumption that they may represent only a small number of strains.

Subject B:

A study of our data on the 360 cultures isolated from Subject B reveals several points of considerable interest. The 30 cultures obtained during the first three weeks all proved to be of O group 8. This group then gave way to O-83 which remained the dominant organism for 7 or 8 weeks only to yield this position to an organism of O group 4. This in turn persisted as the dominant type for 20 weeks, when it too disappeared, not to be seen again during the period of this study. (Table II). In three specimens taken after the disappearance of the O group 4 strain over a period of 11 weeks, were 21 cultures belonging to O group 1. However, the number of specimens collected was too small to permit us to accept this as the dominant O-group during this period. It is of interest that we continued to find a *few* cultures of O-83 at long intervals throughout the tenure of the O-4 cultures. The most plausible explanation of this is that this previously dominant strain had continued to multiply in the bowel through this whole period, but to a large extent overshadowed by the O-4. This view is supported by the fact that most of those later O-83 cultures were identical in their biochemical reactions with the earlier O-83 cultures.

It is interesting that two O-8 cultures (Table II) were found also in specimens collected long after the apparent disappearance of this O group.

The transient strains isolated were all included in "O" groups (1,2,25,36,68,73,112) and accounted for 16.9% of all the cultures isolated from Subject B during the 19 months of the study.

At present approximately 125 "O" groups are known[44]. Our laboratories maintain the coli O serums 1 through 112. Our ungrouped strains might belong to those "O" groups between 112 and 125, or perhaps to a few "O" groups outside of the 125. Agglutination tests performed on 2 1/2 hour autoclaved antigens from some of these cultures, showed that the "O" inagglutinability was not due to the presence of capsules. Time did not permit animal inoculations for further testing of the ungrouped cultures, however, the biochemical reactions indicate a small number of strains. As may be seen in Table VI all except a few of the ungrouped cultures from Subject B, fall into our biochemical Type III.

Summary on Subject A:

1) "O" group 10 was the resident isolated from October 1951 through November 1952, from Subject A. This represents a total of 70.3% of all the cultures isolated from A during the 19 months of this investigation. (Table II).
2) The transient strains isolated were all included in 9 "O" groups and accounted for 13.2% of all cultures isolated from A.
3) As seen in Table VI, the biochemical reactions of the ungrouped cultures indicated a small number of strains.
4) Considerable variation in the biochemical reactions occurred in members of the resident O-10 group.

Summary on Subject B:

1) "O" group 4 gained the dominant position in the bowel of Subject B only after O-6 and O-63 had yielded their positions. During its tenure in the bowel O-4 represented a total of 24.7% of all cultures isolated from B during this investigation. (Table II).
2) The transient strains isolated were all included in 7 "O" groups and accounted for 16.9% of all the cultures from

TABLE VI

Division of Ungrouped Cultures from Cases
A and B into Biochemical Types

		Date of Spec.	Number of Cultures in Biochemical Types														
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XVIII	Totals
A	'51	10- 9	10															
		10-15	5															
		11-12					2											
	'52	3- 3			6													
		3-17														1		
		5-12												1		1		
		9-30										3				2		
		12-28	4		1	5												
	'53	2-23			6			4										
		4-19	7		3													
			26		16	5	2	4				3		1		4		= 61
B	1952	1-21			9													
		2- 4			10													
		2-18		1	6											1		
		2-25			10													
		3- 3			8													
		3-10	1		1												1	
		3-24			7													
		3-31			1													
		4- 7	1		1													
		4-28			3													
		5- 5			7				2									
		5-12			4				6									
		5-19			3				6									
		9-30			7				1									
		10-27			9				1									
			2	1	86				16							1	1	= 107

Subject B during the 19 months of this study.

3) The biochemical reactions of the ungrouped strains show that almost all belong to our biochemical Type III, indicating they could all belong to the same group, serologically.

Discussion:

The results of this investigation show that Subjects A and B with increased intestinal motility do not have any greater number of coli "O" groups at any one time, than do persons with a normal bowel.

In spite of the continued close association of the two subjects in this study, strains of E. coli common to both could be isolated in only two instances. These were cultures, O-8 and O-74 which did not occur in Subjects A and B simultaneously, but weeks apart. Subject B, the husband, had lost his O-8 strain on 11-5-51 (Table II) and his transient O-74 on 11-26-51.

A month later, on 12-3-51, transients O-8 and O-74 were isolated in a single specimen from Subject A. These were never found again during the entire investigation on A. It is of interest that these strains from Subject A differed biochemically from those of Subject B suggesting acquisition of these strains from some source other than B.

Almost a year later O-8 occurred again in Subject B as a single culture in each of two specimens. These differed from each other biochemically in that those isolated on 10-15-51 were late sucrose fermenters and the later O-8 cultures fermented sucrose promptly (Table V).

The members of the resident "O" group 10 isolated from Subject A showed considerable variation in their biochemical reactions. Therefore, there is little or no value in the biochemical reactions as a means of identification of individual strains. However as seen in Table IV and

Supplement .... Table IV

Variation in Biochemical Behavior among Cultures of the Same O-group, Resident O-10 from Case A.

	Date of Spec.	Resident O-10 Distribution among Biochemical Types																
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
1951	10-15	1	4															
	11- 5	8			2													
	11-12	2																
	11-18	6			4													
	11-26		1															
	12-10		7	1					1									
	12-17		10															
1952	1- 7	1	5	2					1	1								
	1-14	7	2		1													
	1-21	3	5						2									
	1-28		9															
	2- 4		7							1		1	1					
	2-11		9						1									
	2-18		10															
	2-25		3		2				3				1	1				
	3- 3		3						1									
	3-10		10															
	3-17	2	5										1			1		
	3-24		8					1	1									
	3-31		10															
	4- 7		9						1									
	4-21	2	8															
	4-28	1	8		1													
	5- 5	2	8															
	5-12	1	6										1					
	5-19	1	8					1										
	6- 2	4	2		2				2									
					Author on Summer vacation													
	9-30		3															
	10-27	2	1	4	2				1									
	11-23		10															
	Totals	43	171	7	14			2	14	2		1	4	1		1	= 260	

Supplemental Chart, a large percentage of the O-10 strains fall into our biochemical Type II.

The resident strains of Subject B also showed considerable variation in the biochemical behavior in cultures belonging to the same O-group, Sup. T. V.

Strains within the same transient "O" groups also differed biochemically. As may be seen in Table IV on Subject A, for example O-group 13, present on 10-22-51, again on 10-26-52, and on 9-30-52 are not the same biochemically either. They existed in the bowel only temperarily and had not multiplied there. The O-1 transient strains isolated from Subject B on three separate occasions 2-2-53, 2-23-53, and on 4-19-53, also differed from each other biochemically.

If one were to inoculate a flask of sucrose broth containing very little other nutriments, with a specimen of feces containing for example, sucrose positive strains of E. coli, together with other strains able to ferment a variety of carbohydrates, the sucrose positive strains would thrive and multiply faster and shortly overgrow all other strains. One would then be able to isolate many sucrose positive cultures from this flask.

The human bowel and contents may be compared to this culture. It seems reasonable to assume that if one continues to isolate, for example, sucrose positive strains from the human bowel, these are not just passing through but are multiplying in the bowel, and are true sucrose fermenters.

Biochemical studies of cultures from Subjects A and B suggested that the fermentative abilities of E. coli strains in the intestinal tract of man are more constant than are those held in storage at room temperature on artificial media. The biochemical reactions of the in vivo strains would appear to be more representative of the true fermentative ability of the strains.

Supplement .... Table V

Variation in Biochemical Behavior among Cultures
of the Same O-group, Resident Strains from Case B.

	Date of Spec.	"O" Group	Resident O-group Distribution among Biochem. Types																
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
1951	10- 9	O- 8	1	2		4				2					1				
	10-15	O- 8	2		8														
	10-22	O- 8	Specimen lost.																
	11- 5	O-83			7				3										
	11-12	O-83			10														
	11-18	O-83			8			1											
	11-26	O-83			8														
	12-10	O-83			10														
	12-17	O-83			9							1							
	1- 7	O- 4	5	1															
		O-83			4														
	1-14	O- 4	10																
		O-83			1														
	1-28	O- 4	5	5															
	2-11	O- 4	10																
	2-18	O- 4		1															
		O-83			1														
1952	3- 3	O- 4	2																
	3-10	O- 4	6																1
	3-24	O-83			3														
	3-31	O- 4	9																
	4-21	O- 4	10																
	4-28	O- 4	7																
	5- 5	O- 4	1																
	5-19	O- 4	1																
	6- 2	O- 4			9			1											
		Author on Summer Vacation																	
	9-30	O- 8			1														
	11-23	O- 4	4																
		O- 8			1														
		O-83							5										
		Totals	73	9	80	4		2	8	2		1			1				1 = 181

It is interesting to consider the possibility that a strain with a constant or true fermentative ability would be the one able to establish itself in the bowel when the particular carbohydrate which it could utilize most readily was present. It would multiply rapidly and to a certain extent crowd out other strains which happened to be present simultaneously. It would far outnumber all others and thus become the resident strain. Those constantly being crowded out would be the transients. As long as the pabulum in the bowel offered that specific carbohydrate readily utilized by the dominant strain it would continue to be the resident in the bowel.

Further studies concerning this influence of the fermentative abilities of in vivo strains on the tenure of the resident "O" group in the bowel will be studied here and reported in a later paper.

A quantitative decrease in the number of E. coli colonies appearing on the eosin methylene blue agar was noted in the cultures from both Subjects A and B, compared with primary isolation plates from persons with a normal bowel, as witnessed over a period of years in clinical bacteriology. Many times only 10 or 20 E. coli colonies grew on the entire E.M.B. primary isolation plates. In one instance no E. coli grew from Subject B specimen of 3-17-52 as may be seen in Table II. It is of interest that no colonies of Aerobacter grew on the primary isolation plates from Subject B, and only an occasional few were noted on those of Subject A. None of these cultures contained any enteric pathogenic organisms during the period of this investigation.

Summary:

A survey has been made of the E. coli cultures isolated from the intestinal tracts of two cases of chronic irritable bowel, over a 19 month period. The 730 cultures thus obtained from A and B have been classified

into groups on the basis of their "O" antigens. This was done in an effort to obtain information on the reason for the sudden loss of resident E. coli strains from the bowel of man.

1) The results from the investigation of intestinal E. coli strains from two individuals with increased intestinal motility, show that these subjects do not have any greater number of coli "O" groups than do persons with a normal bowel.

2) In spite of their frequent diarrheic attacks they have the same kind of an E. coli strain pattern as is found in persons with a normal bowel, that is, each carried a resident strain and a few transients (Table II).

3) The frequent clearing of the bowel did not displace the resident strains from either subject.

4) A quantitative decrease in the number of E. coli colonies growing on the primary isolation plates because of the frequent diarrhea, was evident, compared to studies on normal individuals over a period of years in clinical bacteriology.

5) In spite of the continued close association of the two subjects in this investigation, strains of E. coli common to both could be isolated in only two instances. These cultures, O-6 and O-74, did not occur in Subjects A and B simultaneously, but weeks apart (Table II), and were not the same biochemically (Tables IV and V).

6) It was possible further to subdivide the serological groups into biochemical types. However, subdivision into biochemical types proved to have little or no value in identification of individual strains, since considerable variation in biochemical behavior occurred in cultures belonging to the same "O" group.

7) The presence of various biochemical types within the members of the same resident O-groups indicated the presence of various strains within those groups.

The results from this study of E. coli strains in two cases of increased intestinal motility, throws considerable doubt on the hypothesis that diarrhea causes a loss of the resident E. coli strains from the human bowel.

BIBLIOGRAPHY

1. Breslau. Uber Entstehung und Bedeutung der Darmgase bein neugeborenen Kinde. Monatschr. f. Geburtsh. u. Frauenkr., Berlin, Vol. 28, pp. 1 - 23, 1866.

2. Billroth, T. Untersuchungen über die Vegetationsformen von Coccobacteria, septica und den Antheil welchen sie an der Entstehung und Verbreitung der accidentellen Wundkrankheiten haben. S. 94, Berlin, 1874.

3. Nothnagel, H. Die normal in den menschlichen Darmentleerungen vorkommenden niedersten (pflanzlichen) Organismen. Ztschr. f. Klin. Med., Vol. 3, pp. 275-286, 1881.

4. Escherich, T. Die Darmbakterien des Neugeborenen und Säuglings. Fortschritte der Medicin, Bd. III, s. 515-547, 1885.

5. Escherich, T. Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung. Stuttgart, 1886.

6. Schild, W. Das Auftreten von Bakterien in Darminhalte Neugeborener vor der ersten Nahrungsaufnahme. Zeitschrift für Hygiene und Infectionskrankheiten. Bd XIX, s. 113-129, 1895.

7. Popoff, D. Die Zeit der Erscheinung und die allmählich Verbreitung der Mikroorganismen im Verdauungstraktus der Thiere. Wratsch. No. 39, 1891 Centralblatt f. Bakteriologie, Bd XI, p. 214, 1892.

8. Szegö, K. Die Darmmikroben der Säuglinge und Kinder. Archiv für Kinder heilkunde, Bd. XXII, s. 25-46, 1897.

9. Burrage, S. Bacteria in the Supposedly Sterile Meconium. J. Bact., vol. 13, p. 47, 1927.

10. Hall, I. C. & O'Toole, E. Bacterial Flora of First Specimens of Meconium Passed by Fifty New-Born Infants. Am. J. Dis. Child., vol. 47, pp. 1279-1285, 1934.

11. Hall, I. C. & O'Toole, E. Intestinal Flora in New-Born Infants. Am. J. Dis. Child. vol. 49, pp. 390-402, 1935.

12. Snyder, M. L. The Bacterial Flora of Meconium Specimens Collected from Sixty-four Infants within Four Hours after Delivery. The J. of Pediatrics, vol. 9, pp. 624-632, 1936.

13. Levin, Les Microbes dans les régions Arctiques. Annales de l'Institute Pasteur, Tome XIII, p. 558, 25 Juillet, 1899.

14. Cushing, H. and Livingood, L. Experimental and Surgical Notes upon the Bacteriology of the Upper Portion of the Alimentary Canal, with Observations on the Establishment there of an Amicrobic State as a Preliminary to Operative Procedures on the Stomach and Small Intestine. John Hopkins Hospital Reports, vol. 9, pp. 543-591, 1900.

15. Tracy, R. L. Spoilage of Olives by Colon Bacilli, J. Bact., vol. 28, pp. 249-263, 1934.

16. Jensen, L. B. Microbiology of Meats, 2nd Ed., Garrard Press, Champaign, Illinois, 1945.

17. Kauffmann, F. Zur Serologie der Coli-Gruppe. Acta Path. et Microbiol. Scandinav., vol. 21, pp. 20-45, 1944.

18. Knipschildt, H. E. Under sogelser over Coligruppens Serologi med saerligt Henblik pas Kapselformerne Copenhagen, Nyt Nordisk Forlag. Arnold Busck, 1945 (with an English summary).

19. Vahlne, G. Serological Typing of the Colon Bacteria, Lund, Gleerupska Univ., Bokhandeln, pp. 1-122, 1945.

20. Sears, H. J., Brownlee, I. and Uchiyama, J. Persistence of Individual Strains of Escherichia coli in the Intestinal Tract of Man. J. of Bacteriology, vol. 59, pp. 293-301, 1950.

21. Sears, H. J. and Brownlee, I. Further Observations on the Persistence of Individual strains of Escherichia coli in the Intestinal Tract of Man, J. of Bact., vol. 63, pp. 47-57, 1952.

22. DeBary, A. Die Erscheinungen der Symbiose, Strassburg, 1879.

23. Waksman, S. A. Associative and Antagonistic Effects of Micro-organisms I. Historical Review of Antagonistic Relationships. Soil Science, vol. 43, pp. 51-68, 1937.

24. Waksman, S. A. Antagonistic Relations of Micro-organism. Bacteriological Reviews, vol. 5, pp. 231-274, 1941.

25. Ruchhoft, C. C. Studies on the Longevity of Bacillus typhosus (Eberthella typhi) in Sewage Sludge. Sewage Works Journal, vol. 6, pp. 1054-1067, 1934.

26. Skinner, C. E. and Murray, T. J. The Viability of B. coli and B. aerogenes in Soil. J. Infectious Diseases, vol. 38, pp. 37-41, 1926.

27. Smith, R. P. The Influence of B. coli on the Growth of B. typhosus, with special reference to Enrichment by Brilliant Green in Typhoid Carriers. J. Path. Bact., vol. 26, pp. 122-123, 1923.

28. Vignati, J. Beitrag zur Kenntnis des Typhus-Coil-Antagonisms und Seiner Ausnutzung zur Anreicherung der Typhus bazillen. Zentr. Bakt. Parasitenk., I. Orig., vol. 107, pp. 54-69, 1928.

29. ZoBell, C. E. Bactericidal Action of Sea Water., Proc. Soc. Exp. Biol. and Med., Vol. 34, pp. 113-116, 1936.

30. Gratia, A., and Fredericq, P. Pluralité et Complexité des Colicins. Bull. Soc. Chim. Biol., vol. 29, pp. 354-356, 1947.

31. Hoover, O. H. Antagonisms in the Genus Escherichia in Relation to Antigenic Composition. Unpublished work, Thesis for Master's Degree, U. of O. Medical School, June, 1951.

32. Rosenthal, L. Microbes bacteriolytiques (Lysobactéries) Compt. rend. Soc. Biol, vol. 92, pp. 78-79, 472-474, 1925, vol. 93, pp. 1569-1571, 1925, vol. 95, pp. 10-11, 1926.

33. Kauffmann, F. and Vahlne, G. Uber die Bedeutung des serologischen Formenwechsels für die Bakteriophagenwirkung in der Coli-Gruppe. Acta Path. et Microbiologica Scandinavica. vol. 22, pp. 119-137, 1945.

34. Toft, G. Demonstration of Bacteriophages with effect on capsular strains of B. coli. Acta Path. et Microbiologica Scand., vol. 23, pp. 277-282, 1946.

35. Wallick, H. and Stuart, C. A. Antigenic Relationships of Escherichia Coli Isolated from One Individual. J. Bact., vol. 45, pp. 121-126, 1943.

36. Sears, H. J. Unpublished work, U. of O. Medical School, 1952.

37. Lembke, W. Beitrag zur Bacterienflora des Darms. Archiv. f. Hygiene, Bd. XXVI, S. 293-328, 1896.

38. Kauffmann, F. The Differentiation of Escherichia and Klebsiella Types. Monograph, Charles C. Thomas Co., Springfield, Ill., 1951.

39. Vahlne, Gosta. Serological Typing of the Colon Bacteria. Acta. Path. et Microbiol. Scandinav. Sup. LXII, 1945.

40. Kauffmann, F. and Perch, B. Further Biochemical Tests in the Coli Group. Acta Path. et Microbiol. Scandinav., vol. 25, pp. 507-512, 1948.

41. Kauffmann, F. Enterobacteriaceae. Ejnar Munksgaard, Publisher Copenhagen, 1951.

42. Kauffmann, F. The Serology of the Coli Group. J. of Immunology, vol. 57, pp. 71-99, 1947.

43. Stuart, C. A., Griffin, A. M. and Baker, M. E. Relationships of Coliform Organisms. J. of Bact., vol. 36, pp. 391-410, 1938.

44. Edwards, P. R., and Ewing, W. H. The Status of Serologic Typing in the Family Enterobacteriaceae. Am. J. Pub. Health, vol. 42, pp. 665-671, 1952.