THE INTESTINAL FLORA OF PREMATURE INFANTS

A Thesis

Presented to

the Faculty of the Department of Biology

UNIVERSITY OF HOUSTON

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

John W. Kolaja

August 1953

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Since a review of the literature indicates that there are only a few reports available on the micro-organisms in the intestinal tracts of newborn premature infants, further investigations in this field are warranted. In this study, an attempt was made to determine the time of appearance of intestinal flora in newborn premature infants.

Fecal specimens were obtained by rectal swabs. The scheduled times for the collection of specimens were between six and twelve hours after birth, twelve hours later, and daily thereafter.

The specimens were streaked onto media which were cultured under aerobic and anaerobic conditions for the isolation of intestinal pathogens and normal fecal flora. Differential media were innoculated for the separation of gram positive organisms from the gram negative group. All organisms isolated were identified by colonial characteristics, their appearance on stained smears and biochemical tests. The identifications followed Bergey's Manual of Determinative Bacteriology.

In order to obtain a relatively complete analysis in this particular phase of intestinal bacteriology, the time of appearance of fecal flora can be related to infants' weights, a period of fasting, feedings of different formulae, and the administration of antibiotics.

Over 90 per cent of the first specimens cultured were negative for bacterial growth. But in twenty-four hours, the percentage of positive cultures was just over ninety. Infants weighing between four pounds and four pounds and eight ounces exhibited sterile intestinal tracts longer than infants in other weight ranges. Infants fasted for twenty four hours also exhibited sterile intestinal tracts

longer than those fasted only twelve hours. Antibiotics seem to have an inhibitory effect on the appearance of some organisms even if they are components of the normal fecal flora.

Eight genera were isolated a sufficient number of times to be considered members of normal flora (appearance in 25% of the stools examined being used as a normal). Yeasts, particularly of the genus Monilia, were isolated frequently. And pseudomonas and proteus, both of which are supposedly uncommon to fecal flora, were isolated frequently enough to be considered normal members of the fecal flora.

These established findings should be valuable in the development of intestinal bacteriology of newborn premature infants.

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CHAPTER I

INTRODUCTION

The study of the intestinal flora of infants born at term was begun almost a century ago. Research in this field is abundant, therefore, it is impossible to discuss every investigators work since that time for there is much repetition and only a few major findings. A few of the well established, progressive studies will be discussed.

The appearance of micro-organisms in the intestinal tract of new-born infants, often within ten hours after birth and even before feeding, was observed by Breslau (1866) and was confirmed by Billroth (1874) and Nothnagel (1881).

Escherich (1885) demonstrated that micro-organisms could be grown culturally from fecal samples obtained as early as four hours after birth, even though they were not demonstrable microscopically from those same samples. The first organisms encountered, he called tetradencocci and kettencocci. He stated that while the intestinal flora of the first day is usually simple, in a few days it becomes increasingly complex. The irregular flora, composed of Escherichia coli, Aerobacter aerogenes, Bacillus subtilis, and yeasts, disappeared on or about the fourth day (when the yellow mucoid-acid stool replaced the dark, tarry meconium) and was subsequently replaced by a flora, predominantly composed of slender granular-staining, gram positive, non-sporulating bacilli. He stated that the gram negative organisms Escherichia coli and Aerobacter aero-

genes were obligate members of the milk stool, with the following forms as facultative intestinal bacteria: yellow liquefying bacilli, yellowish-white micrococci (Streptococcus coli brevis) Micrococcus ovalis, white liquefying staphylococci, red yeast, white yeast (Torula-Pasteur), capsulated yeast, and Monilia candida. When cultures on agar plates were made from milk stools, only Escherichia coli and Aerobacter aerogenes were present and gram positive bacteria rarely appeared.

Schmidt (1892) attempted to explain the infrequency of gram positive bacilli by innoculating stools onto acid, alkaline, and fatty media. He concluded that despite the staining differences, the gram positive and gram negative bacilli were growth forms of the same species. Bienstock (1884) isolated gram positive bacilli anaerobically from fecal specimens.

Tissier (1899) reported the isolation from infants feces of the anaerobic Bacillus bifidus communis, a gram positive branching bacillus. Moro (1900) discovered Bacillus acidophilus from the same source.

Logan (1913) upheld Tissier's data that fecal flora was dependent upon diet rather than age. Logan's investigations of bottle-fed infants indicated that Lactobacillus acidophilus was most commonly present when infants received bottle feedings. Lactose fermenters and variable coccal forms were more prevalent in this bottle-fed group than in the infants on breast-fed milk alone. Tissier contended that Clostridium perfringens is a normal

constituent of fecal flora of bottle and breast-fed infants, but Logan isolated the organism only three times from the same source. Hymanson and Hertz (1917) found micro-organisms in nine of thirty-nine specimens of meconium collected by rectal swab shortly after birth. Burrage (1927) reported the cultivation on solid media of bacteria from thirty-eight of one hundred first passage specimens of meconium, the first of which was collected at delivery and contained many bacteria.

Epstein and Jelnik (1932) were the first investigators to report micro-organisms in the intestinal tracts of a significant number of new-born premature infants. However, their study was restricted to one organism, Lactobacillus bifidus. In their group of infants (thirty-five per cent of which were premature) fifty per cent retained the lactobacilli when changed from breast milk to expressive human milk. In the other half of the group, (exactly fifty per cent of which were premature), a gram negative organism of the coli-aerogenes group supplanted it in three to seven days.

Gerstley, et al, (1939) further supplemented Tissier's and Logan's assumptions of the importance of the diet in the appearance of intestinal flora. Their experiments confirmed the work of Brown and Bosworth (1922) and Upton (1929); namely, that it takes approximately two weeks after cow's milk feedings have been discontinued and breast milk given exclusively for the intestinal environment to return again to a state favoring the gram positive flora. A marked difference appeared in the flora within twenty-four hours after the infants were changed from breast feedings to artificial feedings.

Hall and O'Toole (1934) reported that ninety-four per cent of the first specimens passed by infants fail to show bacteria microscopically, but also stated that organisms were present in six per cent of the specimens passed by infants twelve hours old. They demonstrated aerobic bacteria by culture methods in about thirty-eight per cent of the fecal specimens passed by the infants during the first five hours after birth; and that the percentage of specimens containing bacteria rose rapidly to one hundred during the first twenty-four hours of life. The predominating organisms were white micrococci and Escherichia coli, while streptococci and sportulating bacilli were rare.

The same investigators (1935) reported a study of sixty-three specimens of meconium and feces secured from ten new-born infants during the first ten days of life. Seven passed sterile specimens the first day but micro-organisms were present in specimens from all the infants by the second day.

Snyder (1936) published a study of organisms appearing in meconium from sixty-four infants four hours after delivery. Results were checked with skin cultures obtained at the same time. There appeared to be no correlation of the organisms isolated from the two sources.

Snyder (1939) examined the feces of twenty-two infants between two weeks and one year of age. His results showed that while many species of bacteria were isolated, only a few were present enough times to be considered as possible members of the normal fecal flora, even with twenty-five per cent of the stools examined selected the

lowest figure for normal. Davidson (1926) said that the normal intestinal flora is composed of only a half dozen species, but failed to give the species in the order of their appearance. Snyder's work supported Davidson's statement but in his study, the following species occurred in more than twenty-five per cent of the stools examined: Escherichia coli-93.3%, Streptococcus faecalis-78.5%, Clostridium welchi-68.7%, Clostridium teritium-51.6%, Bacteriodies, group I-47%, Aerobacter aerogenes-44%, Streptococcus mitis-29.7%, Macrococcus epidermis-26.4%, and Lactobacillus bifidus-24.4%. He also found that breast milk inhibits the growth of sporulating anaerobes, only four strains of such organisms being recovered from the feces of the breast-fed group. He cited staphylococci to be more prevalent during the early breast-fed period and supplemented breast-fed periods than in later months.

These findings may be compared with those of Duncan and Walker (1942) who discovered a high incidence of Staphylococcus aureus in the milk of nursing mothers and in the throats and intestines of their infants.

Ross (1950) studied feces of infants microscopically, affirming Levi's (1941) work that breast-fed infants have an advantage over artifically-fed in their resistance to various infections. Breast-fed infants produced fecal samples showing almost 100 per cent of the gram positive Lactobacillus bifidus on fecal films. Those of artifically-fed infants exhibited no predominant organisms but about 50 per cent of the bacteria seen were gram positive.

There is evidence, then, that the intestinal tracts of newborn infants may contain bacteria as soon as four hours after birth, and that the appearance of this fecal flora is dependent upon diet rather than age. Lactobacillus acidophilus appears to be the most common organism found in the intestinal tracts of bottle-fed infants, and Lactobacillus bifidus forms the largest percentage of the organisms so found in breast-fed infants. On the third or fourth day, gram negative bacteria began supplementing the lactobacilli, with Escherichia coli and Aerobacter aerogenes being the most common organisms in this group. Although there are other forms of facultative intestinal bacteria, it is generally accepted that normally the fecal flora is composed of a half dozen species.

This study was made to investigate the approximate time of appearance of micro-organisms other than lactobacilli in the intestinal tracts of new-born, premature infants. Lactobacilli have received much attention from previous investigators and the special effort required for their cultivation seemed unjustified in this study.

CHAPTER II

METHODS AND MATERIALS

Fecal specimens were obtained by use of rectal swabs which were prepared by fitting a short piece of glass tubing with fire-polished ends over a cotton applicator stick. This was placed in a test tube, which was then plugged with a cotton ball and sterilized in the autoclave at fifteen pounds pressure for forty-five minutes. When specimens were desired, the anal region of the infant was cleansed with phisoderm, and the glass tube inserted into the rectum just past the anal sphincter. The swab was then run into the rectum, withdrawn, and replaced in the sterile test tube.

All of the specimens were cultured within an hour after collection. Salmonella and Shigella medium was streaked to determine if these pathogens might appear early in the life of premature infants. Desoxycholate agar was used for the isolation of other gram negative organisms. An enrichment broth (Brain Heart Infusion) was innoculated and later was subcultured onto Eosin Methylene Blue agar and Sodium Azide agar for the separation of the gram negative organisms from the gram positive groups. A Thioglycollate broth was innoculated for anaerobic organisms and facultative anerobes. Growth from this medium was then subcultured onto Eosin Methylene Blue agar and Sodium Azide agar and placed under alkaline pyrogallol conditions for anaerobiosis. A Sabouraud's agar slant was streaked for yeasts and fungi.

The media innoculated from the original specimens for the isolation of gram negative pathogens were satisfactorily demonstrated. No

salmonella or shigella were found in the study. The non-lactose fermenting as well as the lactose fermenting organisms were successfully cultured on the Desoxycholate agar when present.

The routines for isolating organisms from the Eosin and Sodium Azide agar plates grown aerobically and anaerobically were identical. In instances where Escherichia coli could not be distinguished from Aerobacter aerogenes by cultural characteristics, biochemical tests were used. When other gram negative organisms were found, they were streaked on Eosin Methylene Blue agar for isolation. Then, if they were non-distinguishable by cultural characteristics, biochemical tests were employed. Identification of the organisms followed Bergey's Manual of Determinative Bacteriology.

The identification of staphylococci from the Sodium Azide agar was done primarily by staining characteristics and colonial morphology. In some instances, subculture onto Loeffler's media for pigment production aided in the separation of species otherwise not separately identifiable.

Streptococci were differentiated according to colonial morphology, staining characteristics, and hemolysis of human blood. Enterococci were identified by their appearance on smear, hemolysis of blood, and the ability to grow in liquid medium containing 6.5% saline.

The anaerobic gram positive bacilli found were identified by microscopic smears from liquid and solid media, hemolysis of blood, spore formation, and biochemical determinations.

The yeasts were identified by growth characteristics and microscopic examination of stained smears and wet preparations.

One hundred and ten newborn, premature infants varying in weight from two pounds and eleven ounces to five pounds composed the group from which fecal swabs were obtained. This study included eight sets of twins.

The original procedure was to obtain specimens of meconium by rectal swab between six and twelve hours after birth and at the end of the following twelve hours. Thereafter, swabs were collected every day. Several of the infants were dismissed in a few days, limiting long range studies to fifty-seven infants for one week, twenty-three for two weeks, nineteen for three weeks, and twelve for four weeks. It must be emphasized that frequently infants were in a weak condition after delivery and it was not advisable to disturb them for rectal swabs at the scheduled time of collection. Therefore, the specific times planned for collection of the specimens could not always be adhered to.

The infants were admitted to separate nurseries depending upon their respective weights and general conditions. Those near the weight limit for normal term infants and appearing normal with good color were cared for in the newborn nursery along with the newborn, full-term infants. The smaller, more premature both in weight and gestation period were placed in the premature nursery.

The separation of infants into the two nurseries is important because of the differing schedules of feeding in each nursery. In the premature nursery, the infants received nothing by mouth for the first twenty-four hours of life. During the second twenty-four hour period, these infants received four feedings of distilled water and four feedings of five per cent dextrose. The babies were not fed when exactly twenty-four hours old, but at the scheduled feeding time which was

nearest to their being one day old. During the third day of life, the infants were started on a one-third strength formula which varied with their respective weights. If an infant weighed less than three pounds and twelve ounces, it was started on either Mead-Johnson formula 410 or 411 depending on the order of the attending pediatrician. Formula 410 consisted of a non-fat milk solution, and dextri-maltose; formula 411 was composed of coconut oil, dextri-maltose and milk components. If an infant weighed more than three pounds and twelve ounces, it was fed one-third strength Alacta, a Mead-Johnson formula of low sodium, high carbohydrate, and low fat content. The fourth day, two-thirds strength formulas were fed and at the beginning of the fifth day, all the infants received full strength formulas.

In the new-born nursery, feedings were begun after the first twelve hours and consisted of distilled water every four hours until the infants were twenty-four hours old. For the following week, they were on a formula of evaporated milk, dextri-maltose, and distilled water. The scheduled feedings were every four hours.

CHAPTER III

RESULTS

The results of this study are found in the following tables which are constructed according to various restrictions and conditions. An interpretation of the tables is found in the discussion.

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
1	3	5.5	White	Male	13	13	13 Hours
2	3	5.5	White	Male	13	13	13 Hours
3	3	12	Colored	Male	6.5	6.5	31 Hours
4	5	8	Colored	Female	6	6	13 Hours
5	5	4	Colored	Female	12	12	19 Hours
6	4	3	Colored	Female	12.5	12.5	14.5 Hours
7	4	7	Colored	Male	12.5	12.5	12.5 Hours
8	4	0	Colored	Male	6.5	6.5	6.5 Hours
9	5	4	Colored	Female	10	10	10 Hours
10	4	12	White	Male	6	6	6 Hours
11	4	12	Colored	Female	7	7	7 Hours
12	5	8	White	Male	15.25	15.25	15.25 Hours
13	5	2.5	White	Female	15	15	15 Hours
14	3	9	Colored	Female	11	11	11 Hours
15	4	5.5	Colored	Male	7.5	7.5	13.25 Hours

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
16	5	0	Colored	Male	11.25	11.25	11.25 Hours
17	4	11	White	Female	12	12	7 Days
18	4	12	Colored	Female	9	9	7 Days
19	2	11	Colored	Male	Positive In 6 Hours (First Culture)		7 Days
20	4	5	Colored	Female	6	6	7 Days
21	4	12	Colored	Female	11	60	7 Days
22	4	10	Colored	Male	27.25	27.25	27.25 Hours
23	3	15	Colored	Male	23	23	7 Days
24	3	12	Colored	Male	17.25	17.25	7 Days
25	4	1	Colored	Male	Positive In 13 Hours (First Culture)		7 Days
26	4	13	White	Female	12.25	12.25	7 Days
27	5	2	Colored	Male	21.75	21.75	1 Day
28	3	13	Colored	Female	6	6	7 Days
29	4	1	White	Female	6	6	7 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
30	5	1	White	Female	10	10	10 Hours
31	4	9.75	Colored	Male	10	10	7 Days
32	4	13	Colored	Female	8.5	8.5	7 Days
33	4	7	White	Female	16.75	16.75	1 Day
34	4	9	White	Female	16.50	16.50	1 Day
35	5	4	White	Male	Positive In 16 Hours (First Culture)		1 Day
36	3	13	White	Female	12	12	7 Days
37	4	2	Colored	Male	Positive In 9 Hours (First Culture)		7 Days
38	5	7.25	Colored	Female	7.25	7.25	1 Day
39	3	14	White	Male	15	15	4 Days
40	3	4	Colored	Male	12	12	1 Day
41	4	0	Colored	Female	6	6	2 Days
42	4	6	Colored	Male	Positive in 11 Hours (First Culture)		7 Days
43	5	2	Colored	Female	20.5	20.5	2 Days
44	4	4	Colored	Female	12.5	12.5	2 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
45	4	3	Colored	Female	10	10	7 Days
46	5	8	Colored	Female	7.5	7.5	2 Days
47	5	7	Colored	Male	12.25	18.5	1 Day
48	4	8	Colored	Female	16	16	7 Days
49	5	2	White	Male	9.75	9.75	2 Days
50	5	6	White	Male	Positive In 6 Hours (First Culture)		1 Day
51	3	4.75	Colored	Male	14.50	14.50	1 Day
52	5	4	Colored	Female	14	14	7 Days
53	5	3.75	Colored	Male	11.25	11.25	1 Day
54	5	1	Colored	Male	11	11	2 Days
55	3	5	Colored	Male	12	12	1 Day
56	3	7.5	Colored	Female	7.75	7.75	3 Days
57	5	6	Colored	Female	20	20	2 Days
58	5	5.5	Colored	Male	9	9	2 Days
59	3	12	Colored	Female	17.5	17.5	7 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
60	4	3	Colored	Female	16.25	16.25	7 Days
61	4	77	Colored	Female	32.75	32.75	7 Days
62	5	1	White	Male	8	8	5 Days
63	5	2	Colored	Female	10.25	10.25	2 Days
64	4	6.75	White	Male	41.50	41.50	7 Days
65	4	5	Colored	Male	8	8	3 Days
66	5	6.25	Colored	Male	11	11	4 Days
67	4	9	Colored	Female	8	8	14 Days
68	3	10	Colored	Female	12.75	12.75	7 Days
69	5	8	Colored	Female	8.25	8.25	2 Days
70	5	8	Colored	Female	13.75	13.75	1 Day
71	5	8	Colored	Female	12	12	2 Days
72	4	13	White	Male	17.50	17.50	30 Days
73	5	8.5	Colored	Female	8	8	2 Days
74	3	7	Colored	Male	11.5	11.5	30 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
75	4	4	White	Male	13.25	18.25	30 Days
76	5	3	White	Male	12	12	30 Days
77	5	1	Colored	Female	10	14.25	1 Day
78	4	0	Colored	Female	24.75	24.75	14 Days
79	4	1.50	Colored	Female	8	8	30 Days
80	4	9	Colored	Female	18.75	18.75	30 Days
81	3	8.5	Colored	Male	16	16	30 Days
82	44	2.5	Colored	Female	22	22	2 Days
83	4	7.5	Colored	Female	Positive in 12.5 Hours (First Culture)		30 Days
84	3	10	Colored	Male	11.25	11.25	30 Days
85	3	12	Colored	Female	10.50	10.50	30 Days
86	4	13	Colored	Male	10.50	10.50	21 Days
87	2	10	Colored	Female	10.50	10.50	21 Days
88	2	11	Colored	Male	12.25	12.25	1 Day
89	3	0	Colored	Male	Positive in 14 Hours (First Culture)		30 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
90	3	5.5	White	Male	51	51	3 Days
91	3	11	Colored	Female	14	14	30 Days
92	5	0	Colored	Female	Positive in 9.5 Hours (First Culture)		1 Day
93	4	7.25	Colored	Male	11.50	11.50	21 Days
94	4	9	Colored	Male	11.25	11.25	21 Days
95	4	4	White	Male	18.50	18.50	1 Day
96	4	12	Colored	Male	Positive in 6 Hours (First Culture)		14 Days
97	4	7	Colored	Male	11.25	11.25	22 Days
98	3	7	Colored	Female	11.75	11.75	22 Days
99	5	3.5	Colored	Female	9.25	9.25	2 Days
100	4	2	Colored	Male	19.25	19.25	21 Days
101	5	6	Colored	Male	Positive in 16.5 (First Culture)	16.25	3 Days
102	55	1	Colored	Female	16.50	16.50	3 Days
103	4	15	Colored	Male	10.25	10.25	2 Days

TABLE I

LENGTH OF TIME THAT THE INTESTINAL TRACT OF NEWBORN PREMATURE INFANTS APPEARED STERILE

CASE	WEIGHT POUNDS	WEIGHT OUNCES	RACE	SEX	HOURS CULTURED NEGATIVE AEROBIC	HOURS CULTURED NEGATIVE ANAEROBIC	TIME CULTURED
104	4	3	White	Male	12	12	14 Days
105	4	11	Colored	Female	25.50	11	14 Days
106	4	1	Colored	Female	25.25	12.25	7 Days
107	4	1	Colored	Female	10	10	7 Days
108	4	3.5	Colored	Female	13.5	13.5	7 Days
109	4	3	Colored	Male	9.5	9.5	7 Days
110	4	11	Colored	Male	16.75	16.75	7 Days

TABLE II

A STUDY OF THE APPEARANCE OF INTESTINAL FLORA IN SEVEN SETS OF TWIN PREMATURE INFANTS

Case	Individual Twin	Hours Cultured Negative		Time Cultured	Day of Appearance of Micro-Organisms								
		Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
1	A	15.25	15.25	1 Day	Patient Dismissed								
	B	Positive in 15 Hours (First Culture)		1 Day					1				1
2	A	18.75	18.75	2 Days	2						2	2	
	B	16.50	16.50	2 Days	2				2				
3	A	17.50	17.50	7 Days	5	4	2		2	7	4		
	B	16.25	16.25	7 Days		2	6		2	2	4		
4	A	11.25	11.25	28 Days	2	3	8	4	2	2	8		4
	B	10.50	10.50	28 Days	2	4	6		4	2	7	2	
5	A	Positive in 13.50 Hours (First Culture)		28 Days		5	6	3	1	4	4		
	B	12.25											

TABLE II

A STUDY OF THE APPEARANCE OF INTESTINAL FLORA IN SEVEN SETS OF TWIN PREMATURE INFANTS

Case	Individual Twin	Hours Cultured Negative		Time Cultured	Day of Appearance of Micro-Organisms								
		Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
6	A	11.50	11.50	21 Days	2	2	10	2		2	4		
	B	11.25	11.25	21 Days	2	7	18			2	2		
7	A	Positive in 16 Hours (First Culture)		3 Days	1	2							
	B	16.50	16.50	3 Days	2	2			2		2		

TABLE III

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 2 Pounds, 11 Ounces to 3 Pounds, 8 Ounces Infants Weight	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
2 Pounds, 11 Ounces	Positive in 6 Hours (First Culture)						1				
3 Pounds, 3 Ounces	9.50	9.50	1					1			
3 Pounds, 4 Ounces	12	12					1				
3 Pounds, 7 Ounces	11.50	11.50				1	1				
3 Pounds, 7 Ounces	11.75	11.75	1				1	1			
3 Pounds, 7.5 Ounces	7.75	7.75		1							1
3 Pounds, 8.5 Ounces	16	16	1		1		1		1		

TABLE IV

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 3 Pounds, 9 Ounces To 4 Pounds, 0 Ounces	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
3 Pounds, 9 Ounces	11	11		1							
3 Pounds, 10 Ounces	12.75	12.75				1	1	1			
3 Pounds, 11 Ounces	14	14	1		1						
3 Pounds, 12 Ounces	17.25	17.25	1								
3 Pounds, 12 Ounces	10.50	10.50	1						1	1	
3 Pounds, 12 Ounces	17.50	17.50			1		1				
3 Pounds, 12 Ounces	6.50	6.50					1			1	
3 Pounds, 13 Ounces	6	6	1	1		1					
3 Pounds, 13 Ounces	12	12	1					1			
3 Pounds, 14 Ounces	15	15	1	1				1			
3 Pounds, 15 Ounces	23	23	1				1	1			

TABLE IV

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 3 Pounds, 9 Ounces To 4 Pounds, 0 Ounces	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
4 Pounds, 0 Ounces	24.75	24.75	1				1	1			
4 Pounds, 0 Ounces	6	6	1				1				

TABLE V

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
4 Pounds, 1 Ounce	Positive in 13.75 (First Culture).				1						
4 Pounds, 1 Ounce	6	6	1			1					
4 Pounds, 1.5 Ounces	8	8					1	1			
4 Pounds, 2 Ounces	19.25	19.25						1			
4 Pounds, 2.5 Ounces	22	22					1				
4 Pounds, 3 Ounces	16.25	16.25		1	1		1	1			
4 Pounds, 3 Ounces	12	12			1						
4 Pounds, 3 Ounces	10	10					1	1	1		
4 Pounds, 3 Ounces	12.50	12.50	1							1	
4 Pounds, 4 Ounces	12.50	12.50	1				1				
4 Pounds, 4 Ounces	13.25	13.25	1	1				1			

TABLE V

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range		Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
		Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
4 Pounds, 1 Ounce To 4 Pounds, 8 Ounces												
4 Pounds,	4 Ounces	18.25	18.25	1								
4 Pounds,	5 Ounces	6	6	1								
4 Pounds,	5 Ounces	8	8	1	1							
4 Pounds,	6 Ounces	Positive in 11 Hours (First Culture)				1						
4 Pounds,	6.75 Ounces	41.50	41.40	1	1			1	1	1		
4 Pounds,	7 Ounces	11.25	11.25	1		1						
4 Pounds,	7 Ounces	11.50	11.50	1	1		1		1	1		
4 Pounds,	7 Ounces	32.25	32.25					1		1		
4 Pounds,	7 Ounces	16.75	16.75	1					1	1		
4 Pounds,	7.5 Ounces	Positive in 12 Hours (First Culture)							1			
4 Pounds	8 Ounces	16	16	1				1	1			

TABLE VI

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 4 Pounds, 9 Ounces to 5 Pounds, 0 Ounces	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
4 Pounds 9 Ounces	18.75	18.75	1					1	1	1	
4 Pounds 9 Ounces	8	8	1					1	1		
4 Pounds 9 Ounces	11.25	11.25	1					1	1		
4 Pounds 9.75 Ounces	10	10	1								
4 Pounds 11 Ounces	16.75	16.75	1						1		
4 Pounds 11 Ounces	25	11	1	1							
4 Pounds 11 Ounces	12	12	1						1		
4 Pounds 12 Ounces	7	7					1				
4 Pounds 12 Ounces	11	11					1				
4 Pounds 12 Ounces	9	9	1								
4 Pounds 12 Ounces	Positive in 6 Hours (First Culture)		1								

TABLE VI

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 4 Pounds, 9 Ounces to 5 Pounds, 0 Ounces	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
4 Pounds 12 Ounces	Positive in 6 Hours (First Culture)		1								
4 Pounds 13 Ounces	17.50	17.50	1	1							
4 Pounds 13 Ounces	10.50	10.50					1				
5 Pounds 0 Ounces	Positive in 9 Hours (First Culture)										

TABLE VII

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 4 Pounds, 9 Ounces to 5 Pounds, 0 Ounces		Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
		Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
5 Pounds	1 Ounce	16.5	16.5	1	1			1		1		
5 Pounds	1 Ounce	10	10	1	1							
5 Pounds	1 Ounce	8	8	1	1							
5 Pounds	2 Ounces	9.75	9.75	1	1							
5 Pounds	2 Ounces	10.25	10.25	1	1			1	1			
5 Pounds	2.5 Ounces	15	15					1				
5 Pounds	3 Ounces	12	12		1			1	1			
5 Pounds	3.5 Ounces	9.25	9.25	1	1					1		
5 Pounds	4 Ounces	12	12					1				
5 Pounds	4 Ounces	Positive in 16 Hours (First Culture)		1	1			1				
5 Pounds	4 Ounces	14	14	1	1							
5 Pounds	5.5 Ounces	9	9	1	1							

TABLE VII

THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS RELATED TO SPECIFIC WEIGHT GROUPS

Weight Range 4 Pounds, 9 Ounces to 5 Pounds, 0 Ounces	Minimal Hours Cultured Negative		Micro-Organisms Present In The First Positive Cultures								
	Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
5 Pounds 6 Ounces	Positive in 6 Hours (First Culture)		1	1							
5 Pounds 6 Ounces	20	20	1	1							
5 Pounds 6 Ounces	Positive in 16.5 Hours (First Culture)		1								
5 Pounds 6.25 Ounces	11	11	1	1	1						
5 Pounds 7 Ounces	12.25	8.50							1		
5 Pounds 7.25 Ounces	7.25	7.25	1	1					1		
5 Pounds 8 Ounces	8.25	8.25		1			1		1		
5 Pounds 8 Ounces	7.25	7.25	1	1							
5 Pounds 8 Ounces	6	6					1				
5 Pounds 8 Ounces	8	8					1	1			
5 Pounds 8 Ounces	12	12					1		1		

TABLE VIII

COMPARISON OF THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS IN VARIOUS WEIGHT RANGES

Infants	Weight Range Lbs. Oz. to Lbs. Oz.	Average Hours of Negative Culture		Per Centage of Infants Containing Specific Organisms in First Positive Cultures								
		Aerobic	Anaerobic	Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diptheroids	Gram Positive Bacilli
6	3 3 to 3 8.5	13	13	50	16.7	16.7	16.7	83.5	33.4	16.7	0	16.7
13	3 9 to 4 0	13.1	13.1	71.1	23.7	15.8	15.8	47.4	31.6	7.9	15.8	0
19	4 1 to 4 8	15.5	15.8	63.6	26.5	15.9	10.6	42.4	47.4	26.5	5.3	0
13	4 9 to 5 0	13.4	12.5	79.0	15.8	0	0	31.6	23.7	31.6	7.9	0
19	5 0 to 5 8	10.9	10.3	63.6	74.2	5.3	0	47.7	15.9	26.5	0	0

TABLE IX

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL TRACT OF PREMATURE INFANTS FASTED FOR THE FIRST TWELVE HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms								
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diptheroids	Gram Positive Bacilli
1	16.75	16.75	2 Days	2				2	2		2	
2	16.50	16.50	2 Days	2				2				
3	Positive in 16 Hours (First Culture)		2 Days	1	2			1				
4	7.25	7.25	2 Days	1	1					1		
5	7.50	7.50	2 Days	2	2							
6	12.25	8.50	1 Day						1			
7	9.75	9.75	2 Days	2	2							
8	Positive in 6 Hours (First Culture)		2 Days	1	1		1		2			
9	14.25	14.25	7 Days	2	2			3	7			
10	20.00	20.00	2 Days	2	2							
11	11.50	11.50	2 Days	1	1							
12	10.25	10.25	2 Days	2	2			2	2			

TABLE IX

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL TRACT OF PREMATURE INFANTS FASTED FOR THE FIRST TWELVE HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms								
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
13	8.50	8.50	2 Days		2			2		2		
14	12.00	12.00	2 Days					1		1		
15	8.0	8.0	2 Days	1								
16	10.25	20.25	2 Days	1	1							
17	Positive in 9.5 Hours (First Culture)		2 Days	1								
18	18.50	18.50	1 Day		1							
19	Positive in 16.50 Hrs. (First Culture)	16.50	3 Days	1	2							
20	16.50	16.50	3 Days	2	2			2		2		

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms									
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
1	6.5	6.5	1 Day						1		1	1	
2	6	6	1 Day						1				
3	12	12	1 Day						1				1
4	12.50	12.50	1 Day						1			1	
5	Positive in 7 Hours (First Culture)		1 Day						1			1	
6	Positive in 15.25 Hours (First Culture)		1 Day						1				1
7	Positive in 11 Hours (First Culture)		1 Day		1								
8	7.50	7.50	1 Day		1								
9	12.50	12.50	7 Days	2	2				4		2		

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative: Aerobic	Anaerobic	Time Cultured	Day of Appearance of Organisms: Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
10	8.75	8.75	7 Days	1					5				
11	Positive in 6 Hours (First Culture)		7 Days	2					1		5		
12	6	6	7 Days	2					7		7		
13	11	60	7 Days	6			4		1			6	
14	23	23	7 Days	3					3		6	5	
15	17.50	17.50	7 Days	2							4	5	
16	Positive in 14 Hours (First Culture)		7 Days	5					4			1	2
17	12.50	12.50	7 Days	1	2						3	3	
18	6	6	7 Days	2				2		5	7		
19	21.75	21.75	7 Days	2	7			2			4		
20	10	10	7 Days		1		2				1		
21	8.50	8.50	7 Days		3				2				

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms									
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
22	12	12	7 Days	1			4			4	1		
23	Positive in 9 Hours (First Culture)		7 Days	1				6	1	1	1	1	
24	15	15	6 Days	2	2		2				2	4	
25	12	12	1 Day						1				
26	6	6	1 Day	1					1				
27	Positive in 11 Hours (First Culture)		7 Days	5			1		3	3			
28	10.25	10.25	6 Days	2		2			2	2			
29	16	16	7 Days	2	7				2	3			
30	15.25	15.25	3 Days	2									2
31	17.25	17.25	9 Days	5	4		2		2	8	4		

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms										
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
32	16.25	16.25	9 Days		2		6		2	2	4		
33	32.75	32.75	9 Days	5	5		5	3	3	4	3		
34	41.50	41.50	7 Days	3	4				3	3			
35	8	8	2 Days	2	2						3		
36	20	20	9	2	3			9		2	2		
37	18.50	18.50	30 Days	2	2	4	14		23	4	7		
38	11.50	11.50	30 Days	3	5			2	2	5	13		14
39	18.25	13.25	30 Days	3	3		16	7	6	2	7		
40	30.75	30.75	14 Days	4	5	3	8		3	3			
41	10	10	30 Days	3	4		6		2	2	3		
42	18.75	24.75	30 Days	2	6		5	20	2	3	9	13	6
43	16	16	30 Days	2	4		2		2	2	3		

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative: Aerobic	Minimal Hours Cultured Negative: Anaerobic	Time Cultured	Day of Appearance of Organisms: Escherichia coli	Aerobacter Aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
44	Positive in 12.5 (First Culture)	12.5	30 Days	1	3		5	5	2	2	2	5	
45	11.25	11.25	21 Days	2	3		4	4	2	2	8		4
46	10.50	10.50	21 Days	2	4	8	6		4	2	4	2	
47	10.25	10.25	18 Days	4	15				2	3	5	4	3
48	Positive in 12 Hours (First Culture)		30 Days	8	6		7	3	1	4	3		
49	Positive in 12 Hours (First Culture)		30 Days	12	2		1	4		4	2		
50	6	6	30 Days	1	9		1		3	5	2		5
51	11.50	11.50	22 Days	1	1		11	1		1			
52	11.25	11.25	22 Days	1	6		16			1	1		
53	Positive in 6 Hours (First Culture)		14 Days	1	6		8			2			

TABLE X

THE APPEARANCE OF MICRO-ORGANISMS IN THE INTESTINAL FLORA OF PREMATURE INFANTS FASTED FOR THE FIRST TWENTY-FOUR HOURS OF LIFE

Case	Minimal Hours Cultured Negative		Time Cultured	Day of Appearance of Organisms									
	Aerobic	Anaerobic		Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli
54	11.25	11.25	21 Days	3	3		2			3	3		
55	11.75	11.75	19 Days	2	3		10		2	2			4
56	19.25	19.25	14 Days	2	7		6	4	3	3			
57	26.25	11.75	13 Days	2	2					3			3
58	12.25	12.25	12 Days	7	6	2	9		7	7			
59	10	10	9 Days	5	4		5				2		

TABLE XI

THE EFFECTS OF ANTIBIOTIC THERAPY ON THE APPEARANCE OF INTESTINAL FLORA IN PREMATURE INFANTS

Case	Weight Lbs.	Weight Oz.	Hours Cultured Negative Aerobic	Hours Cultured Negative Anaerobic	Time Cultured	Time Antibiotic Administered	Organisms Present in the Flora Prior to Antibiotic Therapy: Escherichia coli	Aerobacter aerogenes	Pseudomonas	Proteus	Staphylococci	Enterococci	Streptococci	Day of Appearance of Micro-Organisms in the Intestinal Post Antibiotic Therapy: Escherichia coli	Aerobacter aerogenes	Pseudomonas	Proteus	Staphylococci	Enterococci	Streptococci
1	5	1	9.5	9.5	6 Days	At Birth								1	1		5	5		
2	5	6	11	11	3 Days	At Birth								2	2		2	3	3	
3	3	10	12.75	12.75	7 Days	On 4th Day			1		1		1		1		1	1		1
4	5	3	12.25	12.25	30 Days	On 3rd Day		1			1	1		6		8	10			
5	4	6	12	12	14 Days	On 3rd Day			1			1		6	10		6			
6	3	15	13.5	13.5	7 Days	On 3rd Day					1		1	6	6				5	
7	3	4	9.5	9.5	7 Days	On 2nd Day	1								4		7		5	3
8	4	9	6	6	7 Days	At Birth								2	4				2	
9	4	11	12.5	12.5	2 Days	At Birth								1				1		
Average Day of Appearance of Specific Micro-Organisms in the Intestinal Flora of Premature Infants Post Antibiotic Therapy														3.5	4.6	8	6	3.5	5	4

TABLE XII

THE APPEARANCE AND SUCCESSION OF INTESTINAL FLORA IN SEVENTEEN PREMATURE INFANTS REPORTED DAILY FOR ONE WEEK

	PER CENTAGE OF INFANTS EXHIBITING SPECIFIC ORGANISMS IN FECAL CULTURES										
Day	Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli	Monilia
1	11.6	11.6	0	0	0	11.6	11.6	5.8	11.6	0	0
2	75.4	17.4	0	5.8	17.4	34.8	40.6	11.6	23.2	11.6	17.4
3	100	23.2	11.6	0	23.2	40.6	63.8	17.4	29	11.6	17.4
4	100	23.2	5.8	11.6	23.2	34.8	75.4	5.8	29	17.4	0
5	100	40.6	0	5.8	23.2	40.6	87	11.6	11.6	5.8	23.2
6	100	23.2	0	11.6	29	34.8	87	11.6	17.4	5.8	17.4
7	100	34.8	0	11.6	23.2	34.8	100	17.4	17.4	5.8	11.6

TABLE XIII

THE APPEARANCE AND SUCCESSION OF INTESTINAL FLORA IN TEN PREMATURE INFANTS REPORTED ON ALTERNATE DAYS FOR ONE MONTH

	Per Centage of Infants Exhibiting Specific Organism in Fecal Cultures										
Day	Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli	Monilia
2	50	40	0	20	20	70	50	20	0	0	20
4	70	50	0	10	30	80	100	40	0	0	30
6	80	80	0	40	70	70	100	40	10	20	50
8	60	70	0	30	40	40	100	40	10	10	50
10	80	80	0	60	50	60	90	60	10	0	50
12	70	100	0	60	30	70	100	30	0	0	60
14	80	90	0	70	50	60	100	50	10	0	60
16	80	90	0	70	30	30	100	50	0	10	70
18	80	80	0	70	30	20	100	40	0	0	60
20	90	90	0	40	30	40	90	40	0	0	60
22	80	100	0	50	50	30	90	40	0	0	70

TABLE XIII

THE APPEARANCE AND SUCCESSION OF INTESTINAL FLORA IN TEN PREMATURE INFANTS REPORTED ON ALTERNATE DAYS FOR ONE MONTH

	Per Centage of Infants Exhibiting Specific Organism in Fecal Cultures										
Day	Escherichia coli	Aerobacter aerogenes	Paracolon	Proteus	Pseudomonas	Staphylococci	Enterococci	Streptococci	Diphtheroids	Gram Positive Bacilli	Monilia
24	90	90	0	60	30	20	80	40	0	0	70
26	70	90	0	50	50	20	90	30	0	0	70
28	80	100	0	50	40	20	80	30	0	0	70
30	80	100	0	50	50	20	90	20	0	0	70

CHAPTER IV

DISCUSSION

The results of this study are presented in table forms. The first series of these charts is a summary of the newborn infants studied, indicating their weight, race, sex, the time specimens were collected and the minimal hours their intestinal tracts were observed to be negative of bacteria. In eleven instances, the first specimens obtained contained bacteria, the earliest positive culture occurring at six hours. In four of the cases, aerobic organisms were isolated before the anerobic; and in two instances, anaerobic organisms appeared before aerobic. Organisms isolated from these four aerobic cultures were Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus, and Streptocci. From the two anaerobic cultures Alpha hemolytic streptococci, Escherichia coli, and Aerobacter aerogenes were isolated.

The second table is concerned with a study of seven sets of twins. In three of the sets, one infant exhibited positive meconium cultures before its twin. There appears to be no actual correlation of the fact that the twins were so closely related in gestation period and prenatal environment to the time that specific micro-organisms appeared in the intestinal tract of either twin. However, Escherichia coli, Aerobacter aerogenes and Staphylococcus aureus, in that respective order, were the most common organisms first invading the intestinal tracts.

The findings of the study were separated into six groups based on the infants' weights, and differing in minimum and maximum by a half pound. Summation of the observed minimal hours that negative results were obtained showed that the intestinal tracts of infants weighing between five pounds and five and one-half pounds were invaded by micro-organisms earlier than those of lighter weight babies. Infants ranging in weight from four pounds to four pounds and eight ounces exhibited average negative cultures for the longest periods. Overall percentages indicated that the intestinal flora generally began with Escherichia coli, staphylococci, or Aerobacter aerogenes in that respective order or in combinations with one another.

Charts were developed to compare the periods of fasting to the differences in the time of appearance of micro-organisms in the intestinal flora. Of twenty newborn premature infants fasted for twelve hours, twenty per cent of the first specimens collected gave positive results on culture. Of the remaining eighty per cent, the average number of minimal hours that negative results were obtained was twelve and one-half for aerobic micro-organisms and thirteen and three-fourths for anaerobic ones. The facultative aerobes Escherichia coli, Aerobacter aerogenes, and Staphylococcus aureus, were the most commonly appearing organisms during the first and second days. Enterococci were found in forty-eight per cent of the specimens after four days.

Of the fifty-nine infants fasted for twenty-four hours, 18.7% yielded aerobic and anaerobic organisms on culture of the first

specimen. The other 81.3% showed average minimal negative aerobic cultures for thirteen and three-fourths hours and just over fourteen and three-fourths hours for anaerobes. This is approximately an hour longer than the group fasted for only twelve hours. It seems as if the times that feedings are begun have little effect on the time of appearance of bacteria.

In the report of the appearance of intestinal flora post-antibiotic therapy, the specific antibiotics used were Gantrisin, Streptomycin, and Penicillin. Of nine cases studied, five infants presented positive fecal cultures prior to the administration of the antibiotic, the organisms isolated being common to intestinal flora. However, post-antibiotic therapy, these organisms were not cultured. It is possible that the organisms exhibited a marked sensitivity to the antibiotics. Another noticeable result was the delayed appearance of the colon-aerogenes group post-antibiotic therapy.

The results illustrating the particular constituents of the flora at definite times are illustrative of a significant number of individual cases and should be valuable in determining the type stools passed at a specific time.

As previously stated, Hall and O'Toole (1934) cultured aerobic bacteria from thirty-eight per cent of the specimens passed during the first five hours and found that the percentages rose rapidly to one hundred during the first twenty-four hours of life. The predominating organisms they observed were white micrococci and Escherichia coli, while streptococci and sporulating bacilli were rare.

In this study, aerobic organisms were cultured from only three per cent of the specimens obtained during the first six hours and the percentage rose to ninety-one in twenty-four hours. Escherichia coli and staphylococci were the predominant organisms with Aerobacter aerogenes and streptococci being found frequently. Sporulating bacilli were rarely isolated, being found from one day to two weeks after birth.

Snyder, (1939) selecting twenty-five per cent as the lowest figure, found six species composing normal intestinal flora, but this study showed eight genera which appeared in at least twenty-five per cent of the specimens examined. These organisms were Streptococcus faecalis- 80.1%, Escherichia coli- 75.8%, Aerobacter aerogenes- 58.1%, Monilia candida- 36.7%, staphylococci- 35.7%, proteus-29.8%, streptococci- 28%, and pseudomonas- 20.2%. The organisms isolated by Snyder (1939) and in common with these findings were Escherichia coli- 93.3%, Streptococcus faecalis- 78.5%, and Aerobacter aerogenes- 44%. Although Dudgeon stated that proteus and pseudomonas are not common components of the fecal flora, the organisms were isolated often.

In twenty-one cases, a species of proteus overgrew the other bacterial colonies on both Eosin Methylene Blue agar and Sodium Azide agar. However, successive cultures revealed complete disappearance of this organism in nineteen of the cases.

A species of fusiform bacilli and Bacteriodes were isolated once.

Gram positive bacilli were isolated in only eleven cases, appearing

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any time from the first day to two weeks. The cases can be sub-divided into two of Bacillus subtilis, two of Clostridium fissum, three of Clostridium tertium, and four of Clostridium welchi.

CHAPTER V

SUMMARY

The reports containing the most important findings in the intestinal bacteriology of infants were reviewed. In this study, an attempt was made to determine the time of appearance of intestinal flora in newborn premature infants. Fecal specimens were obtained by rectal swabs from one hundred and ten new-born premature infants. The scheduled times for the collection of the specimens were between six and twelve hours after birth, twelve hours later, and once a day after the first day. Occasionally the infants were in a weakened condition after delivery and the specimens could not be collected at the scheduled times.

The specimens were streaked onto media which were cultured under aerobic and anaerobic conditions for the isolation of intestinal pathogens and normal fecal flora. Differential media were innoculated for the separation of gram positive organisms from the gram negative group. Some species of organisms were isolated which may be considered as intestinal pathogens. The gram negative bacteria found which could not be differentiated by cultural characteristics were identified by biochemical determinations.

Gram positive cocci were frequently present but gram positive bacilli were rarely isolated. Differentation of the cocci and bacilli into genera and species was by cultural characteristics, staining characteristics, and biochemical tests. Lactobacilli were not studied.

The results of the study are tabled, illustrating the appearance of intestinal flora in new-born, twin premature infants, infants within a certain weight range, infants fasted for different periods of time, and infants on antibiotic therapy. Tables were also developed to show the succession of flora up to a month after birth.

Of the 110 cases studied, eleven had bacteria in the first specimens examined. In some instances, specimens contained aerobic organisms before anaerobic ones, while in others, the reverse was found.

Although the organisms isolated from the intestinal tracts of the twin infants were common to one another, the time of appearance varied.

There were distinct variations in the time of appearance of bacteria in the fecal flora of infants in various weight ranges, infants fasted for different periods of time, and infants fed different formulas. There were noted effects in the appearance and succession of bacteria in the fecal flora post-antibiotic therapy.

Eight genera were isolated in twenty-five per cent of the specimens examined. Yeasts appeared frequently, while proteus and pseudomonas, organisms supposedly rarely found in feces, were isolated frequently enough to be considered normal components of the fecal flora.

These established findings should be valuable in the development of intestinal bacteriology in newborn premature infants.

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