A Study of B. coli mutabile from an Outbreak of Diarrhea in the New-born*

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DURING the winter of 1933–1934 a succession of cases of a hitherto unrecognized diarrhea occurred among the new-born in the Memphis General Hospital. Forty-seven per cent of the infants affected died.

The disease made its appearance on the 5th to the 9th day of life, and there was no relation to sex, race, prenatal history, or state of nutrition. Since the outbreak occurred during the height of the economic depression, the lack of correlation with the nutritional state is worthy of notice.

The onset of illness was characterized by an elevation of temperature or marked loss of weight occurring within 24 hours. This was followed by a diarrhea which increased in intensity until the stools numbered 10 to 20 per day. The stools were watery, yellowish or yellowish green of color and, surprisingly, free from blood, pus, or mucus.

Vomiting usually followed the feedings, the vomitus consisting of curds of yellowish, partially digested food. Dehydration, septicemia, otitis media, and alkalosis were complicating factors. In cases ending fatally, the infants became cyanotic, respiration was rapid and shallow, and the eyes were sunken.

The physical findings were essentially negative. No medication seemed effective, and in 4 to 9 days the babies either died or gradually improved.

At necropsy the positive findings were dehydration, acute cloudy swelling of the visceral organs, otitis media, and, in several cases, bronchopneumonia. The intestinal tract was negative on gross and microscopic examination.

Extensive bacteriological studies were carried out in the hope of determining the causative agent. Cultures were made from feces, urine, blood; and nose, throat, and ear discharges of the sick babies. Five autopsies were performed and blood, exudates, and swabs from the visceral organs were inoculated to suitable media. The feedings were repeatedly cultured, as were swabs from the nipples of the bottles in order to detect food or hand contaminations. The throat and fecal flora of the mothers and attending nurses were studied. With the possible exception of the fecal cultures the findings were varied and inconsistent, and permitted no conclusions as to a specific causative agent.

Blood cultures from 10 of the babies yielded *B. coli, Staphylococcus albus,*

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and pneumococci, with *B. coli* as the organism of highest incidence. The possibilities of terminal invasion and skin contamination were recognized. *B. coli* and *Staphylococcus albus* were obtained from the urine cultures; *B. coli,* streptococci, staphylococci, pneumococci from nose and throat swabbings; and staphylococci, *B. coli* and pneumococci from cultures from the ear. *B. coli* was the most common organism found in middle ear infections. The most significant bacteriologic findings at autopsy were the frequent isolations of *B. coli,* and these results will be discussed later.

Repeated culturing of the yellowish watery stools consistently yielded slow lactose fermenting bacilli which proved to be *B. coli mutabile* in a high percentage of cases, and the study of these cultures instigated this report.

METHOD OF EXAMINATION OF FECES AND FINDINGS

The specimens of feces from all babies were obtained by introducing a sterile proctoscope and swabbing the rectum. The swabs were immediately washed off in tubes containing 2 c.c. of sterile physiological saline. Smears were stained by Gram's method. As a rule, the smears showed a mixture of organisms—*B. bifidus,* enterococci, Gram-negative bacilli, etc. The stools of normal breast fed babies contained *B. bifidus* as the predominating organism, over 90 per cent of the organisms were of this type, while the stools of healthy babies artificially fed showed a high proportion of Gram-negative bacilli. Regardless of the type of feeding, the stools of babies suffering from diarrhea gave a high percentage of Gram-negative bacilli, with Gram-positive organisms present in small numbers. The Gram-negative bacilli in the diarrheal stools were single or paired, with rounded ends.

Serial dilutions of the initial fecal suspensions were made so that the last tube was water clear. The three highest dilutions were spread on dry Endo's plates, by bent glass rods. Fecal suspensions from sick babies were also inoculated on blood, and plain agar plates, brain medium, and nutrient broth. All cultures were made in duplicate and grown under aerobic and anaerobic conditions.

The Endo's plates showed the most interesting and significant results. Three chief types of colonies were observed—typical *B. coli* colonies, flat and metallic; small pin-point colonies which proved to be enterococci; and colorless colonies characteristic of the typhoid-dysentery group. The colorless colonies, which when present outnumbered the other types on all Endo's plates, were opalescent, regular in outline, raised, typically smooth, and measured 1 to 2 mm. in diameter. When transplanted to Russell's double sugar a paratyphoid reaction was obtained with active production of acid and gas in the butt and no change in the slant. After 2–3 days at room temperature, secondary colonies appeared on the slant; these were raised, yellowish, circumscribed elevations in the midst of the mass growth. When dilute suspensions of 24 hour cultures on Russell's double sugar were spread on Endo's plates, only colorless non-lactose fermenting colonies were obtained. Within 48 to 72 hours a number of these originally colorless colonies developed red metallic papillæ. We then recognized the organism as *B. coli mutabile* and were able to demonstrate the cycle of colony form described by Neisser (1906) [1] and Massini (1907).[2]

Transplants were made to Russell's double sugar and plain agar, and stored while we made the examinations of feces of healthy and diseased adults. Specimens of stools were collected on sterile swabs, a saline suspension was

made, and spread on Endo's plates. These plates were observed for non-lactose fermenting colonies and subcultures were made.

INCIDENCE

The stools of 27 sick babies were cultured and in 18 (67 per cent) *B. coli mutabile* organisms were present in large numbers. As shown in Table I, we also cultured the stools of 41 normal babies and of 118 healthy and diseased adults to ascertain the frequency of this organism and other slow lactose fermenters in normal and pathological conditions of the gastro-intestinal tract. Of 21 normal babies, studied at the time of the epidemic, we isolated *B. coli mutabile* from the stools of 5 (24 per cent). Six months later, when the outbreak of diarrhea had entirely subsided, we examined the stools of 20 other normal babies and did not find this organism in any specimen. Five (10 per cent) of the stools from 47 normal individuals were positive for slow lactose fermenting Gram-negative bacilli, and 1 (2 per cent) proved to be *B. coli mutabile.* Examination of the stools of 28 adults suffering from diseases in which diarrhea may be an outstanding symptom (typhoid fever, dysentery, food poisoning) yielded 9 strains of slow lactose fermenting Gram-negative bacilli, 2 (7 per cent) of which fulfilled the cyclic criteria of this "mutabile" organism. Of 43 parturition patients 9 (21 per cent) showed slow lactose fermenting bacilli, 3 (5 per cent) of which were *B. coli mutabile.*

It is seen that slow lactose fermenting Gram-negative bacilli are increased in pathologic conditions in which diarrhea is a part of the picture, an observation frequently reported. While 32 per cent of sick adults showed such organisms in their stools, the incidence of slow lactose fermenting Gram-negative bacilli in the stools of babies suffering with diarrhea increased to more than twice this figure. All of these organisms proved to be *B. coli mutabile.* Kriebel [3] has reviewed the literature on this question and described such organisms which she isolated from food handlers and persons suffering from diarrhea and vomiting of unknown origin. We made no attempt to classify these miscellaneous slow lactose fermenting bacilli and only studied the organisms which were typical *B. coli mutabile.* The incidence of these unidentified slow lactose fermenting Gram-negative bacilli was increased in the parturition group.

Autopsy cultures yielded a variety of organisms. *B. coli mutabile* was found in 3 of the 5 cases; in 2 this organism was recovered from the heart blood, spleen, ileum, jejunum, colon, and middle ears. In each case in which a pure culture of *B. coli muta-*

TABLE I

SHOWING THE INCIDENCE OF B. COLI MUTABILE IN THE STOOLS OF 186 DISEASED AND NORMAL BABIES AND ADULTS

Group	*Number*	*Slow Lactose Fermenting Gram-negative Bacilli*	*B. coli mutabile Present*	*Negative for Both*
Sick babies	27	18 (67%)	18 (67%)	9 (33%)
Normal babies	41	5 (12%)	5 (12%)	36 (88%)
Normal adults	47	5 (10%)	1 (2%)	42 (90%)
Sick adults	28	9 (32%)	2 (7%)	19 (68%)
Parturition	43	9 (21%)	3 (7%)	34 (79%)

bile was obtained from one ear, cultures from the other ear showed a mixed flora.

While we are reluctant to assign a fixed etiological rôle to this organism, the high percentage of isolations from the stools of infants showing diarrhea, the low incidence in stools of normal babies and normal and sick adults is regarded as highly significant. Additional evidence is offered by the absence of gross and microscopic changes in the intestinal tract and the isolation of this organism from secondary foci of infection at autopsy. Finally, the cultural and serological relationship found to exist among these organisms affords the best bacteriologic evidence of their possible etiological rôle.

OBSERVATIONS ON B. COLI MUTABILE IN RELATION TO LACTOSE

Suspensions from the edge of the colorless mother colony when spread on Endo's plates gave rise to 100 per cent colorless colonies. Within 3 days, approximately one-fourth of these colonies had developed red metallic papillæ, and after 10 days all showed this phenomenon. In most cases, the earliest papillæ appeared near the center of the colony. Papillæ usually developed 1 to 2 days earlier at incubator than at room temperature, but the final number was not different.

When a saline suspension of these metallic papillæ was spread on Endo's agar, both red and white colonies developed. The red typical *B. coli* type predominated; as a rule, less than a third of the colonies were colorless. The white colony showed the S characteristics; the red colony was flat and granular and suggestive of the R type. The lactose fermenting colon-like colony bred true while the colorless non-lactose fermenting colony reproduced the mutabile cycle.

It was thus possible to obtain a red strain which was stable and a white strain which was unstable in that it continued to give rise to some red descendants. We have designated the lactose fermenting organisms as "red" and the non-lactose fermenting organisms as "white," unless otherwise indicated. All efforts to obtain a stable white strain have failed. We tried repeated platings of both young and old colonies—reasoning that very young colonies might be expected to yield only white descendants before red variants had been given off in response to the lactose stimulation, and that variation in old colonies would not occur after active cell division had ceased, but all white colonies, young or old, have continued to yield red papillæ on Endo's plates and apparently all non-lactose fermenting cells are able to give rise to lactose fermenting descendants under the stimulus provided by the lactose. We also tried plating after storage on plain agar, but so far have obtained no stable non-lactose fermenting cultures.

Transplanting of the white strain to Russell's double sugar medium at intervals of 1 or 2 days maintained the paratyphoid-like reaction. If transplantation were delayed for 10 to 14 days, until numerous secondary colonies had developed, a typical colon reaction was produced on the Russell's agar.

Serial passage of the white strain in lactose broth speeded up the rate of lactose utilization. Four to 7 days were initially required for production of acid and gas in 1 per cent lactose broth, but after 7 to 10 passages at 24 hour intervals, it occurred in 48 hours. This is in accordance with the findings of Jones,[4] Kennedy,[5] Kriebel,[3] and others on atypical colon bacilli.

The utilization of lactose was also influenced by the type of culture container. Kennedy[5] states that his slow lactose fermenting organisms broke down lactose more rapidly when grown

in toxin flasks, and we found this to be true. For example, 1 per cent lactose broth of pH 7.4 with brom-thymol-blue as an indicator was distributed in 40 c.c. amounts in the following types of containers: 1,000, 250, 125, and 50 c.c. flasks, and a large test tube 200 x 25 mm. A regulation test tube, 120 x 18 mm., containing 20 c.c. of broth served as a control.

All containers received the same inoculum of white organisms prepared by scraping off a single non-lactose fermenting colony from an Endo's plate inoculated 15 hours previously, and suspending the growth in sterile saline. Whereas the indicator showed an acid reaction greater than pH 6.1 in the 3 larger containers by the end of 48 hours, the 50 c.c. flask and the 2 tubes were still neutral. After 72 hours the organisms growing in the 50 c.c. flask had produced sufficient acid to change the color of the broth to yellow while the 2 tubes remained unchanged until 96 hours had elapsed.

When samples from the various sized containers were withdrawn at intervals and spread on Endo's plates the findings varied. The original inoculum and 24 hour samples from all containers yielded only non-lactose fermenting colonies and produced a typical paratyphoid reaction on Russell's double sugar. After 72 hours, samples from the tubes still gave only non-lactose fermenting colonies while Endo's plates inoculated from all 3 flasks showed both red and white colonies. A typical colon reaction was produced on Russell's double sugar. Thus the utilization of lactose was shown to be a result of dissociation; of the production of a sufficient number of red variants to break down lactose, rather than an acquired or latent use of this sugar by the white organisms. It is doubtful if the white forms can ever utilize lactose, but under the stimulus provided by this sugar lactose splitting descendants are produced with the resultant acid and gas.

Jones *et al.*,[4] offer data to show that apparently slow fermentation of lactose by certain strains of *B. coli* is in reality due to production of sufficient ammonia in the early hours of growth to neutralize the acid formed from the beginning. Nungester and Anderson[6] have described a series of variants derived from a colon-like bacillus. Some of these variants produced non-lactose fermenting colonies on Andrade's indicator agar but when grown in lactose broth reversion to lactose fermenting forms took place. Kriebel[3] also states that cultivation of Gram-negative non-lactose fermenting intestinal bacilli in 5 per cent lactose solution stimulated dissociation into lactose fermenting variants.

This dissociation of white to red was correlated with the utilization of lactose. Nutrient broth of pH 7.4 was prepared and 300 c.c. placed in each of 2 flasks of 500 c.c. capacity. Ten per cent lactose solution which has been separately sterilized was added to the broth to give a final concentration of 1 per cent. A single non-lactose fermenting colony from an Endo's plate which had been inoculated with the Garrett strain 15 hours previously was suspended in saline and 1 flask of broth inoculated. The other flask received a comparable inoculum of a lactose fermenting colony of the Garrett strain taken from an Endo plate inoculated with the red form at the same time. Samples were taken immediately after inoculation; part was diluted and used for streaking of dry Endo's plates, the remainder for the quantitative determination of lactose. This procedure was repeated after 8, 19½, 25, 31, 41, 48, and 96 hours' incubation. All plates from the flask containing the culture of white organisms up to the 31 hour period

yielded only white or non-lactose fermenting colonies. The plates inoculated with dilutions of the 31 hour sample were the first to show lactose fermenting organisms. Twelve per cent of these colonies were red metallic colon-like. The plates inoculated with dilutions of the sample taken after 41 hours' incubation gave approximately an equal number of red and white colonies while the plates from the 48 hour sample showed a 75 per cent red and 25 per cent white distribution. After 96 hours, 90 per cent of the colonies were red.

TABLE II

EFFECT OF LACTOSE ON DEVELOPMENT OF RED FORMS

Hours After Inoculation	*Percentage of Colonies on Endo's Plates*	
	White	*Red*
0	100	0
8	100	0
19½	100	0
25	100	0
31	88	12
41	50	50
48	25	75
96	10	90

The plates from the flasks inoculated with the red form consistently yielded only red lactose fermenting colonies. Details of the utilization of lactose are not included here, but while the red form actively broke down lactose from the beginning, the white form did not show definite evidence of using this sugar until 31 hours had elapsed. In other words, utilization of lactose is coincident with the appearance of red variants and lactose apparently has a stimulating effect on the production of such forms, a conclusion reached by early workers. Lewis [7] has recently disputed this conclusion and maintains that variation occurs under any condition suitable for growth. In support of this statement he offers data obtained from plating out cultures grown in various media. He was always able to demonstrate some variant cells, recognized on the basis of size, in colonies grown on any medium by means of densely seeded agar plates. According to him, sugars and alcohols serve as selective agents but do not specifically incite variation. We cannot agree with this since we have not been able to demonstrate the development of lactose fermenting organisms in plain broth or broth containing other sugars than lactose.

When flasks of the same size and containing equal amounts of 1 per cent lactose, 1 per cent dextrose, and plain broths received the same inoculum of white organisms and were grown under the same conditions, dissociation to the red form occurred only in the presence of lactose. Endo's plates streaked from samples of the dextrose and plain broth at intervals over a 5 day period initially gave rise to only white colonies. These in time showed lactose fermenting papillæ. With the exception of one culture in 1 per cent sucrose broth, Nungester [6] was not able to effect reversion of his non-lactose fermenting variant to the lactose fermenting form in any medium other than lactose broth.

Daily transfer for 10 days in 1 per cent dextrose, maltose, saccharose, mannite, broths did not alter the picture when these cultures were subcultured to Endo's plates.

It was also found that the time of coming into contact with the stimulating effect of lactose did not greatly influence the end results. Equal quantities of a suspension of Garrett white organisms were introduced into 2 flasks, one containing 100 c.c. of plain broth and the other 100 c.c. of 1 per cent lactose broth. Conditions of growth were the same. After 24 hours samples from both flasks gave rise only to non-lactose fermenting colonies. After 48 hours the plating from the

lactose broth culture showed that dissociation had taken place and approximately 20 per cent of the colonies were red. The sample from the plain broth yielded only non-lactose fermenting colonies. After the 48 hour sample had been withdrawn from the plain broth culture, sufficient 10 per cent lactose was added to give a final concentration of 1 per cent and the flasks returned to the incubator. After an additional incubation period of 48 hours Endo's plates were spread with samples from the two flasks. A comparable distribution of red and white forms appeared—approximately 90 per cent of the colonies on both plates were of the red lactose fermenting variety. We had thought that late contact with the lactose might not incite variation.

CULTURAL REACTION

Nungester [6] points out that any conclusions regarding fermentation of carbohydrates in liquid mediums by variants of a series must take into consideration the possibilities of variation occurring during the period of observation. He found that certain of his variants differed in their ability to use sugars (maltose and lactose) when grown in liquid and solid mediums containing the same concentration of these carbohydrates. Platings from the culture grown in lactose broth gave rise to a mixture of non-lactose and lactose fermenting colonies.

As previously stated, we repeatedly demonstrated that fermentation of lactose by the white forms of our *B. coli mutabile* strains was brought about by the lactose fermenting forms which developed in response to the stimulus afforded by the sugar.

We also found that apparently slow fermentations of salicin and raffinose were in reality dissociation phenomena with the production of organisms capable of utilizing these sugars in a highly specific fashion.

Rapid fermentation of sugars was never accompanied by any dissociative process. All strains of *B. coli mutabile*—both white and red—produced acid and gas within 18 hours when grown in broths containing the following sugars in 1 per cent concentration —dextrose, maltose, mannite, saccharose, xylose, arabinose, sorbite, rhamnose, levulose. Equally active fermentation was demonstrated on sodium-sulphite-fuchsin agar (3 per cent) containing the same concentration of these sugars—only red metallic colonies forming. Apparently all organisms readily attacked these sugars.

All strains of *B. coli mutabile*—both lactose red and white—failed to break down inosite, melizitose, cellobiose and adonite after 14 days incubation.

Dulcite, salicin, and raffinose were irregularly fermented. All strains except 1 (Shea white) fermented dulcite within 5 days; all strains—both red and white—fermented salicin within 5 days. All 18 red forms produced acid and gas in raffinose broth in 3 to 4 days, but 8 corresponding white forms (Shea, Whitney, Lee, Russell, Byrd, Phillips, Garrett, Rimmer) failed to produce any change in this medium even after 14 to 20 days incubation.

These inconsistent results led us to study the reactions of 2 of our strains, Rimmer and Garrett, toward these sugars under conditions of liquid and solid media. Nutrient broth and sodium-sulphite-fuchsin agar (3 per cent) containing 1 per cent amounts of dulcite, salicin, and raffinose were prepared. These media were inoculated at the same time with suspensions of Rimmer, red and white, and Garrett, red and white. The inoculums were prepared by scraping off isolated colonies from Endo's plates, inoculated the previous day, and suspending the

TABLE III

SHOWING THE REACTIONS OF GARRETT AND RIMMER (LACTOSE RED AND WHITE) IN BROTH AND ON SODIUM-SULPHITE-FUCHSIN AGAR CONTAINING THE SAME SUGARS

Strain	Dulcite		Salicin		Raffinose	
	Broth	Agar	Broth	Agar	Broth	Agar
Garrett White	A.G.-4 da.	Colorless colonies in all cases. No metallic papillæ. Sub-cultures from broth yielded only colorless colonies.	*A.G.-3 da.	Originally colorless colonies all of which gave rise to metallic papillæ in 4 days	Neg.-20 da.	Only colorless colonies; no metallic papillæ
Garrett Red	A.G.-3 da.		*A.G.-3 da.		*A.G.-4 da.	Colorless colonies with metallic papillæ 2 days
Rimmer White	A.G.-3 da.		*A.G.-3 da.		Neg.-20 da.	Only colorless colonies; no metallic papillæ
Rimmer Red	A.G.-3 da.		*A.G.-3 da.		*A.G.-5 da.	Colorless colonies with metallic papillæ 4 days

* Sub-cultures to agar produced both white and red colonies.

growth in a small amount of saline. A summary of these results is given in Table III.

Dulcite agar plates inoculated with these four organisms, Garrett, white and red, Rimmer, white and red, showed only colorless colonies. Even after 5 days there was no change in the general appearance nor had metallic papillæ developed. Dulcite broth, however, was fermented by all 4 organisms in 3 to 4 days but when these cultures were spread on dulcite agar plates only colorless non-dulcite fermenting colonies developed. Apparently, dulcite affords an example of true slow fermentation.

Salicin agar plates inoculated with the 4 organisms originally yielded only colorless colonies. After 4 days all colonies were studded with metallic papillæ. The salicin broth was broken down in 3 days and when these cultures were spread on salicin sodium-sulphite-fuchsin agar, both white and red colonies appeared. Here we have an example of true dissociation with salicin red forms developing in liquid and solid medium in response to the stimulus afforded by the sugar.

In the case of raffinose we demonstrated a third type of reaction. Raffinose broth was not fermented by either Garrett, white, or Rimmer, white, even after 20 days, but both Garrett, red, and Rimmer, red, formed acid and gas in 4 to 5 days. When these red cultures were spread on raffinose agar both white and red colonies were demonstrated. Inoculation of the raffinose agar plates from the original inoculum produced colorless colonies. After 2 days Garrett red colonies showed many metallic

papillæ and after 4 days the Rimmer red colonies had also developed metallic papillæ. In this case we have an example of specialized fermentation of raffinose by the lactose red organisms due to the production of raffinose red descendants.

We plan to extend these studies to other strains. It is possible that we may be able to demonstrate dissociation to dulcite, and since some of the white forms did ferment raffinose we would expect to obtain both white and red colonies on raffinose agar upon sub-culture.

In no case was the reaction of these organisms toward lactose altered. Salicin, red and white, and raffinose, red and white, organisms when subcultured to Endo's plates produced colonies characteristic of the original type. In other words, variation to lactose was not altered by contact with other sugars.

It seems possible that such a dissociation phenomenon may often occur and that slow fermentation of sugars should be investigated from this standpoint. It is not unlikely that certain difficulties in classification of some of these intestinal organisms could thus be eliminated.

An acidity developing in 2 to 4 days with the production of curds characterized the growth of all strains in litmus milk; no peptonization occurred.

Indol was formed in Dunham's peptone broth by the red and white forms of all strains.

Nitrates were reduced to nitrites and ammonia formed in nitrate broth. The methyl red reaction was positive, the Voges-Proskauer reaction negative.

Gelatin was not liquefied after 14 days.

AGGLUTINATION TESTS

Agglutination tests were carried out with the sera of 43 patients—5 sick babies, 13 healthy babies, 13 mothers, 10 sick adults, and 2 normal adults. For antigens we used heat killed saline suspensions of *B. coli mutabile,* Rimmer and Garrett, and *B. coli communior* from feces. We also immunized rabbits with the white and red forms of 9 strains of *B. coli mutabile* and tested their sera for agglutinins for homologous and related strains of *B. coli mutabile, B. coli communior, B. typhosus, B. paratyphosus* A and B. The results are summarized in Tables IV and V.

It is obvious that agglutinations on sera of babies do not offer proof of the etiological rôle of this organism. A higher percentage of the sera of sick babies than of well babies agglutinated this organism but our series is too small for conclusions. Fothergill [8] was only able to offer serological evidence of pathogenicity in 2 cases of his series of babies suffering from diarrhea the stools of whom yielded atypical paratyphoid-like organisms. In view of the age of the infants it must be remembered that any agglutinins may not have been formed in

TABLE IV

RESULTS OF AGGLUTINATION TESTS ON SERA OF 43 PATIENTS WITH B. COLI MUTABILE ANTIGEN (RIMMER)

		Highest Dilution of Serum Showing Agglutination					
Group	*Number*	*Neg.*	*1:20*	*1:40*	*1:80*	*1:160*	*1:320*
Sick babies	5	3	1	..	1	..	..
Normal babies	13	8	3	1	1	..	..
Mothers	13	1	..	1	5	6	..
Sick adults	10	2	..	2	3	3	1
Normal adults	2	..	..	1	1	..	..

response to an infection but may have been passively acquired from the mother.

Rabbits were immunized by introducing the antigen into the marginal ear veins. By giving the rabbits 2 series of 5 injections at 1 day intervals with an intervening rest period of 5 days we were able to produce high titers of agglutinins. We can offer no exact results—it may be stated that some of our strains were highly toxic to rabbits when injected in the fresh state and we used heated cultures for most inoculations.

While rabbits were immunized with 9 cultures and the sera tested with heat killed antigens prepared from all strains, no attempt is made to present the complete results because of the involved charts necessary.

A striking serological relationship was demonstrated among all strains of *B. coli mutabile* isolated from sick babies. The sera of rabbits immunized with any one strain agglutinated all antigens to approximately the same titer. No marked antigenic differences between the white and red organisms were apparent. In most cases the red organisms were agglutinated to higher titers than the white —even by white sera. This happened after every effort had been made to prepare red antigens in a manner that would eliminate any tendency toward spontaneous clumping because of the granular character of growth.

No close antigenic relationship existed between our *B. coli mutabile* strains and *B. coli communior* isolated from feces during routine examination. Likewise there was no marked cross-agglutination with typhoid or paratyphoid organisms.

Table V presents a summary of some of our serological tests.

SUMMARY

1. *B. coli mutabile* was isolated from the stools of 67 per cent of new-born babies suffering from an unrecognized type of diarrhea.

2. Stable lactose fermenting and unstable non-lactose fermenting (in that lactose fermenting descendants continued to be given off) could be derived from all cultures.

3. Dissociation to the lactose fermenting form occurred under stimulation of contact with lactose. Lactose exerted a specific effect since no other condition of growth produced the same result.

4. Utilization of salicin and raffinose was shown to be similar to that of lactose and depended upon the development of variants capable of fermenting these sugars.

5. Biologically the organisms are closely related to *B. coli communior*, but no close antigenic relationship was demonstrated.

6. All strains of *B. coli mutabile* isolated from sick babies were found to form a homologous group when tested with sera of rabbits immunized with red and white forms.

7. Substantial proof that *B. coli mutabile*

TABLE V

SHOWING RESULTS OF AGGLUTINATION TESTS WITH SERA OF RABBITS IMMUNIZED WITH B. COLI MUTABILE AND OTHER INTESTINAL ORGANISMS

Sera	Titer to Which the Following Antigens Were Agglutinated											
	Russell White	*Russell Red*	*Terrell White*	*Terrell Red*	*Garrett White*	*Garrett Red*	*Byrd White*	*Byrd Red*	*Para A*	*Para B*	*Typhoid*	*Coli Communior*
Russell White	10240	10240	5120	5120	1280	10240	10240	10240*	40	40	40	80
Russell Red	10240	10240	10240	10240	1280	5120	5120	10240*	40	40	40	80
Terrell White	1280	5120	2560	5120	640	5120	2560	5120	40	40	80	160
Terrell Red	2560	10240	2560	10240	1280	10240	5120	10240	40	40	40	40
Paratyphoid A	40	40	40	40	40	40	40	40	5120	320	640	40
Paratyphoid B	40	80	40	40	40	80	40	160	320	10240*	1280	160
Typhoid	80	80	40	80	40	40	40	40	320	320	10240	80
Coli communior	80	160	160	80	80	80	160	160	40	40	40	320

* Titer not reached

was the cause of the outbreak of infant diarrhea is presented, but it is admitted that the predominance of these organisms may have been secondary and an unrecognized organism the primary cause.

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Oh Enema!

Un petit clystere insinuatif, préparatif et rémollient pour amollir, humecter, et refraîcher les entrailles de Monsieur.
—Molière

IT is said that the enema habit was introduced into England from France early in the nineteenth century. I have searched my library of English health books which goes back to 1589 and find the first references, with the exception of one author, in 1807 and in 1828. The exception is Francis Bacon, Lord Verulam (1550–1626), in whose writings I have found three references to the " clyster " which was the English word for enema in his time and is, you will note, practically the same word as used by Molière. But Lord Bacon was unusually erudite and there is no doubt that the enema was more popular at that time in France than in England. Sir John Sinclair (1807) notes that, on page 284 of the Manual of Health, " there is an amusing story connected with the subject of lavements, regarding the lively and amiable Duchess of Burgundy who was the Rosalind of the court of Louis XIV and who was accustomed to take them previous to her going to the theater."

Unfortunately I have never been able to find that Manual of Health. But the word lavement which is the modern French for enema has had an unfortunate influence on our practice. In the Anglo Saxon tradition a washing demands soap and so to the ancient clyster or glyster soap was added, as it never had been before, and colons suffered in consequence. Doctor Hurst believes this evil practice still to be limited to the British Isles. Alas we know better.—J. Rosslyn Earp, Dr.P.H., *New Mexico Health Officer,* Aug., 1935.