A STUDY OF MUTABILE STRAINS OF ESCHERICHIA COLI

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A STUDY OF MUTABILE STRAINS OF ESCHERICHIA COLI

Introduction

The term "mutabile" was first applied to some strains of Escherichia coli that produced a certain metabolic variation towards lactose. In lactose broth containing an indicator, E. coli mutabile shows evidence of acid formation only after about three days of incubation at 37 degrees Centigrade. On lactose containing agar plates, the colonies develop papillae or secondary colonies after about three days incubation, which when picked off into lactose broth give acid and gas in 24 hours. When plated out on lactose containing medium again, these papillae do not themselves produce secondary colonies. They consist of organisms that are culturally identical with the typical E. coli colonies on the various differential plating mediums used for this group. Rapid lactose fermentation is a stable characteristic of the variant.

Other genera besides Escherichia have been found to contain members that show a similar "mutabile" phenomenon of variation. A similar variation was found to take place in relation to other carbohydrates. The mechanism of this metabolic variability has been extensively studied, but as yet, is little understood. Because of the peculiar position of Escherichia coli mutabile in relation to lactose fermentation, this organism has offered some difficulties in classification. Some have

classified it with the paratyphoid group, others with the colon group, and still others with a heterogeneous paracolon group of bacilli.

This organism with its "mutabile" phenomenon of variation gives rise to problems of more than theoretical interest. It presents many practical problems of interest to the bacteriologist dealing with intestinal bacilli, particularly in connection with the pathogenic gram negative bacilli in faecal specimens. It is the special purpose of this paper to throw light on these practical problems.

We have been impressed with the frequency of mutabile strains of E. coli in faecal specimens from normal individuals as well as from those suffering from intestinal disturbances. They appear to constitute a large proportion of those organisms frequently found in stool specimens that must be differentiated from the well defined pathogens. During the course of a routine stool examination for intestinal pathogens, E. coli mutabile at first appears to be one of the Salmonella group of bacteria. Dextrose is fermented with the formation of acid and gas, but there is no evidence of lactose fermentation in 24 hours. On further investigation, however, there will appear certain differences from the Salmonella, and lactose eventually will be attacked. Unless this organism is kept in mind, and the possibility of delayed fermentation of lactose is recognized and understood, its identification may be unnecessarily delayed.

In this study a large number of stool specimens have been examined to determine the frequency with which E. coli mutabile

is actually present in faecal specimens. The strains isolated have been studied thoroughly and quite fully described. Finally, a routine procedure has been devised for their rapid and certain identification.

HISTORICAL BACKGROUND

The history of this interesting organism is somewhat difficult to trace, because its identity is frequently masked under different names. It has been variously called "B. coli mutabile", "paracolon" bacillus, "metacolon" bacillus, "coliform" bacillus, "slow lactose fermenter", "pseudo-paratyphoid", "paratyphoid-like", "B. neopolitanus Emmerich", "Bact. imperfectum", "B. columbense", "B. khartoumense", "B. wesenbergi", "B. guimai", and "B. asiaticum".

Organisms that appeared to be similar to the typical B. coli in most respects, but differed in one way or another; and yet could not be classified with the paratyphoid group were described very early in the literature concerning the development of the intestinal group of bacteria. In 1893, Gilbert and Lion described bacilli that simulated B. coli, except that they did not ferment lactose. They were indol positive or negative, and either motile or non-motile. Gilbert in 1895 introduced the term "paracolon" for this group. This term has been introduced into bacteriological terminology, but unfortunately has been used in different senses by different workers. This name was applied to the very questionable organisms that are not true paratyphoids, but more nearly simulate E. coli. The E. coli mutabile has frequently been included in this group. As recently as 1935, Sandiford prefers the term "Paracolon" Bacillus for the mutabile forms of E. coli. Since the identity of E. coli mutabile has so fre-

quently been overlooked and unrecognized, and the organism itself so often inadequately described, some of its history may well be buried in the literature where it is difficult to find, or so confusing that its significance is difficult to evaluate. The liberty has been taken in order to make this summary as complete as possible, to include some references which appear to be concerned with E. coli mutabile, altho not considered as such by the authors.

The first description of E. coli mutabile was recorded in 1906 by M. Neisser, who gave it the name "mutabile". He described the organism as producing colorless colonies on Endo agar. On the third or fourth day small papillae developed on the top of the colonies. On picking off such an elevation and transplanting to Endo agar, both red "fuchsinglanz" and colorless colonies resulted. The red colonies bred true, that is, they reproduced only red colonies on subsequent transfers to Endo agar. The colorless colonies, however, continued to throw off red forms. Neisser stated that there were no intermediate stages. The mother and variant were pathogenically, culturally, and agglutinatively alike.

Massini, the following year, 1907, described an apparently similar organism which he obtained from the stool of a mild case of gastro-enteritis. It grew on Endo agar like the typhoid bacillus except that the colony was less transparent. It became redder in a few days but never metallic like typical

E. coli. The whole plate took on the rose pink shade of the colonies, and the latter retained it until they died. There was noted around some older large colonies, a light area about one centimeter in diameter. The papillae, which he terms "knotchen" came on the second or third day; most commonly as tiny colorless knobs gradually turning red. They were most numerous in the center of the colony. Sometimes as many as 200 papillae occurred in the large colonies, while in the smaller, closely lying colonies, none were seen. He never observed papillae on top of the "knotchen". Growth in broth was uniformly turbid with some deposit no pellicle formation. Litmus milk turned faintly red on the second day, redder on the third, and was coagulated within 8 to 10 days. Indol was not produced. Dextrose, maltose, galactose, levulose, and mannitol were fermented readily with the formation of both acid and gas. Sacharose was not fermented. Inoculation from a colony on Endo agar within 24 hours gave only white colonies, but inoculation from a 48 hour colony gave both white and red colonies. Inoculation from a red colony gave only red colonies. Papillae appeared only on medium containing lactose. The lactose fermenting variant retained the character of rapid fermentation of lactose even when cultured for long periods on non-lactose containing medium. Agglutinative characters, and pathogenicity of the two varieties proved to be alike. This very first and quite complete description of the B. coli mutabile by Massini has been repeatedly verified

and still stands as one of the best descriptions of this organism found in the literature to the present day.

Twort in 1907 recorded variants in fermentation of other organisms of the typhi-coli group. E. typhi produced secondary colonies on dulcite containing medium which when subcultured gave variants fermenting dulcite rapidly. Twort, by growing certain organisms on various sugars not already fermented by them, and subculturing them every 14 days over a long period of time, was able to induce fermentation of the sugar on which the organism was "trained". In this manner he reported to have "trained" several organisms to ferment a sugar which had previously been unattacked. The paratyphoid bacilli could be made to ferment saccharose. E. typhi could be made to ferment dulcitol and lactose. Dysentery Flexner-Kruse could be made to ferment saccharose; and Dys. Kruse, lactose as well as saccharose. This work of "training" bacteria to ferment carbohydrates has never been completely confirmed.

Arnold Burk, 1908, described an organism isolated from the stool of a case of food poisoning which resembled the B. coli mutabile of Neisser and Massini. The colonies on Endo agar were colorless but formed characteristic knob like elevations after 3 to 10 days incubation at room temperature. The knobs when subcultured to Endo agar produced red and white colonies. The red colonies were rapid lactose fermenters forming acid and gas, and retained the character over a period

of 5 months observation. The red colonies did not produce secondary papillae nor did they produce a variant of any sort.

Reiner Muller, 1908 - 1909, in line with former reports of the behavior of this organism, studies its reaction on 18 different carbohydrates; also studying other different types of organisms in these same sugars. He found that B. typhi formed papillae which he called "knöpfe", literally translated, buttons, on isodulcite. Some of these papillae were red on isodulcaite agar and on subculture gave both red and white colonies; the red ones being able to ferment isodulcite, not producing secondary colonies and breeding constantly red. These papillae appeared at the earliest in 48 hours, generally 3 to 6 days. He investigated 50 strains of typhoid bacilli and from all of them he obtained the isodulcite papillae and the rapidly fermenting variant, corresponding to that obtained from B. coli mutabile on lactose. Of 300 strains of other bacilli in the typhi-coli group he found only one other that behaved in a similar manner on isodulcite, viz., a single strain of Flexner dysentery bacillus. Paratyphoid B. gave papillae on raffinose agar, but the papillae could not be shown to ferment the raffinose. Müller suggested that this phenomenon of secondary colony formation and variation in fermentation of the particular sugar might be regarded as definitely specific for the variety of micro-organisms exhibiting the character. All of the typhoid bacilli uniformly exhibited this character on isodulcite, and thus it may be regarded as a very specific test

in the identification of the organism.

Burri and Duggeli, 1909, described several organisms identified as B. coli that acquired the power to ferment saccharose in a similar manner as B. coli mutabile ferments lactose.

Hubener, 1909, examined 400 stool specimens from normal individuals. Among the organisms similar to but not identical with the B. coli, he describes three organisms which were similar to Neisser's B. coli mutabile, but apparently differed in some of the biochemical reactions. On Endo agar always red and white colonies appeared. From the red colonies typical B. coli were consistently subcultured, fermenting lactose readily in 24 hours. The pale colonies, however, fermented lactose in 2 to 6 days and produced secondary colonies on lactose agar within 14 days. The description is beyond doubt that of B. coli mutabile, but it was not recognized by the author as such.

Seiffert, 1909, in a rather extensive study of the paratyphoid bacilli from stools of normal human beings and normal swine, reports 3 paracolon strains isolated from typhoid fever patients. These organisms may very well have been B. coli mutabile from their description, but were not identified as such by the investigator.

Sauerbeck, 1909, confirmed Massini's description of B. coli mutabile. He considered that there was some difference between the papillae formed, some being white and others red on Endo agar. All of the secondary colonies did not give purely red colonies when transplanted to Endo agar. The per-

son's serum agglutinated its own strain of B. coli mutabile and several other strains in a dilution of 1-25 to 1-100.

Morgan and Ledingham, 1909, encountered the organisms in human feces and divided them into three groups on the basis of cultural reactions. They reported mutabile strains of B. coli as numerous in cases of diarrhoea, but regarded them as non-pathogenic or of little pathogenic significance.

Burri, 1910, gave the name "Bacterium imperfectum" to an organism showing the lactose variation phenomenon. He considered it to be an uncommon occurrence. The papillae were again described as "knöpfe" and gave the usual lactose variation. He found that transplantation from a 4 to 5 day lactose broth culture into a fresh lactose broth medium would give fermentation with formation of acid and gas in 24 hours. He pointed out that there was a wide variation in the amount of gas produced by identical cultures of the same strain. The variant, when grown in pure culture, produced greatest amount of gas. Burri considered the development of fermentation ability not a sudden but a gradual change, and that it was due not to the appearance of an entirely new function but to the activation by the lactose of a latent ability. This problem has as yet not been solved, and the issue was reopened recently by I. M. Lewis. (1933).

Kowalenko, 1910, made a significant contribution in that he used single celled cultures and was able to confirm Massini's work. Thereby he was able to eliminate the possibility of con-

tamination as playing any part in the behavior of the cultures. He observed cultures at ice box temperature, room temperature, and incubator temperature, for a period of two and a half months. The lactose fermenting variant died off during this time in the cultures maintained at room temperature and incubator temperature, but remained alive at ice box temperature. The mother organism retained the same cultural characteristics as before and was again able to throw off fermenting variants on further subculture on lactose. The mother organism and the variant were found to be agglutinatevly homologous.

Wilson, 1910, isolated an organism from a typhoid carrier that fermented lactose in 7 to 24 days. It was a member of the colonaerogenes group. Wilson believed that this organism formed a connecting link between E. typhi and E. coli. The lactose splitting enzyme of the strain studied by Wilson did not develop at 37° C., but apparently developed slowly at 22° C. There was complete disappearance of lactose at 22° C. and little if any at 37° C. On Conradi Drigalski plates, the colonies remained blue at 37° and slowly turned red at 22°. This organism behaved somewhat differently on lactose from the typical E. coli mutabile, but the late lactose fermentation warrants its mention in this historical summary. Unfortunately, it was not described more completely to allow further discussion.

Penfold, 1910 - 1911, made some rather extensive studies on fermentation variations of the intestinal group of bacilli.

In 1910 he tested Twort's strain of B. typhi which fermented lactose. He confirmed Twort's observations and found the organism to be a true typhoid bacillus. On lactose agar plates it gave both fermenting and non-fermenting colonies. Penfold attempted to "train" other strains of typhi to ferment lactose but was totally unsuccessful. He showed that B. typhi fermented dulcitol in about 10 days; the colonies on dulcitol agar producing secondary papillae after about 5 days which were both red and white and some of which were capable, and others not, of fermenting the sugar rapidly. He confirmed R. Müller's observation of secondary colony formation of B. typhi on isodulcite, but did not find that they fermented the isodulcite readily as was Müller's experience. After considerable "training", however, they could be made to ferment the sugar. He concluded that papillary formation arising in carbohydrate medium on colonies of members of the typhi-coli group may indicate either variation by slow degrees as proported by Burri--or by mutation as proported originally by Massini. The two types of variation were exemplified in Twort's strain of typhoid bacillus showing variation toward dulcite, isodulcite and lactose and also sorbitol. Formation of papillae simply indicated that the variation affects only a very few individuals of a colony to a great extent. It does not guarantee permanency of the character. Penfold believed that if the papillae arise early and repeated subcultures on the carbohydrate are not necessary to produce them, the evi-

dence suggests that the character developed more suddenly and will have considerable permanency; the opposite of this being also true, that the character is less permanent and develops more gradually in those particular in those particular instances where secondary colonies arise late and are difficult to produce. Penfold did not accept R. Müller's view that the formation of papillae on certain carbohydrates could be regarded as a definetly specific character and that the ability of an organism to vary in any particular direction may be of considerable value in the differential diagnosis of the organism. Penfold continued his work in 1912 and confirmed the single cell technic of gaining pure cultures of B. coli mutabile that continue to throw off variants in exactly the same way as strains which had been obtained by the ordinary plating methods. Further experiments on "training" various other members of the coli-typhoid group to ferment certain carbohydrates were unsuccessful. This substantiated the view that such powers to vary were innate characteristics of certain species and could not be trained into the species by environmental changes. In the 1912 report Penfold recorded a complete biochemical study of 24 strains of B. coli mutabile.

Theysen, 1911, in a preliminary report entitled, "Studien über functionelle Anpassung bei Bakterien" described 8 strains of intestinal bacilli showing variations in fermentation. Four strains were typical B. coli that gave sucrose fermenting variants in a similar way as B. coli mutabile pro-

duced lactose variants. Two strains that gave acid and gas on maltose and glucose gave both lactose and sucrose fermenting variants. One strain was identical with Burri's B. imperfectum--and one strain was apparently identical with Massini's B. coli mutabile. Thaysen, however, placed all of these organisms in the paratyphoid group.

Sobernheim and Seligman, 1911, described several strains of coliform bacilli producing secondary colonies on lactose agar which when subcultured produced lactose fermenting colonies. These organisms were undoubtedly B. coli mutabile strains, not recognized as such in the report.

Baerthlein, 1912, continued the active investigation into the mechanism of variation. In a rather extensive study he observed two distinct types of colonies formed by the organism. One was an opaque colony composed of rather plump bacilli. The other was a very transparent colony composed of slender bacilli. Each of the colony forms produced papillae and lactose fermenting variants, but the variants were in each case of the same colony type as the mother organism. Until this time all investigators had agreed that the variant was stable and did not revert to the mother form. Baerthlein, however, set out to prove that atavistic reversion occurred. After 6 days of daily transplants on plain agar tubes the transparent red variant when plated out began to produce the white mother type of colony. This atavistic return to the mother colony occurred in 7 days with the opaque strain. This

observation has not been confirmed by other investigators. Baerthlein also found that after several weeks growth of the transparent colonies on plain agar and then transplanting to a fresh agar tube, both transparent colonies and opaque colonies were obtained. Under the same conditions opaque colonies reverted to transparent colonies. The variants, both opaque and transparent lactose fermenters, were inoculated into the blood stream of white mice. On death of the mouse only the same type of organism that was injected was recovered from the heart's blood.

Cecil Reeves, 1911, in a series of experiments to attempt to cause reversion of the fermenting variant, failed to show that the various biochemical properties of the coli strains were not stable.

Several reports on investigations into the stability of the variation were published at about this when Lentz, 1912, and Bernhardt, 1912, report reversion of the variants to the mother organism by animal inoculation.

Bernhardt and Markoff, 1912, worked with a variation of a dysentery bacillus toward maltose, in every way analogous to the lactose variation of E. coli mutabile. The variant was fed to healthy Rhesus monkeys. Subsequently the mother organism as well as the variant was isolated from the stool. An attempt to produce a similar type of reversion with E. coli mutabile proved entirely unsuccessful.

J. Henderson Smith, 1918, in a discussion of the differentiation of organisms of the coli-typhoid group, emphasizes the importance of recognizing the frequent variations toward carbohydrates that occur in this group. This frequent variability does not, however, invalidate the use of carbohydrate tests in their differentiation, for the variations do not occur in a haphazard fashion, but along certain directions which are definite for each organism. Carbohydrate variations among other members of the coli-typhi group as well as of B. coli mutabile were discussed and Smith agreed with R. Müller that an organism produces with greater readiness variants towards some carbohydrates than toward others, and the more disposed an organism is to produce a particular variant, the more readily does it take the new character permanently.

Many references in the literature on paratyphoid-like bacilli or psuedo-paratyphoid like or paracolon bacilli record organisms which might very possibly have been B. coli mutabile as far as the descriptions go, assuming that either the worker overlooked the possibility, or failed to give a complete description.

Gildemeister and Baerthlein, 1915, however, were very familiar with the mutabile variation. In a short report describing a group of "paratyphoids" which were indol positive, but were antigenically heterogeneous, they classify this interesting group of organisms as intermediate between the hog

cholera bacillus and B. coli mutabile. The basis for this position is not convincing and the descriptions of the organisms is not adequately complete to permit criticism.

Investigations were unimportant from this time, 1915, until about 1920. Study of the mutabile strains of the colon bacilli was then again resumed. These later works are numerous and will be discussed under the various headings, according to their content.

DISTRIBUTION OF B. COLI MUTABILE

Incidence of B. coli mutabile in stools. Experimental work.
A series of 454 stool specimens were examined for slow lactose and non-lactose fermenting gram negative bacilli, with particular emphasis on the search for mutabile forms of the coliform bacilli.

SPECIMENS EXAMINED The series of 454 stools are divided into four groups:

Group 1. 120 specimens Healthy food handlers.
Group 2. 140 specimens Relatively healthy adults.
Group 3. 105 specimens Adult patients of the Multnomah Hosp.
Group 4. 89 specimens Children from the Doernbecher Hospital.

Group 1 includes healthy persons, chiefly young adults, receiving the routine examination of all food handlers in the city of Portland. This examination includes periodic stool examinations for any possible carrier condition. These specimens were made available thru the courtesy of the City Bureau of Health.

Group 2. The faecal specimens of this group came from ambulatory individuals, relatively healthy, but seeking treatment for hay fever, asthma, or other allergic conditions.

Group 3. Adult patients of the Multnomah County Hospital comprised this group. Males and females of all ages above 14, suffering from a wide range of both chronic and acute diseases were included.

Group 4. This series of specimens was from the Doern-

becher hospital for Children, admitting children below the age of 14. All types of cases were included, both surgical and medical, chronic and acute.

No specimens were grossly dysenteric. None of the patients were known to have a primary intestinal infection. The complete medical record of each patient is available, but a tabulation of the clinical findings in correlation with the bacteriologic findings was not deemed of particular value in this investigation.

TECHNIC OF THE STOOL EXAMINATIONS

The fecal specimens of group 1 including the food handler's series, were plated on Endo agar by the City Bacteriologist as soon as the specimens were received. They were then stored in the refrigerator over night and collected early the following morning and brought to the laboratory where they were again plated out on Endo agar. At no time were non-lactose fermenters found by the City Bacteriologist when they were not also discovered on the cultures made the following day. None of the specimens proved to harbor pathogenic organisms of any public health significance. The Endo agar used was prepared from the dessicated Difco product.

The stool specimens of groups 2, 3, and 4 were collected in selective or restraining mediums. Into two wide mouth bottles containing respectively, 15 cc. of Stress' 30% glycerine in 0.85% NaCl and the Haven's tetrated brilliant green bile mixture were placed well selected portions of stool about the size

of a bean. These were kept at room temperature. Platings onto Endo agar were made in 24, 48 and 72 hours.

After 24 hours incubation at 37° on Endo agar, pale or suspicious metallic colonies were picked and inoculated into Russell's double sugar agar slants. The medium contained 0.1% dextrose and 1.0% lactose in 1.5% extract agar containing 1% Andrades indicator. It was sterilized in the autoclave at 10 pounds pressure for 30 minutes. The number of colonies picked varied with the number and type present. All of the suspicious colonies were picked, or when they were very numerous, at least 12. The Russell's cultures not giving B. coli reactions were again inoculated onto Endo agar plates in order to study the colonies more thoroughly and to insure purification of the cultures.

Motility tests were then made and inoculations to various carbohydrates and differential mediums depending upon the results of the Russell's agar and original Endo plates. The original Endo plate culture and all subsequent plates, as well as the Russell's slant cultures were kept in the 37° incubator for from one to three weeks, occasionally longer depending upon the completion of the identification and cultural reactions. The incubator was saturated with moisture to prevent dessication of the cultures.

TABLE I

SUMMARY OF STOOL EXAMINATIONS

GROUP NO.	1		2		3		4	
	Normal food handlers		Ambulatory patients with Allergic symptoms		Adult hospital patients		Children hospital patients	
No. of cases	120		140		105		89	
No. on which suspicious colonies were present	32	26.7%	49	35%	32	30.5%	40	45%
Suspicious colonies yielding significant organisms	18	15%	22	15%	22	21%	30	33%
Mutabile forms of Escherichia	11	9.2	10	7.1	11	10.5	14	15.6
Mutabile forms of Aerogenes					2		1	
Shig. paradys. Dispar	2		2		3		7	
Salmonella like			2		1			
Salmonella Morgani							2	
Alkaligenes fecalis					2		2	
Pseudomonas aeruginosa			1		1		2	
Doubtful	5		7		2		2	

RESULTS:

The number of pale colonies which appeared suspicious enough to be picked off was included in the table to show how frequently such colonies appear on routine examinations using Endo agar. From group one to four respectively the percentage of specimens giving pale colonies was 26. 7%, 35.0%, 30.5%, and 45.0%; an average of 34.3% of the total number of specimens examined.

The number of specimens yielding either non lactose or slow lactose fermenting bacilli as expressed in percent were respectively from group one to four; 15%, 15%, 21%, 35.6%; or an average of 21.2%. The difference between 34.3% and 21.2% or 13.1% is made up of suspicious colonies which proved on further investigation to be some organism of no significance. These usually proved to be Escherichia, Aerobacter, or Staphylococci.

By far the most frequently encountered organism of this group of non-lactose or late lactose fermenting coliform bacilli was the mutabile form of E. coli. 9.2% of group 1 and 7.1% of group 2 yielded mutabile forms. Since both these groups may be considered as coming from healthy adult persons, the average of 8.2% of 260 faecal specimens gives the incidence of E. coli mutabile in healthy adults in this limited series.

The third group, County Hospital patients, shows a slightly higher incidence of 10.5% of 105 specimens; the fourth group,

of ill children shows a still higher incidence of 15.6% of 89 specimens yielding mutabile strains. This high incidence alone makes this organism an important bacillus with which every bacteriology technologist must be thoroughly familiar to be able to differentiate it speedily and conclusively from the members of the Salmonella group which it at first so closely simulates.

The full description of the mutabile forms of E. coli is given in the section discussing its metabolic properties.

SUMMARY OF LITERATURE ON INCIDENCE OF E. COLI MUTABILE

Mutabile forms of E. coli in stools have been frequently reported in the literature. As has been pointed out before, these organisms have been given various names and have been included sometimes in the colon-paracolon group and sometimes in the paratyphoid group. Because of this confusion in classification and nomenclature, the actual incidence of E. coli mutabile is difficult to ascertain from the early literature. There are many inadequate descriptions of the organism, still further, adding to the confusion.

There are frequently early reports of paracolon bacilli in the stools of both normal individuals and those suffering with diarrhoea. Some of the organisms included in this paracolon group almost surely are mutabile forms of E. coli. Their pathogenic significance has been variously interpreted.

Hubener, 1909, and Morgan and Ledingham, 1909, found

mutabile strains in stools of healthy individuals. Penfold, 1912, cultured 50 stools of healthy individuals suspected of being typhoid carriers, and found that 34 of the 50 plates showed non-lactose fermenting colonies, in 24 hours. But on further incubation for 7 days, 21 of these plates showed definite papillae typical of the mutabile strains, which on subculture produced typical rapid lactose fermenting variants. Penfold concluded that "lactose mutating" organisms were comparatively common in the gut. These 21 organisms were carefully described and shown definitely to be Massini's B. coli mutabile. This organism masquerading under other names was probably frequently found in normal individuals by other early workers.

Many investigations in more recent years have confirmed the frequency of mutabile strains in faecal specimens of both normal individuals and those suffering from various types of disorders wholly unrelated to any pathogenic effect that the organism might assume.

In 1924 Trawinski reports 18 paratyphoid like organisms isolated from healthy persons. They were not identified as mutabile strains, but some, if not all, were most surely mutabile strains. Their descriptions, however, seem to us to probably include some mutabile forms.

Dudgeon, 1926, examined faecal specimens from 200 cases and recovered slow lactose fermenting B. coli from 2% of the specimens. He believed that they came from the caecum more frequently than from the lower bowel.

Stewart, 1926, examined stools from normal individuals and from a group of 253 patients having a mental illness and various intestinal disorders and found 4% of the normals and 28% of the mental cases to contain "paracolon mutabile" bacilli.

Cruickshank, 1928, states that slow lactose fermenting B. coli and other coliform bacilli are "frequently" found in the intestines of normal persons.

Fothergill, 1929, investigated 104 cases of infant summer diarrhoea. He divided the cases into two series. In the first of 49 cases he found: Atypical paratyphoids, 22.4%; Saccharose fermenters 12.2%; Morgan's bacilli, 24.5%. In the second series of 55 cases he found: Atypical paratyphoids 65.4%; Sucrose fermenters 38.1%; Morgan's bacilli 25.5%. He did not identify the atypical paratyphoids or saccharose fermenters as mutabile types. From the description of them, however, many of them, if not all of them appear to be mutabile forms.

A more extensive investigation by McBroom, 1930, of a group of faecal specimens from 126 persons--29 healthy, and 97 suffering from varied disorders--revealed 33% of the entire group contained "intermediate organisms". Of this group there were 37 paracolons, 11 "para-proteus", and 5 "para-typhoid-like". The paracolons are atypical lactose fermenters and appear to be mutabile strains, although the investigator did not recognize them as such.

Kriebel, 1934, studied stool specimens from 127 food handlers. 25 or 20% produced non-lactose fermenting colonies resembling pathogenic bacteria. Of the 25, 23 were late lactose fermenters. She did not call them mutabile forms and recorded them as non-lactose fermenters in the chart listing the fermentation reactions.

Dulaney and Mitchelson, 1935, examined stools of 20 normal babies, but found no B. coli mutabile forms. In 18 out of 27 cases of severe diarrhoea in infants 5 to 9 days old, E. coli mutabile were found in large numbers as the only possible pathogen. They also found slow lactose fermenting forms in 5 or 10% of stools from 47 normal adults. One or 2% of these was definitely identified as E. coli mutabile. Stools from 28 sick adults were examined, all suffering from diseases in which diarrhoea may be considered an outstanding symptom (e.g. typhoid fever, dysentery, food poisoning). Nine strains of slow lactose fermenting gram negative bacilli were recovered of which 2 fulfilled the cyclic criteria of the mutabile form of E. coli. Of 43 parturient patients, 9 showed slow lactose fermenting bacilli in the faeces, of which 5 were shown to be E. coli mutabile. The investigators conclude that slow lactose fermenting gram negative bacilli are increased in pathological conditions in which diarrhoea is a part of the picture. This observation has been frequently reported. While 36% of sick adults showed slow lactose fermenters in the stools, the incidence of the late lactose fermen-

ters in the stools of babies suffering from diarrhoea increased to more than twice this figure. It should be pointed out that comparatively few of the late lactose fermenters in this series were identified as mutabile forms.

In the series reported, all of the organisms that fermented lactose slowly with the formation of both acid and gas could be identified as mutabile forms of Escherichia or Aerobacter. No doubt can remain as to their identity. The criteria of identification used was the production of secondary colonies on lactose agar and the ability to produce a variant that ferments lactose rapidly with the production of both acid and gas.

Several conclusions arise from a survey of the literature on the incidence of E. coli mutabile and from a survey of the investigation of 454 stools for E. coli mutabile and other bacilli not giving rapid lactose fermentation. First, it is obvious that E. coli mutabile are relatively common in the stools of normal persons--roughly in about one-tenth of all stool specimens. This organism ranks first in the frequency of producing suspicious colonies on lactose differential medium. This fact, namely, that the pale colonies are more likely to be E. coli mutabile than any other organism makes it very important that they be correctly identified and surely differentiated from any pathogenic organism which may be well recognized of etiologial significance and that the criteria of identification be thoroughly understood. The

cyclic penomenon of the production of lactose fermenting variants by this organism makes the identification relatively simple, as we shall attempt to point out in the next section. But this criteria of identification must be well understood. The great frequency with which mutabile strains of E. coli must be recognized in the routine examination of stools would warrant a greater emphasis upon this organism in the bacteriology text books and other references to which a technician would ordinarily refer.

DISTRIBUTION OF E. COLI MUTABILE IN NATURE

E. coli mutabile has been found in faeces from many animals as well as from human beings. It has also been isolated from water, soil, sewage, milk, cheese, and other foods.

E. coli mutabile was isolated in this laboratory by Sears from well water.

In 1914 Kligler found 4 "Paracoli"; 2 of which were isolated from well water and 2 from subsoil. He did not identify them as mutabile strains, but from their description they are very probably E. coli mutabile.

Bronfenbrenner and Davis, 1918, found a great many strains of E. coli-like organism in foods. A great many specimens of food were investigated with a view to studying them as a possible vehicle for spreading infection if handled by carriers. The organisms described were late fermenters of

lactose with the production of acid and gas. They were not identified as E. coli mutabile.

Koser, 1920, found atypical similar organisms in canned ripe olives.

Hagan, 1925, in a study of bracken poisoning in cattle isolated E. coli mutabile from pond water and from faecal specimens of cows.

Hoder and Singer, 1927, isolated late lactose fermenting E. coli from water and from stools of typhoid patients.

Kennedy, Cummings, and Morrow, 1932, described a late lactose fermenting bacillus from water. This was not considered to be a mutabile strain by the authors. No secondary colonies were described.

Jones, Orcutt, and Little, 1932, found late lactose fermenting E. coli on several occasions in faecal specimens of cows suffering from diarrhoea. They indicated that such organisms had been previously reported as found also in the faeces of horses and swine. Faeces from 50 normal cows drawn from 5 herds yielded only one atypical lactose fermenting colon bacillus. They pointed out that these organisms were of interest especially to those concerned in the matters of public health, since they may readily gain access to milk from faecal contamination of the cows and at first be mistaken for paratyphoid bacilli.

In 1934, Lewis reported 2 strains of E. coli mutabile from cheese; also Aerob. cloacae mutabile strains from water and soil; and B. acidi lactici mutabile from water. He also

described strains of E. communior mutabile, E. coscoroba mutabile, and E. coli mutabile and Escherichia lactici mutabile from human faeces.

In summary, it may be pointed out that the distribution of the mutabile strains of Escherichia and Aerobacter is similar to that of the typical members of these genera. That the mutabile strains have probably the same significance as typical E. coli in public health affairs is also a very important consideration to workers in that field, and offers many practical laboratory problems.

As Jones, Orcutt, and Little, 1932, pointed out, these organisms may be mistaken for paratyphoid bacilli, because they do not readily ferment lactose. Also, because of this, they may be entirely missed in a search for E. coli as evidence of faecal contamination. Therefore, they become of some importance and interest to all Bacteriologists dealing with intestinal bacilli.

DESCRIPTION OF E. COLI MUTABILE

The original descriptions of E. coli mutabile by Neisser, 1906, and Massini, 1907, have never been improved upon as to their clarity and accuracy. Many subsequent investigators have, of course, added new material, and made the knowledge of this organism more complete. In this section we will describe only the mutabile strains of Escherichia. As has been pointed out previously, mutabile variations are frequently encountered in other genera, but the type of metabolic variation is similar.

MORPHOLOGY

The Escherichia coli mutabile is a "coliform" bacillus, morphologically similar in every way to the typical E. coli. The cells are from 1 to 5 micra long and from 0.4 to 0.6 micra wide, with parallel sides and rounded ends. The predominating form is a short rod. Certain strains, however, vary greatly in regards to size and shape of the individual cells. Some are almost coccal in form, others show unusually long forms. The cells stain evenly, form no spores and show no granules. They stain negative with the Gram method and are non-acid-fast. From this description it is evident that neither shape, size, structure, nor arrangement of the bacterial cells, affords an adequate method of differentiation from other intestinal bacilli.

MOTILITY

The motility of E. coli mutabile is very variable. Some strains show fairly active motility of a majority of cells, others show motility of only a relatively small number of cells; or sluggish motility of a great number of cells, while others are entirely non-motile. Most of the strains we have studied show rather sluggish motility of many of the cells, but by no means the majority of them. Table No. 2 summarizing the cultural characteristics of a group of mutabile strains includes the motility findings. The results are entirely analagous to the motility of E. coli.

Jordan, Caldwell, and Reiter, 1934, in a study of motility of intestinal bacilli, showed that motility varied with the environment and with different strains of the same species. They showed that standard cultural characteristics and biochemical reactions do not appear to be correlated with the presence or absence of motility, and that motility is not a character that can be used for exact delineation of species or varieties of bacteria. We have, therefore, not attributed much significance to the motility findings of the organisms studied.

GROWTH CHARACTERISTICS

Growth of the mutabile strains of E. coli is abundant on the ordinary nutrient medium used in the laboratory, without the aid of any accessory substances. Growth is aerobic

or facultative anaerobic, though usually less abundant under the latter conditions. Growth at 37° C is much more rapid and abundant than at room temperature or about 20°. Cultures of E. coli mutabile in either broth or solid medium continue to grow without being transplanted over an unusually long period of time. Apparently the stationary phase and also the phase of decline is prolonged, making the old cultures very heavy ones. Old colonies continue to grow for many days and become very large. A study of the growth and death curve of mutabile would thus be exceedingly interesting. This problem was studied by Jarl, 1935, in this laboratory and is discussed in the chapter on Mechanism of Variation.

In broth, growth is abundant. With smooth strains there is uniform turbidity with a fine deposit of growth on the bottom of the tube.

COLONIES ON PLAIN AGAR The E. coli mutabile colonies on plain agar appear essentially like typical E. coli colonies. If the strain is a mutabile type of Aerobacter, the colonies simulate the Aerobacter type. The smooth E. coli mutabile colony is circular, raised, low convex, smooth, shiny, colorless or whitish grey; 2 to 3 mm. in diameter in 24 hours, with a finely granular structure. The consistency is butyrous and the growth emulsifies readily. At first the colony edge is entire and circular. The well isolated colonies continue to grow and increase in size, the edge becoming irregular to undulate, the center becoming increasingly more opaque, until the colony reaches a diameter of 6 to 10 mm. This continuous

growth is very characteristic.

An important observation which helps to differentiate the mutabile strains from the characteristic typhi, paratyphi, dysentery colonies is that the mutabile colonies are slightly but definitely more opaque than the usual pathogenic intestinal bacillus type of colony. This differense increases with the age of the colony, the mutabile continuing to become more opaque. This point is often a great help in giving the searcher for intestinal pathogens a lead of its being B. coli mutabile and not one of the well recognized pathogens.

The rough colony of B. coli mutabile, and there are rough strains, yields a coarsely granular form, more dull, and flat, with edges less regular and more effuse, and the entire colony more opaque.

COLONIES ON ENDO AGAR On Endo Agar the young colonies are perfectly colorless, but in about 36 hours and occasionally as early as 24 hours, a faint pink color appears in the center, more opaque portion of the colony. The color gradually increases in density. In 72 hours, and sometimes as early as 48 hours, there is a sufficient pink coloration to rule out a frankly non-lactose fermenting pathogenic organism. Sometimes the pink color is rather prominent in 24 hours. There is a great deal of variation in this point among the different strains, some strains remaining remarkably pale. There is always some slight amount of increased pink color, however, although it often occurs late and is certainly no greater than the pink that one frequently encounters with some of the

intestinal pathogens. Shig. parodys. Sonne and at times even some strains of Shig. dysenterical produce colonies on Endo agar that have a definite pink tinge. This color is not produced by the pathogenic paratyphoid bacilli, so that after the glucose fermentation reaction is known, this slight central blush may be of great help. It must be borne in mind, however, that the preparation of Endo agar, the environmental conditions and the method of handling this medium materially influence the appearance of the plates and also that of the growth on the plates. The pink of the mutabile colonies always appears in the center more opaque portion first. By the time a definite pink is recognized there usually will have appeared some secondary colonies also. These are described in detail in the section on that subject. Because of these several characteristics, namely, that of the helpful pink opaque center and the typical secondary colonies, which both appear usually at sometime after the first 24 hourse of incubation. The original Endo agar plates should not be discarded as soon as the suspicious colonies are transplanted into a differential medium, such as Russell's double sugar agar, but should be placed back into the incubator and observed at frequent intervals. Careful daily observation should be made for secondary colonies. The plates should not be discarded until the organisms have been identified as completely as possible. An incubator environment saturated with moisture is desirable.

SECONDARY COLONIES: The presence of secondary colonies or papillae on lactose agar is one of the characteristic and constant findings of all strains of mutabile forms of Escherichia. The secondary colonies have been described by the earliest workers as well as by a great many investigators since that time. They have been variously called daughter colonies, secondary colonies, papillae, and by the Germans, "knöpfe", and "knötchen".

The typical secondary colonies occur only on lactose containing medium. One must not, however, forget that a great many organisms within as well as outside of the intestinal bacillary group produce papillae on plain agar and various other types of solid mediums. When these secondary colonies are explained in the same manner as are those of the mutabile group there is a problem that is as yet not definitely determined. Evidence seems to point, however, toward the influence of other factors than those involved in the mutabile variation. There seems to be agreement that the secondary colonies of mutabile strains are produced only on medium containing lactose, and that the papillae contain in them the rapid lactose fermenting variant.

The appearance of secondary colonies on Endo agar only, will be described. Other differential lactose containing mediums also produce the typical papillae. Of these were tested: lactose litmus agar, Conradi-Drigalski medium,

McConkey's medium, brom cresol, purple agar, Levine's eosine methylene blue agar, Kligler lead acetate agar, and desoxycholate - citrate agar containing lactose. On each of them, typical secondary colonies were readily obtained.

Secondary colonies are present with most of the strains after about three days incubation at 37° C., though there is a great deal of variation in the length of time, growth is necessary before they appear. Some strains yield secondary colonies only after ten days incubation. With the use of a low power magnifying lens, very small colorless secondary colonies can often be seen in 48 hours. They are frequently first seen along the heavy growth where the colonies are confluent. There are all variations between these intervals. Growth at room temperature is slower than at incubator temperature; the secondary colony formation being delayed from one to three days later than their appearance at incubator temperature.

There is also a great deal of variation in the size, shape and color of the papillae. They may be barely visible and quite colorless, or they may have a diameter up to 1 mm. and be colorless. Any of the secondary colonies may be various shades of pink to very red with or without the metallic sheen.

The number of secondary colonies is also very variable. This depends somewhat upon the length of time the culture has been incubated. After four days incubation the secondary colonies may be nonexistent or there may be 40 in one single

colony. Some strains produce not more than three or four secondary colonies on the unisolated mother colony. We have, however, found strains on which there were over 100 secondary colonies on a large mother colony. Very often the secondary colonies are largest where there are few in number, but this observation is not consistent with all strains, for some secondary colonies as large as one mm. in diameter are found when 40 others are present on the same colony. The absolute number of papillae seems to have very little relationship to their size. They grow from very tiny colorless ones to very large ones, gradually becoming deeper red. There may be all colors and sizes of papillae on a single colony.

If either a colorless or red secondary colony is picked off and plated on Endo agar, there will appear both white and red colonies. If one is very careful in picking off only the secondary colony and attempting to get no organisms from the mother colony, there will be very few colorless colonies, more than 75% will be red. These red colonies produce no papillae, but the white ones continue to produce papillae. In lactose broth, a secondary colony inoculation if carefully done will usually produce fermentation with formation of abundant acid and gas in 24 hours. The red colony breeds true, that is on further subculture no more white colonies are formed, and the organism appears in every respect like a typical colon bacillus.

In our work we have never found a late lactose fermenting gram negative intestinal bacillus in which we could not demon-

strate the typical mutabile type of variation; that is, formation of secondary colonies on lactose containing agar that contained variants fermenting lactose rapidly. There are many reports of late lactose fermenting organisms similar to the colon bacilli, which do not mention secondary colony formation at all. This is unfortunate, since it leaves the full identification of such strains incomplete. The secondary colonies are not always readily demonstrated, and often require very careful observation and several trials. Secondary colonies sometimes appear very late and are sometimes very few in number, and at first are very small. On merely casual observation of young cultures they may be entirely missed. They sometimes appear first as a few small projections in the confluent growth before they have appeared as well defined papillae on the isolated colonies. Since this secondary colony formation with the production of lactose fermenting variants is the salient characteristic of mutabile strains, this cyclic phenomenon must be demonstrated before the identification can be completed.

Gyorgy, 1920, reported that the "majority" of his paracolon strains gave secondary colonies on lactose agar with fermenting papillae.

In 1932 Kennedy and Cummings found 8 out of 21 strains of "atypical lactose fermenters" that produced secondary colonies on Endo agar within 5 days. No further comment was made. Five days observation time is not long enough to

detect all of the daughter colony producing strains.

Jones, Orcutt and Little, 19[illegible]2, found no secondary colonies using brom-cresol purple agar on any of his 30 cultures isolated from cows that gave late lactose fermentation.

Dulaney and Mitchelson, 1935, reported 6 out of 25 slow lactose fermenting coliform bacilli that could be definitely identified as E. coli mutabile by the production of the variant in the secondary colony. Sandiford, 1935, stated that "many" of his cultures produced papillae, but makes no further mention of them.

In this study and in our experience in general, so far, all of the apparently late lactose fermenting bacilli that give acid and gas on dextrose, could be identified as mutabile forms of one or the other of the more or less well defined species of Escerichia or Aerobacter by the demonstration of the mutabile phenomenon. The careful search for secondary colonies and the subsequent attempt to demonstrate a rapid lactose fermenting variant is an important procedure to carry out on all gram negative intestinal bacilli, that at first simulate the Salmonella group, but by agglutination do not prove to be an organism that conforms with its well recognized members.

BIOCHEMICAL ACTIVITIES OF MUTABILE FORMS OF ESCHERICHIA COLI

The biochemical reactions of E. coli mutabile have been reviewed by many investigators, most recent of which is the very excellent study by Kristensen et al, 1935. Other investigators that have reported cultural reactions of mutabile strains based on the study of a series of organisms are: Penfold, 1911 - 1912; Castellani et al, 1916 - 1920; Bronfennbrenner and Davis, 1918; György, 1920; Trawinski, 1924; Dudgeon, 1926; Mikkelsen, 1927; Fothergill, 1929; Hungester and Anderson, 1931; Kennedy and Cummings, 1932; Jones, Orcutt, and Little, 1932; Lewis, 1934; Kriebel, 1934; Sandiford, 1935; Belliger, 1935; Dulaney and Michelson, 1935. No one series conforms in every respect to any other series reported. Our series also differs in some details from all others. 46 mutabile strains were studied. In all of these the typical mutabile cyclic phenomenon could be demonstrated, and a rapid lactose fermenting variant could be isolated. Five of these proved to be indol negative, and were mutabile forms of either Aerobacter or an intermediate group between Escherichia and Aerobacter. The other 42 strains were indol positive and proved to be mutabile forms of Escherichia. The results of the Escherichia group only will be given in this study.

TECHNIC The tests were made in 5 inch Durham fermentation tubes. Their composition was 1% bacto peptone, 0.5% C.P. sodium chloride, 0.3% Bacto beef extract, 1% Andrade's indicator

TABLE II

Cultural Characteristics Possessed by all

Members of the Mutabile Strains of Escherichia

Lactose	A.G. "late"	(production of a variant fermenting lactose rapidly with the formation of both acid and gas)
Dextrose	A.G.	Indol +
Galactose	A.G.	Inositol -
Mannose	A.G.	Inulin -
Levulose	A.G.	Gelatine -
Maltose	A.G.	Nitrate reduction +
Mannitol	A.G.	
Arabinose	A.G.	
Milk	Acid and late coagulation	

A.G. = Fermentation with acid and gas

\- = No reaction

\+ = Positive reaction

(by volume) in distilled water. The lactose, maltose, mannitol, dextrose and saccharose were added in amounts of 1% to the medium before it was sterilized. The other carbohydrates were prepared in concentrated solutions in distilled water, sterilized, and added to each test tube aseptically in amounts to make the final concentration 0.5%. The tubes contained the extract broth plus Andrade's indicator and had been already autoclaved. All sterilization was performed in the autoclave at 10 pounds pressure for 30 minutes. Inoculations were made from actively growing 24 hour plain agar slant cultures and incubated at 37° C. Results were tabulated at frequent intervals and observations were extended over a three week's period, or longer where necessary.

<u>RESULTS</u> The series is composed of 42 strains, all of faecal origin. In table II are listed the biochemical characteristics that all of these strains have in common. In table III are recorded all of the cultural reactions that varied in the different species.

TABLE III

CULTURAL CHARACTERISTICS IN WHICH THERE WERE VARIATIONS AMONG THE STRAINS OF THE SERIES OF MUTABILE FORMS OF ESCHERICHIA.

No. of type giving these reactions.	No. of strains giving these reactions.	Saccharose	Salicin	Dulcitol	Raffinose	Sorbitol	Rhamnose	Xylose	Adonitol	Motility +	Motility -
1	1	+	+	+	+	+	+	+	-		1
2	1	+	+	+	-	+	+	+	-		1
3	1	+	+	-	+	+	+	+	-	1	
4	1	+	+	-	+	+	-	+	-		1
5	11	+	-	+	+	+	+	+	-	6	5
6	1	+	-	+	+	-	+	+	-		1
7	1	+	-	+	+	+	-	+	-		1
8	1	+	-	+	+	+	+	+	+		1
9	2	+	-	+	-	+	+	+	-	1	1
10	2	+	-	-	+	+	+	+	-		1
11	1	+	-	-	-	+	+	+	-	2	
12	1	+	-	-	+	-	+	+	-		1
13	1	+	-	-	-	-	+	+	-		1
14	1	-	+	+	-	+	+	+	-		1
15	2	-	+	-	-	+	+	+	-	2	
16	12	-	-	+	-	+	+	+	-	8	4
17	1	-	-	+	-	+	+	-	-	1	
18	1	-	-	-	-	+	+	+	-	1	

+ = Fermentation with the production of acid and gas.

- = No fermentation.

Dextrine fermentation was difficult to interpret. Some strains gave no fermentation; most of them, however, caused a faint acid reaction, causing the indicator to turn pink, but the reactions did not go to completion. Glycerine was also very variable. In our hands the Koser's Citrate medium, which should regularly fail to support the growth of the typical Escherichia members, was also variable. At times it gave growth after several days incubation. We were unable to interpret the results.

In Table III there is no indication of the slow fermentation reactions. Frequently rhamnose, xylose, and salicin did not show a vivid acid reaction after the first 24 hour's incubation. The reaction usually went to completion in 2 to 3 days, although rarely it required a longer time. The greatest amount of variation in fermention is found in saccharose and raffinose. The mechanism of this "late" fermentation appears to be exactly the same as in respect to lactose. Saccharose, or raffinose variants are produced which attack the corresponding sugar rapidly with the production of acid and gas. On sucrose or raffinose agar secondary colonies are formed exactly like those produced on lactose agar. Of the 25 strains that fermented saccharose, 16 gave the typical type of "late" saccharose fermentation. Of these 16 strains, 10 showed the typical "late" fermentation of raffinose in three weeks time. There probably would be a closer correlation between these two sugars if the search for a raffinose variant had been more thorough.

Motility tests were done on 24 hour broth cultures. It should be pointed out that a negative test does not mean that the organism is definitely nonmotile; at another occasion and in different medium under different environmental conditions the same strain may prove to be motile.

Milk was always rendered acid and coagulation took place in 5 to 10 days.

DISCUSSION A study of the reactions charted in Table III reveals that the biochemical characteristics of the mutabile strains in this series are in every way similar to those of the typical members of the Escherichia group, except for the "late" fermentation of lactose. The lactose reaction is explained by the production by the mother organism of a daughter variant that is itself able to ferment lactose rapidly with the production of both acid and gas. "Late" lactose fermentation is described in the section to follow, and discussed more fully in the last section on the mechanism of variation.

The fact that mutability is a descriptive term that can be applied to certain strains, e.g. the mutabile strains of virtually any member of the Escherichia group is an important guide to the interpretation of these biochemical reactions. Lewis, 1934, described mutabile forms of Aerobacter cloacae, Escherichia coli, Escherichia coscorobe, and Escherichia acidi lactici. He pointed out that many species of Aerobacter and Escherichia may have mutabile forms.

Biochemical tests have in the past been the basis for sub-

dividing these two groups. Serologically they appear to be heterogeneous. With the present more complete knowledge of antigenic components and methods of technic of testing these components it appears likely that a more fundamental study of these organisms in this light, may demonstrate more exact subdivisions and relationships.

Levine in 1918 studied 333 coli-like bacilli isolated from the soil, sewage, and faeces of various animals. He showed that the action of the colon group on carbohydrates, alcohols, and glucosides showed the greatest differences among the individual members. If a large number of these carbon compounds were employed and separate groups formed from each separate combination of reactions, the result would be an exceedingly large number of different groups. He pointed out that when 8 descriptive characters are used, 256 combinations are possible; 10 characters, 1024; with 16, 56, 256, and so on. Yet there should be no objection to the multiplication of species if the specific differences, however, small, are stable. For practical purposes, however, a simpler, more workable classification must be followed and too much stress cannot be placed on minor differences in fermentative ability.

In the present series we are concerned with the Escherichia group of mutabile forms. Escherichia generally occurs in large numbers in the intestines of man, whereas the Aerobacter group is found only in small numbers in the faeces and is frequently present in soil and on grains.

The mutabile strains of Aerobacter are, therefore, very infrequent in stool examinations. In this series isolated from 454 stools, 42 were mutabile strains of Escherichia and 5 were mutabile strains of Aerobacter. The differentiation of the Aerobacter types from the well recognized pathogens offers no great difficulty.

It should be pointed out, however, that so called intermediate" forms between Escherichia and Aerobacter occur. To this group no definite names has as yet been accorded. The group appears culturally to fall more nearly in the Escherichia, but resembles the Aerogenes group as regards habitat. Therefore, they are relatively infrequent in faecal specimens.

There are certain biochemical characteristics of the Escherichia group that are quite constant. These are: Acid and Gas formation of Dextrose and the hexoses, Maltose, and Mannitol. Indol formed from tryptophane broth, Methyl Red positive, Voges-Proskauer negative, no liquefaction of gelatine, Hydrogen Sulphide not produced, no fermentation of Inositol, fermentation usually of Xylose, arabinose, rhamnoese.

Winslow, Kligler and Rothberg, 1919, gave an excellent review of the classification of the whole coli-typhoid group. Many of the divisions of the colon group made by Levine, 1917, are approved. They conclude that the following types of Escherichia as recognized by Levine are sufficiently well established and constant to merit separate consideration as species or varieties.

Table IV. FERMENTATIVE TYPES OF ESCHERICHIA (LEVINE)

	Sacch.	Salicin	Dulcite	Adonite	Motility
B. neopolitanum	AG	AG	0	0	0
B. coli communior	AG	0	AG	0	+
B. coscoroba	AG	0	AG	0	0
B. immobile	0	AG	AG	0	0
B. coli commune	0	AG	AG	0	+
B. acidi lactici	0	0	0	AG	0
B. grünthal	0	0	0	AG	+

B. coscoroba differs only from communior in being non-motile. Likewise B. immobile differs only from B. coli commune in being non-motile. B. grünthal differs from B. acidi lactici in being motile. Winslow et al suggest that the presence or absence of motility does not justify differentiation into separate types. They suggest, therefore, the recognition of 4 species of lactose fermenters producing both acid and gas. These four are underscored above, namely: B. neopolitanum, B. coli communior, B. coli commune, and B. acidi lactici.

The great amount of variability in motility in one strain at various times has been the experience of every student of these organisms. Jordan, 1934, demonstrated quite conclusively that this characteristic can not be used for differential purposes in this group.

Jackson, 1911, used dulcitol along with sucrose as the two primary criteria in classification. Winslow pointed out that in his collection there were both dulcite fermenters and

nonfermenters among all four of the types classified according to sucrose and salicin. These dulcite subgroups and others which may be obtained when using other fermentative characteristics, may be regarded as varieties of the corresponding four species mentioned.

Table V correlates the fermentative types of the mutabile strains with Winslow's species, based on the fermentation of saccharose and salicin. It is of some interest and significance that each of the varieties is found in the mutabile form.

TABLE V

FERMENTATIVE TYPES OF ESCHERICHIA COLI, WITH THE CORRESPONDING MUTABILE TYPES.

	Saccharose	Salicin	Dulcitol	Corresponding types of mutabile strains (see table B
Escherichia neopolitana	+	+	+	1, 2.
	+	+	-	3, 4.
Escherichia coli communis	+	-	+	5#, 6, 7, 8, 9.
	+	-	-	10, 11, 12, 13.
Escherichia coli communior	-	+	+	14
	-	+	-	15
Escherichia acidi lactici	-	-	+	16#, 17.
	-	-	-	18

This type includes more than 10 of our strains.

\+ = fermentation with acid and gas

\- = no fermentation

In table VI the fermentative types are diagrammatically represented. The number of strains in each group is recorded. More than half of our series of mutabile forms fell into two groups. They have all fermentation reactions in common except sucrose and its closely related carbohydrate, raffinose. All of them fermented, sorbitol, xylose, rhamnose, and dulcitol; and failed to ferment salicin, and adonitol. Eleven fermented sucrose and raffinose, and 12 failed to ferment both of these sugars. These organisms could not be correlated as to origin, as they came from both adults and children, normal and otherwise. It would be of some interest to know whether the E.colon bacilli of this region were chiefly of these two varieties.

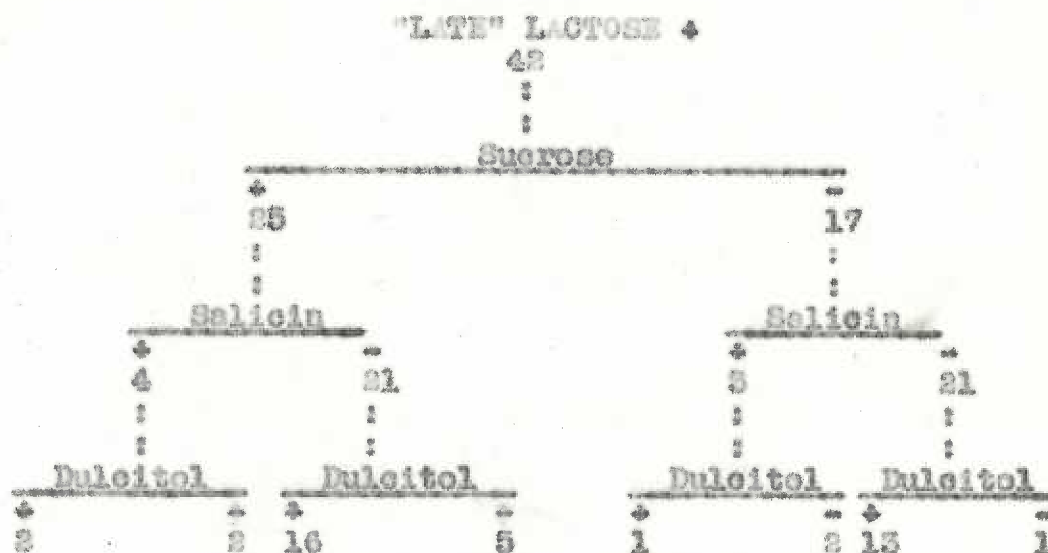

TABLE VI. DIAGRAMMAIC REPRESENTATION OF FERMENTATIVE TYPES WITH WITH NUMBER OF STRAINS IN EACH DIVISION.

TABLE VII.

CULTURAL REACTIONS OF ESCHERICHIA COLI MUTABILE

(A Summary of Tables A and B)

Dextrose	A.G.
Galactose	A.G.
Mannose	A.G.
Levulose	A.G.
Maltose	A.G.
Lactose	A.G. "late"
Saccharose	A.G. rapid, "late" or -
Raffinose	A.G. rapid, "late" or -
Xylose	A.G. rarely -
Arabinose	A.G.
Rhamnose	A.G. rarely -
Mannitol	A.G.
Dulcitol	A.G. or -
Sorbitol	A.G. infrequently -
Inositol	-
Adonitol	- rarely A.G.
Salicin	A.G. or -
Inulin	-
Dextrin	A.G. or -
Glycerin	A.G. or -
Litmus milk	A coagulation "late"
Indol	+
Gelatine	-
V.P.	-
M.R.	+
Motility	- or +

LATE LACTOSE FERMENTATION

Of the many different biochemical tests, the fermentation of lactose is by far the most interesting and significant in this study; so much so that it warrants special consideration.

The great distinction between the non-pathogenic colon group and the pathogenic typhoid- paratyphoid- dysentery group is that a typical member of the former ferments lactose with the production of acid and gas whereas the latter group is unable to attack lactose (or as is the case with Shigella paradysentery Sonne attacks lactose with the production of acid only). Lactose fermention has been the natural cleavage line in the intestinal group of bacilli for 50 years. Under discussion, however, we have an organism which appears to be in an intermediate position between the two large divisions; an organism which itself apparently does not ferment lactose, but which is able to produce offspring which are able to ferment lactose readily with the production of both acid and gas. This variant offspring is in no way different from the typical colon bacillus. This mutabile organism appears to span the gap between the two large groups, with one offspring in the generally pathogenic nonlactose fermenting group, and the other offspring nonpathogenic lactose fermenting group. There is some doubt, therefore, that lactose fermentation should be accepted as such an important classificatory character.

Intestinal bacilli that attack lactose can readily and correctly be divided into two groups, one fermenting lactose with the formation of acid only and the other with the formation of both acid and gas. Likewise the "late" lactose fermenting organism producing acid only are to be differentiated from the "late" lactose fermenting organisms producing both acid and gas.

FERMENTATION WITH THE PRODUCTION OF ACID ONLY Shigella paradysenteria Sonne (Sonne, 1914, 1915) and Shigella paradysentery dispar of Andrews (Andrews 1918)are both lactose fermenting bacilli producing acid only, some of them giving the evidence of lactose fermentation late. Both have been carefully studied by Nelson, 1930, and by Koser, Reiter, Bortniker, and Swingle, 1930, who showed sufficient evidence that they occupy definite positions in the Shigella group. These organisms are not considered further, in this study, except to point out that they are also lactose fermenters and that they produce variants analogous to those produced by mutabile strains. The mother organisms give pale colonies on Endo agar which do not ferment lactose readily. They produce secondary colonies after several days incubation. The secondary colonies when picked off to Endo agar give red colonies which ferment lactose readily.

The Sonne type produces variants also toward saccharose and raffinose quite uniformly. (Sears and Schoolnik, 1936). It is pathogenic to man, producing a mild form of dysentery chiefly in young children. One such epidemic is recently des-

cribed by Sears, Bilderback, Ashley, Rohner, 1924.

<u>"LATE" LACTOSE FERMENTATION WITH THE PRODUCTION OF BOTH ACID AND GAS</u> All the organisms that we have encountered giving "late" fermentation of lactose with the production of acid and gas proved to be mutabile forms.

E. coli mutabile when inoculated into lactose broth in Durham fermentation tubes containing Andrade's indicator grows abundantly, forming a homogeneous cloudy growth. In from 5 to 6 hours there frequently is present a definite pink coloration of the entire culture. This blush fades rapidly and on examination in 24 hours is entirely absent or the tube is definitely pink in the inner inverted tube, the culture on the outside being colorless. This initial pink blush in the fermentation tube during the early growth of the organism is not always present. The reason for it has not been determined. It was absent in the cultures of the Salmonella group that were tested. We have seen it only with mutabile strains of the colon bacilli. The early pink color in the inner tube may not develop until 48 hours, sometimes 72 hours, or it may be delayed as long as 7 days, and even 14 days. But once the color has appeared it increases in density to a deep rose within several more days. The medium outside the inverted tube changes color much more slowly and in some cases, never becomes definitely pink or red at all. Usually by the third or fourth day the outer medium becomes pale pink and progressively increases to a deep rose color. There rarely appears the

very bright red color that is given uniformly by the typical E. coli. In many cultures of mutabile strains there is never more evidence of lactose fermentation than the pink change in the inside inverted tube. Gas usually appears in the inverted tube soon after it begins to show the effects of sufficient acidity to turn the Andrade's indicator pink. The amount of gas produced varies widely with different strains and with different cultures of one single strain. There may be no gas formed in some cultures, or a tiny bubble of gas may appear within 48 hours , which gradually increases in amount to about 25% of the length of the inverted tube. Cultures taken from the same colony from plain agar may produce the widest of variations.

Thousands of lactose broth cultures have been observed systematically over long periods of time including thousands of tubes made from one single strain as well as many from a large number of different strains. In table No. are the results of the lactose fermentation of 100 lactose broth cultures of one strain of E. coli mutabile. The rapidity of acid and gas formation on lactose, as manifested by the fermentation tube varies tremendously. One strain (our S1?) produced a deep rose color and a large bubble of gas in 24 hours on lactose broth. This acidity reverted to an alkaline reaction in about 6 days. This same culture produced secondary colonies on Endo agar from which were obtained variants that fermented lactose with the formation of a bright red color and 25% of

gas exactly as the regular colon bacillus. This tube never reverted back to an alkaline reaction. The variant when plated out did not produce secondary colonies. In other strains the lactose fermentation tubes produced acid and gas in from 3 to 10 days. An occasional tube never produced gas at all over a month's time of observation. Explanation of these very variable reactions of the mutabile organism on ordinary lactose fermentation tubes involves a great many known and unknown factors of variation.

In a "selection" experiment in which a large number of lactose fermentation tubes were inoculated with one strain of B. coli mutabile, an opportunity was afforded to study the great variation in the manner and length of time in which lactose fermentation took place. Our B. coli mutabile strain number 269f was used. In its original description there was recorded, fermentation of lactose with the formation of acid and gas in 5 days. This organism had never been in contact with lactose. It was being transplanted frequently on plain agar slants. From a 24 hour plain agar plate culture a single well isolated colony was inoculated into a plain agar slant. After 24 hours incubation at 37°C, the growth was emulsified in broth and again plated out on three plain agar plates. These plates were allowed to incubate for 24 hours after which time one hundred colonies were picked off, and each inoculated into lactose broth. This medium contained 1% lactose, Pfahnstiehl's, and 1% Andrade's indicator in ordinary

beef extract broth, sterilized altogether in the autoclave at 10 pounds pressure for 30 minutes. Exactly the same portion of broth having the cultures were incubated at 37°C and observed daily over a period of a month. The results are tabulated in the following chart No. They illustrate the wide variation in the behavior of one strain of the mutabile type of organism, in spite of an attempt to control the environmental factors to keep them as nearly uniform as possible.

TABLE VIII

DIAGRAMMATIC REPRESENTATION OF FERMENTATIVE VARIABILITY OF ONE STRAIN OF E. COLI MUTABILE

Days of incubation	2	3	4	5	6	7	10	13	15	20	28
Negative	82	59									
Pink in inner tube	16	39	7								
Red thruout	2	2	96								
Red and acid			37	98	94	11	7	1	1	1	1
Alkaline in outer tube						78	70	44			
Comlete reversion to alkalinity								19	55	52	51
Gas				2	6	11	23	25	44	47	48

Numbers indicate the number of tubes showing that particular reaction.

It should be emphasized that the observation of a paratyphoid like organism on lactose broth will frequently give early leads to its identification as a mutabile strain of E. coli. There are two observations which should always be looked for. First; an initial pink blush of the fermentation tube in the course of 3 - 6 hours incubation. Second; the early evidence of some fermentation of lactose appearing as a pink color in the inverted tube. This pink appears first in the bottom of the tube in from 24 to 48 hours and gradually increases in intensity. The non-lactose fermenting Salmonella do not produce these early changes on properly prepared and controlled lactose broth medium.

ANTIGENIC CHARACTERS

The organisms of the colon group of bacilli are generally considered to be remarkably heterogeneous in antigenic make-up. This is found to be true also with the so called "paracolon" bacilli and with the mutabile forms of the colon bacilli. Some investigators have attempted to show by agglutination and absorption, very definite serological relationships between certain members of the colon and paracolon groups of bacilli, previously segregated either by fermentation and biochemical characteristics or by their pathogenic properties. Among the investigators succeeding in demonstrating such relationships are Sieke, 1921; Meyer and Lowenberg, 1924; Dudgeon and Pulvertaft, 1927; and Gundel, 1930-1931.

Mackie, 1913-1914, used the complement fixation test in studying the antigenic relationship of the coli group and also of the non-lactose fermenting coliform bacilli. He stated that "The fact that such gram negative bacilli i.e. the non lactose fermenting coliform bacilli behave in complement deviation experiments very similar to other typical E. coli strongly suggests that lactose fermentation which has always been considered the most important criterion of an E. coli can hardly be taken as of any more significance than other sugar reaction".

Herrold and Culver, 1919, isolated 45 paracolon bacilli from urine. Agglutination tests showed some definite relationship among them, enabling them to be divided into four groups.

Dulaney and Mitchelson, 1935, in a study of B. coli mutabile strains isolated from infants having diarrhoea, prepared high titer rabbit serums from 9 strains. Each serum agglutinated every other organism to a high titer, (details not given), showing conclusively a very close antigenic relationship of the group he studied. No antigenic relationship to B. coli communior nor to the typhoid-paratyphoid group could be demonstrated.

Sandiford, 1936, applied the term paracolon to a group of organisms culturally resembling B. coli except for fermenting lactose atypically and showing no relationship in being agglutinated by a typhoid-paratyphoid serum. Individual strains of these organisms do show, however, a small degree of common antigen. Nine high titer serums made with different strains agglutinated all of the 41 strains.

Kriebel, 1934, found one out of 25 strains of slow lactose fermenting coliform bacilli to give a fine partial agglutination on a slide in paratyphoid A, para-B, and typhoid serum. All other strains were negative with these serums. This result seems to indicate some common O antigen.

Kristensen, et al, 1935, studied 1004 organisms isolated from feces, none of which fermented lactose in 24 hours, but did ferment mannite with the formation of both acid and gas, but were not typical Salmonella. They found that 164 strains were agglutinated with O serum of the Salmonella group more or

less strongly. From this it appears that there is some relationship to the Salmonella group. An H antigen in common with the Salmonella group could not be demonstrated.

There appears to be quite complete agreement that the mother organism and the variant of any one particular strain of E. coli mutabile are antigenically identical. That is our result, and the result of numerous other workers. Jones, Orcutt and Little, 1932 showed by their agglutination tests that the mother strain of slow fermenting mutabile organism apparently possessed a somewhat different antigen complex than the variant, but the apparent difference could be entirely explained on quantitative grounds. More thorough and comprehensive investigation of the antigenic structure of E. coli mutabile should be made.

PATHOGENICITY OF E. COLI MUTABILE

SURVEY OF THE LITERATURE The literature lacks convincing evidence that the mutabile forms of E. coli have any pathological significance when found in the stool. Many attempts have been made to correlate possible disease conditions with the finding of this organism in the intestinal tract. They have been frequently isolated from the faeces of persons suffering with enteric like infections, and from acute cases of enteritis. Occasionally the organism has been isolated from the blood stream. In many instances, however, it has been cultivated from stools of perfectly normal individuals. It is doubtful,

whether E. coli mutabile has any real significance as a primary infecting agent of the enteric and dysenteric groups of diseases, though it can possess pathogenic potentialities when invading other tissues besides the intestinal tract.

Gilbert and Lion, 1893, in their first description of the paracolon bacilli considered that the organisms were possibly of clinical significance in infantile diarrhoea, but probably of no significance in adult diarrhoea.

Morgan and Ledingham, 1908 - 1909, concluded from their investigations that non-lactose fermenters increase in numbers in the intestines in diarrhoea, and that the paracolon group was of little pathogenic importance.

Castellani, 1912 - 1913, in numerous reports of para-typhoid-like organisms assigned them the cause of certain intestinal disorders. The evidence presented for his conclusions is not convincing. The organism that he terms B. asiaticum was later identified by Sandiford as a slow lactose fermenting E. coli. Others in his group that are probably similar are B. guimai, B. khartoumense, B. columbense, and possibly B. wesenbergi. A summary and discussion of his results would be exceedingly complex and misleading. In Castellani's work reference is made to investigations made by Khaled of Egypt, 1922 - 1923. He found B. asiaticum in 10% of the suspected enterica infections. The organism was present in 1 out of 50 normal stools examined. Castellanni stated that he had never found this organism in normal stools, though doubtless there are carriers.

Gyorgy, 1920, suggested that the mutabile forms played a considerable part in diarrhoea in both men and calves.

Stewart, 1926, as a result of the examination of the stools from 253 mental hospital patients concluded that while the paracolon mutabile group may possibly cause some secondary ills in an already disordered intestine, their presence has a symptomatic rather than an etiologic significance.

In 1926 Dudgeon stated that his group of slow lactose fermenters may give rise to intestinal disorders but does not give convincing support of his view.

Fothergill, 1929, considered that his inagglutinable paratyphoids were probably of etiological significance in infant diarrhoea, since these "atypical paratyphoids" are found mot frequently in the early fall and summer and this is also the season for the greatest incidence of "acute nutritional disturbances". Agglutination with the patient's own serum in 11 cases (50%) of series, was up to 1 - 320 and in one case 1 - 2,560.

McBroom, 1930, found 41% "intermediate organisms" from healthy persons and 39% from cases suffering from ulcers and various intestinal disorders. The total series was only 126. Her work does not substantiate an etiological relationship of the late lactose fermenting E. coli with intestinal disturbances.

Jones, Orcutt, and Little, 1932, found mutabile strains of E. coli throughout the small intestine of a cow slaughtered

during an attack of diarrhoea. He examined faecal specimens from 50 normal cows taken from 5 herds, and found the paracolon bacillus on only one occasion. From this it would appear that such organisms are infrequent in normal cows. A late lactose fermenting E. coli was isolated from all of the stool specimens examined from cows during an epidemic of infectious diarrhoea. In later epidiemics their appearance was irregular and during 1930 and 1931 they were entirely absent. Although the mutabile strains may be the predominating organism in certain of the intestinal disorders in cattle, little evidence was brought forth to incriminate them as the etiologic agent in such maladies and, therefore, they cannot be considered to be the cause of the disease.

Dulaney and Mitchelson, 1935, studied an outbreak of dysentery in infants. Stools were examined from 27 infants between the ages of 5 and 9 days. All of the infants were having an elevation in temperature, loss of weight and were passing from 10 to 20 stools per day. The stools were watery, yellowish, or green, but free from blood, pus, or mucus. He isolated 18 E. coli mutabile organisms, as the only abnormal stool organism. They were present in relatively large numbers. All strains were culturally similar. They were isolated from the heart's blood, spleen, ileum, jejunum, colon, and middle ear from 2 out of 5 post mortems. Stools from 41 normal infants revealed 5 or 12% to contain mutabile forms of E. coli. In adults the incidence was still lower; 2% of 47 adults; 7%

of 28 sick adults and 3% of 43 perturients yielding E. coli mutabile. Dulaney and Mitchelson state, "While we are reluctant to assign a fixed etiological role to this organism, the high percentage of isolations from the stools of infants showing diarrhoea, 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 at autopsy. Finally, the cultural and serological relationship found to exist among these organisms affords the best bacteriological evidence of their possible etiologic role."

Sandiford, 1936, added no support to the view that the presence of paracolon bacilli (among which he includes mutabile forms) in a stool has any pathogenic significance. Both blood cultures and agglutination tests in patients carrying them were negative, and an examination of 174 controls or normals showed that there was no significant difference in the percentage of stools showing them among three groups studied, dysenteric, enteric, and normal, when one took into consideration the relative findings of the total non-lactose fermenters therein. He further points out that any increase in paracolon found in diarrhoeal conditions is simply part of a general increase in nonlactose fermenting. Investigation might show that this apparent increase of non-lactose fermenters is fallacious and represents only part of a general increase in the total

flora of a disordered bowel.

From this survey of the literature it is quite evident that there has been consistent lack of evidence to attribute any pathological significance to mutabile strains of E. coli in the stool. That it is, however, a potential pathogen is to be expected in view of the ability of E. coli and other coliform bacilli to act as pathogens outside their normal habitat, the intestinal tract. We have in numerous instances been able to isolate mutabile strains of E. coli from urine in cases of urinary tract infection, the specimens being obtained directly from the ureters by catheterization. In the literature one encounters numerous reports of the isolation of this organism from urine in cases of urinary tract infections.

Herrold and Culver, 1919, found 43 paracolon organisms isolated from urine in cases of acute infections. Most or all of these may be mutabile forms, although not designated such by the investigators.

Dudgeon, Wordley and Bawtree, 1922, isolated slow lactose fermenting colon bacilli from the urine of 3 cases of exceptionally severe cases of urinary tract infection. Later Dudgeon, 1923 - 1924, and Dudgeon and Pulvertaft, 1927, described 49 coliform organisms, apparently mutabile forms, which were isolated from the urine of patients with acute infections of the urinary tract and in many of which also a diagnosis of enterica had been made.

Fothergill, 1929, described 2 such paracolon bacilli

from urine of acute pyelonephritis cases.

Wesler, 1931, described 5 strains of slow lactose fermenting E. coli from the urinary tract infections that agglutinated with the patients serum in dilutions of 1-160 to 1-640. All strains were culturally similar.

Nungester, 1931, found a lactose variable coli like organism from a case of empyema of the gall bladder.

Sandiford, 1935, isolated paracolon bacilli in 4.4% of 203 specimens of urine examined. In these cases they were usually found in pure culture. His paracolon group included mutabile forms of E. coli.

EXPERIMENTAL EVIDENCE Although the problem of the etiologic role of the mutabile strains of E. coli was not attacked as such in our own investigations, we have some data concerning the problem. Several preliminary animal experiments were undertaken. All results were negative. Animal inoculation experiments are best at the start with the difficulty of producing intestinal disturbances in laboratory animals that are pathologically analogous to those produced in man.

The incidence of E. coli, as shown by the results set down in the previous section, in stools of adults and children, normal and sick was found to be roughly 10%. This recognized frequency of the organisms in stools generally, does not necessarily preclude any pathogenic role. As Castellani points out these individuals may be carriers. A criterion that a pathogenic organism may not be present in normal in-

dividuals, only in thos affected by the illness does not hold, but the significance of the organism when found associated with some intestinal disorder, must be interpreted with a great deal of care. The works of Dulaney, and before him, of Fothergill, and even Gilbert and Lion, seem to point to a definite relationship between the mutabile organism and infantile diarrhoea. It must be conceded, however, that the exact relationship has not been worked out and that an etiologic role cannot as yet be given to this organism in infantile diarrhoea.

An indirect method of determining some relationship between the possible infection caused by an organism, is to study the reaction of the host, manifested in the specific antibody response. At various occasions we have tested human serums for agglutinins agains the mutabile forms of E. coli, isolated from that particular individual. The results are very variable. The reactions are usually negative, or agglutination occurs in low dilutions of the patient's serum.

TABLE IX.

Agglutination reactions of E. coli mutabile
with their homologous serums.

Antigen	Serum a	Serum b	Serum c	Serum d
A	1-80			
B		neg.		
C			1-20	
D				neg.

Table X shows the results of the agglutination tests of four human serums with the mutabile strain isolated from the intestinal tract of the same individual, from which the serum was obtained. Organism A with its homologous serum is agglutinated at a titer of 1 to 80. Organism B and D gave no agglutination with their homologous serums. The lowest dilution made in the case of B. was 1-20 and in D was 1-10. Organism C was agglutinated in a dilution of 1 to 20, but not in 1 to 40. Strain A was our number 104. The organism was a typical one. It was isolated from a stool that was normal in appearance. The majority of the colonies on the plate culture made from the stool were mutabile forms of E. coli. The patient was suffering from a mild allergic condition. This organism was used as an antigen against six serums that were obtained at random from the routine serological run. The results are recorded in the following table.

TABLE X

Agglutination reactions of one strain of E. coli mutabile with various serums.

Serum dilutions	1-10	1-20	1-40	1-80	1-160
Serum A	++++	++++	++++	++++	-
1	++++	++++	++++	++++	+++
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	++++	++++	++++	-	-

The organism was agglutinated by its homologous serum A to a titer of 1-80. Serum 1 produced fairly heavy agglutination which we called three plus (+++) in the highest dilution of 1-160. This is higher than was produced by the homologous serum. One other serum tested, No. 6, gave good agglutination in 1-40. Four serums, 2, 3, 4, and 5, produced no agglutination in the lowest dilution, 1-10. These results would indicate that any such reaction would be of no significance to determine any etiologic role of E. coli mutabile isolated from a patient having some intestinal disturbance. These results are in every way analogous to those one might get with a colon bacillus as the antigen.

In summary of the problem of the etiology of mutabile forms of E. coli, it may be stated that there is at present insufficient evidence that they have any pathologic significance when found in the stool. Their etiological significance seems to be analogous to that of the colon bacilli.

CLASSIFICATION

The mutabile strains of E. coli have been classified in many different ways. This fact in itself is evidence that certain problems and difficulties exist in evaluating their behavior in relation to their position in the systematic scheme of bacterial classification. The organism is difficult to trace in the literature because of the wide variation of terms under which it has been described. The following names are a list of those applied to mutabile forms of E. coli, or groups under which it has been included and described:

B. coli mutabile
paracolon bacillus
metacolon bacillus
coliform bacillus
slow lactose fermenting bacillus
paratyphoid like bacillus
pseudo-paratyphoid bacillus
B. neopolitanus Emmerich

It has been classified in the Escherichia group following the species name, simply by mutabile, e.g. Escherichia, coli communior mutabile.

Several investigators have given it other specific names, e.g. B. asiaticum, although the identity is not always very certain from the original descriptions.

This rather confusing variety of terms can readily be grouped into four definite trends of nomenclature: First, classification with the paratyphoid group; second, with an intermediate so called paracolon group; third, with the colon group or Escherichia; and fourth, designating the organism by a new specific term.

Relatively few investigators have considered the mutabile strains under the paratyphoid group. Among these, Watts, 1919, called it a "pseudo-paratyphoid" bacillus and Fothergill, 1929, called it an "atypical paratyphoid". Trawinski, 1924, simply called it "a nonlactose fermenting coliform bacillus". Gildemeister and Baerthlein, 1915, included it under the term, "paratyphoid-like" bacilli.

Those investigators who placed them into a paracolon group are numerous. However, as was pointed out previously, the paracolon group itself is not well defined. The consensus of opinion, however, is that a paracolon bacillus is a gram negative bacillus that occurs in the intestinal tract, ferments with acid and gas, dextrose, mannitol, maltose, does not liquify gelatine, and behaves antigenically individual. It does not agglutinate with serums prepared against any well recognized typhoid-paratyphoid organisms. Some investigators have included as part of their description that they do not ferment lactose--others that they must ferment lactose atypically, that is late, slowly, or give off lactose fermenting variants. Some have simply placed into this group all of the coli-like and paratyphoid-like organisms that could not be conveniently classified in any of one of the distinct subgroups. The term itself conveys the definite understanding that the organisms are perhaps more closely related to the colon bacilli than to the typhoid-paratyphoid group of bacilli. The investigators placing these organisms into a paracolon group are:

Gilbert and Lions, 1893-95; Seiffert, 1909, Herrold and Culver, 1919; Jordan and Irons, 1918; Gyorgi, 1920; Wilson, 1929; McBroom, 1930; Sandiford, 1935; Bolliger, 1935.

The majority of investigators have chosen to link this organism even more closely with the colon bacilli. From the first description of the organism as B. coli mutabile, by Neisser and Massini, it has been most often considered as belonging to the colon group of bacteria or very closely allied to it. The following reports give the organism a position in the colon group:

Sauerbeck, 1909; Müller, 1908; Morgan and Ledingham, 1909; Burk, 1908; Hubener, 1909; Kowalenko, 1910; Wilson, 1910; Thaysen, 1911; Penfold, 1912; Baerthlein, 1913; Dudgeon, 1926, "slow lactose fermenting B. coli"; Wasler, 1931, classifies them as Escerichia, because they ferment lactose; Hungester, 1931 "B. coli like organism"; Jones, Orcutt and Little simply called it atypical slow lactose fermenting B. coli; Kennedy and Cummings, 1932, Bacterium because they ferment lactose but are a connecting link between Salmonella and Escherichia; Hershey and Bronfenbrenner, 1936, Escherichia.

Some investigators preferred to coin new names: Bact. imperfectum, Burri, 1910 (because he believed the organism did not throw off a "mutation" suddenly, and the term mutabile carried that idea with it); B. neopolitanus Emmerich, Stewart, 1926. Castellani coined a number of new terms, but unfortunately from the descriptions given it can not be definitely de-

cided whether they are identical with the mutabile forms of E. coli, but to us the following appear so: B. asiaticum; B. columbensis, B. khartoumense, B. guinsi, B. wesenbergi.

Bolliger, 1935, introduced a large number of new terms, which are not included here because we feel that too much stress has been laid on minor differences in fermentative ability, and such new names only add to the confusion already present. It seems useless to assign to each of the strains a separate species until it is shown whether they are a heterogeneous group or not.

The confusion in classification lies in attempting to estimate the significance of the variation phenomenon. The fermentation of lactose has long been a rather fundamental differential criterion. The value of the variations in relation to classification of bacteria, in general, including many variations besides the mutabile type, is doubtful in the light of our present knowledge. One shouldn't have the impression, however, that such organisms are so variable that classification is precluded or that the variations change in an irregular unpatterened fashion. On the contrary, the mutabile forms have such remarkably constant variations that the variations in themselves might conceivably be used as a means of identification. Certainly with our present knowledge of the mutabile forms of E. coli the manner of variation towards lactose fermentation is sufficiently constant to make it of great value in identification. The characteristics upon which we rely for identification, however, are not a priori those on

which we can base their classification.

As has been pointed out earlier, the mutabile variation occurs in relation to other genera besides Escherichia, and to many if not all of the species in these genera. The variant itself, can readily be placed in a well recognized division of its genus in the system of classification, and becomes a stable member of that group. The mutabile form of Escherichia coli uniformly produces an organism that is identical as far has been determinatable with the typical E. coli. The mother mutabile organism, certainly must have close correlation with its own daughter organism. Since the daughter organism is a typical colon bacillus, in the genus Escherichia, the mother organism should most naturally belong to this genus.

We prefer to use the term "mutabile" purely in a descriptive sense, placing it after the name of the variant organism, which can usually be readily classified. It thus can be applied to any group or species of organism showing the particular phenomenon of variation. This avoids the dangerous procedure of assessing this property the importance of a differential criterion of classification.

Great advances are being made in differentiating certain types and varieties of intestinal bacteria by the use of comprehensive antigenic studies. This has not as yet been applied to the colon bacteria or to the mutabile forms of E. coli. Antigenic analysis provides at present the greatest hope as the best available method for accurate determination of the natural relationships of the mutabile strains with other closely related intestinal bacilli.

RECOMMENDED PROCEDURE FOR THE EXAMINATION OF STOOL SPECIMENS FOR PATHOGENIC BACILLI

COLLECTION OF THE SPECIMEN A satisfactory specimen is the first criterion for obtaining dependable results in the diagnostic laboratory. The stool specimen should be placed into a clean container and brought immediately to the laboratory, where it is received and immediately cultured. If there is any delay in inoculating the specimen, there will be a rapid overgrowth of the colon bacilli. A delay of even a few hours increases the difficulties of isolation of certain of the pathogenic bacilli with consequent reduced chances of a positive result. Where there is any possibility for delay, therefore, it should be placed immediately into some medium restraining the growth of the colon bacilli, but having no injurious effect upon the pathogen. Since ideal conditions are rarely present, it is usually desirable to routinely use some means of preserving the samples. Many restraining mediums have been described and have proved satisfactory. The method we prefer is the use of two different mediums, in saline 30% glycerine solution, Strauss and Spindle, 1928, and Haven's Brilliant-green-bile mixture, Havens and Irwin, 1932. A carefully selected portion of the stool, preferably a portion containing mucous or blood, about the size of a bean, is placed into each of two wide mouthed bottles, containing about 15 cc. of the two mediums, respectively. The bottles are shaken to emulsify the faeces and sent to the laboratory. They are allowed to remain at room temperature.

INOCULATION OF THE SPECIMEN A differential plating medium is essential for the isolation of the possible pathogen. Its value depends on the fact that the normal intestinal flora consists largely of lactose fermenting bacteria, while the significant pathogens do not attack this sugar readily. Therefore, the presence of lactose and an indicator provides a method for the immediate elimination of a large portion of the flora--that portion that attacks lactose. Any colonies that do not show up as containing non-lactose fermenting organisms are further examined and from these the pathogen will be discoverable. There are many mediums that have been enthusiastically recommended. None are perfect. Many have certain desirable points. More important than the medium chosen is the personal factor of becoming perfectly familiar with the reactions and behavior of the particular medium, under varied conditions, and with the many different types of intestinal organisms. Whatever the differential plating medium chosen, therefore, it should be used consistently. In our hands we still prefer Endo agar. The indicator gives definite color reactions, growth of the pathogens is not materially inhibited, and there is no marked diffusion of the color change through the medium.

The specimen is inoculated to fresh plates, preferably several, in a manner to insure the growth of well isolated colonies. It is of prime importance that the colonies be well isolated. Even if typhoid bacilli are present, they will not

grow or their growth will not be detected on the surface, if the medium is preempted by the rapidly growing colon bacillus.

Platings from the glycerine and brilliant green bile mixtures should be made after standing at room temperature for 24, 48 and 72 hours. Occasionally non-lactose fermenting colonies will appear in the 72 hour inoculation and not in the first 24 hour plating.

After 24 hours incubation, the colonies should be examined primarily for their color. Any colorless or pale pink colonies should be regarded as suspicious and should be picked off and studied further. The colonies must, of course, also be carefully examined for all other morphological characters whether opaque, transparent, mucoid, chromogenic, etc. The plates should be returned to the incubator and reexamined on subsequent days. To avoid dessication of the plate, the incubator is saturated with moisture, or the plates are placed into a moist chamber within the incubator. They should not be destroyed for one or two weeks, or until the organisms have been identified. Often a leading clue as to the type of organism encountered can be obtained from the appearance of the colony. It is well to remember, however, that there can be a great deal of variation in the colony types of any one particular strain of organisms, and that too much emphasis can not be placed on colony form alone. The colonial variation may be transient, affect all or part of the colonies, affect only one generation, or it may be permanent.

SUBCULTURE FROM PLATES A large number of suspicious colonies are picked off, using a long needle, and inoculated into Russell's double sugar (1.0% lactose - 0.1% dextrose) agar slants, making a stab through the base of the tube and streaking over the surface of the slant. It is absolutely essential that single well isolated colonies only be picked, so that pure cultures will result. The Russell's medium must be carefully prepared, using only reliably pure sugars, and sterilizing the medium in the autoclave at a pressure of not over 10 pounds. At least 12 cultures should be made, or as many as there are well isolated pale colonies from which to inoculate. This is very important in view of the frequency of finding several different types of non-lactose fermenters in one stool, and the possibility of finding a pathogen along with a mutabile form of E. coli.

The cultures are examined at 24, 48, and 72 hour intervals. It is usually wise to plate out again from the Russell's medium to insure the purity of the culture and to allow further study of the colony characteristics. The reactions on Russell's agar help to determine into what general group the organism falls.

REACTIONS OF RUSSELL'S AGAR CULTURES

Acid only in base - - - - - - -	Eberthella typhi
	Shigella dysenteriae paradysenteriae
Same or acid throughout - - - -	Shig. paradys. Sonne or Dispar (Staphylococci can be ruled out by making a strain of the culture)
Acid and gas in base only - - -	Salmonella group e.g. Sal. paratyphi Sal. aertrycke Sal. Schottmulleri Sal. enteritidis Sal. Morgani and others Mutabile strains of Escherichia Proteus vulgaris
Acid and gas throughout	Escherichia coli Aerobacter aerogenes

The culture on Russell's agar should be examined not only for fermentation reactions, but also for the character of the growth. This often furnishes a basis for the rapid elimination of many of the bacteria that are of no clinical or epidemiological significance. Staphylococci have a very opaque characteristic growth. Pseudomonas aeruginosa or B. pyocyaneous, in which pigment production is slight or late and is inhibited or masked on the Endo agar are fairly common on the plate cultures. Proteus vulgaris gives a paratyphoid reaction and shows the swarming or spreading characteristic, but this is often delayed and is usually inhibited on the Endo agar

cultures. The Aerobacter group of bacilli can usually be ruled out by the appearance of the colony, a large, raised, pink to red, mucoid mushy type, and a similar mushy growth on Russell's medium. Their acid reaction is frequently masked by their very active alkaline production. Quite frequently also ordinary colon bacilli are picked off to Russell's because their colonies had not as yet produced a sufficient acid reaction on Endo's agar to become red.

INOCULATIONS FROM RUSSELL'S MEDIUM

ACID ONLY IN THE BASE A gram stain and a test for motility should be made. Motile organisms are probably typhoid bacilli, nonmotile ones belong to the dysentery group, or are nonmotile forms of the typhoid bacilli. The preliminary species differentiation can be accomplished by a few cultural reactions. Inoculations should be made to lactose, mannitol, saccharose, tryptophane broth for the indol test, and milk. Other sugars may be employed, but because of the great variability of results, are not of great value. Dulcitol and xylose are useful in the recognition of the B. alkalescense. Rhamnose, arabinose, and sorbitol are of interest, but are not essential for their recognition. Table XI charts the differential characteristics of this group.

TABLE XII

DIFFERENTIAL CHARACTERISTICS OF IMPORTANT ORGANISMS GIVING ACID IN THE BASE OF RUSSELL'S MEDIUM

	Motility	Lactose	Mannitol	Saccharose	Dulcitol	Indol	Rhamnose	Arabinose	Xylose	Sorbitol	Milk
Eberthella typhi	+	-	A	-	-	-	-	-	A	A	A to N
Shig. dysenteriae	-	-	-	-	-	-	-	-	-	-	A to N
Shig. ambigua (Schmitz)	-	-	-	-	-	+	A	-	-	A	A to N
Shig. paradys. Flexner	-	-	A	-	-	+ or -	- or A	- or A	- or A	A	A to Alk
Shig. paradys. Sonne	-	A late	A	A late	-	-	A	A	A	-	A to C
Shig. dispar	-	A late	A	A late	-	+	A	A	A	A	A to C
Shig. alkalescens	-	-	A	-	A	+	A	-	A	A	Alk

A = Acid - = negative + = positive N = neutral

Alk = Alkaline C = coagulation

Conclusive identification is, of course, always by serological tests, using the culture as the antigen and reliable known high titer serums. Both an H and O serum should be used in the identification of the typhoid bacillus.

ACID AND GAS IN THE BASE OF THE MEDIUM Always make another Endo agar plate. Make a gram stain. A motility test is of interest but not essential. Inoculate further to tryptophane broth, lactose, mannitol, sucrose, dulcitol, rhamnose, sorbitol, and xylose.

The Salmonella group is particularly characterized by marked variations in biochemical reactions. This fact should not discourage one from doing the reactions that may be of some help. If they do not conform to a well defined group at first, the organism should not be discarded as unidentifiable, but the reactions should be repeated after some time, since many of the intestinal bacilli have variable reactions particularly when first isolated. Tartrate agar, Jordan and Harmon, 1928, and citrate agar, Simmons, 1926 are very useful mediums for differentiation within the Salmonella group. There are many strains of Salmonella which we have not included in the following table.

TABLE XII

DIFFERENTIAL CHARACTERISTICS OF IMPORTANT ORGANISMS
GIVING ACID AND GAS IN THE BASE OF RUSSELL'S MEDIUM

	Indol	Lactose	Mannitol	Saccharose	Dulcitol	Xylose	Sorbitol	Rhamnose	Tartrate	Citrate
Salmonella paratyphi	-	-	AG	-	AG	-	AG	AG	-	-
" Schottmulleri	-	-	AG	-	AG	AG	AG	AG	-	+
" enteritidis	-	-	AG	-	AG	AG	AG	AG	+	+
" aertrycke	-	-	AG	-	AG	AG	AG	AG	+	+
" Morgani	+	-	-	-	-	-	-	-	-	+ +
Proteus vulgaris	+ -	-	-	AG	-	AG	-	-	+	+ +
Escherichia coli mutabile	+	+ late	AG	- or AG	- or AG	AG	- or AG	AG	+	-

AG = acid and gas - = negative + = positive

There are many other members of the Salmonella group. Their identification is made purely on serologic studies. Topley and Wilson, 1936, p551, give an excellent review of their classification and antigenic structure.

THE DIFFERENTIATION OF E. COLI MUTABILE FROM THE WELL RECOGNIZED PATHOGENS--ESPECIALLY FROM MEMBERS OF THE SALMONELLA GROUP.

1. Colony appearance is frequently the first clue. The colony is larger and more opaque. It is not entirely colorless, but frequently has a definite pink center.

2. Secondary colonies, colorless to deep red, appear from the third day on. By careful examination of some strains, they can be detected in 48 hours.

3. Indol is produced by all of the mutabile forms of Escherichia. None of the paratyphoid bacilli form indol except Morgan's bacillus and this can be differentiated readily by the fact that manitol is not fermented by the Morgan's bacillus, and is readily attacked by E. coli mutabile.

4. Lactose fermentation tubes are routinely inoculated and frequently begin to show a definite pink change in the inverted tube in 2 to 3 days. Sometimes, of course, this change does not occur until 10 days.

5. Litmus milk may be inoculated and prove to be quite helpful. The mutabile strains of E. coli produce acid and later coagulation, while the paratyphoid strains of E. coli produce an initial acid reaction which later reverts to neutrality or alkalinity.

6. Sucrose is fermented by many of the strains of the mutabile forms. Sometimes it is fermented "late" in a similar manner as lactose. The mother organism also throws off a rapid sucrose fermenting variant. This is likewise true in relation

to raffinose.

7. If there appears to be any question whether the organism is a member of the Aerobacter group, buffered dextrose phosphate broth should be inoculated and the Methyl red and Voges-Proskauer test done. The Aerobacter strains are M.R. negative and V.P. positive, whereas the Escherichia strains are M.R. positive and V.P. negative. The presence of intermediate strains, between Escherichia and Aerobacter must be borne in mind.

8. Proteus vulgaris sometimes does not reveal any swarming until inoculated onto a moist agar slant. Gelatine liquifaction helps to prove the identity of Proteus.

9. The pigment of the pyocyaneous bacillus is sometimes missed until the culture is inoculated to a plain agar slant.

If both paratyphoid bacilli and matabile forms of E. coli are present, isolation of the former organism is made more difficult. In such cases careful examination of the colonies and transplantation of a large enough number of them will enable the isolation and identification of both types. The value of careful examination of well isolated colonies on repeated occasions can not be emphasized too strongly.

The final identification of the pathogenic paratyphoid bacilli must always be made by serologic tests. Agglutinations should be made with both the "H" and "O" serums which have been proven reliable. Since there is so little known about the antigenic structure of the E. coli mutabile at present, only negative results with the paratyphoid serums are

of any help. It appears, that there is some common "O" agglutinin of the mutabile forms with the paratyphoid group.

THE MECHANISM OF VARIATION

"Variation" is a vague descriptive term of certain bacterial phenomena which manifest themselves in many different ways. The underlying mechanism involved is as vague as the term itself. This discussion will be limited to the metabolic variation characterized by mutabile forms of E. coli. This "mutabile" phenomenon has already been described at length. The theories thus far advanced do not satisfactorily or completely explain all the phases of the behavior of an organism that gives rise to this mutabile variation. The theories are not well enough supported by experimental proof to be accepted without much further confirmation. Such theories are necessarily based on experimentation beset with great difficulties. In the approach to the investigation of this variation phenomena an attempt is made to find the correct explanation for the behavior of a single organism, and all the factors affecting it. This organism should be visualized as one in the midst of many millions or billions of bacilli, all descendants of a single mother organism, all developing in a similar environment. The billions of organisms are reproducing their own kind. For some reason, however, this one bacillus gives rise to an offspring that varies in

respect, from the other offspring, that is, in its ability to attack lactose with the production of certain metabolic products. This variant offspring continues to reproduce its kind. Experiments by direct observation are impossible. Indirect procedures must be devised, the results of which are difficult to interpret. Interferences from indirect experimental methods are beset with dangers.

THEORIES ADVANCED

Various interpretations have been put forward, accounting for the mutabile variation. The first one suggested was that of contamination. The fermenting papillae on the non-fermenting colony suggested the presence of two organisms in intimate relation or symbiosis, difficult to separate by ordinary plating. Contamination as the cause of the variant has, however, been definitely ruled out. The demonstration of similar agglutination properties in the case of the original and variant strains, the similarity of all cultural tests, with the exception of the one or two affected in the variation process, the fact that all the members of a species without exception may show the mutation when grown, in a particular carbohydrate, and finally the demonstration of the process in cases in which the culture was started from a single cell, disposed of this objection and established on a firm basis this mutabile process as occurring in bacteria.

Massini, 1907, regarded the mutabile variation as a mutation, occurring suddenly and not reverting. Some bacilli in the colony acquire the new ability to attack lactose and retain it. The bacteria had before only the power to attack

the two hydrolytic products of lactose, namely glucose and galactose. By growth on lactose certain specially adapted germs acquire the new characteristic. This new type occurs only on lactose andis not brought about by other influences. Even very small amounts of lactose are sufficient. These were also the conclusions of Neisser, 1906.

Numerous later investigators agreed with Massini. Among them were R. Müller, 1911, Penfold, 1910-1912, Baerthlein, 1912, and others. Penfold concluded from his work that there were other types of variations that did not occur suddenly, but developed by slow degrees. Those developing by slow degrees were not usually as stable as the other typical mutabile types. Müller guarded his conclusion, stating that the variation may be a mutation, occurring suddenly, but that the insufficient experimental evidence to support this is a "warning" that it may not be a mutation.

Twort, 1907, was the first to consider the variation a gradual one, that could be induced by long periods of growth on the particular carbohydrate that one wished the organism to ferment. For example, a strain of typhoid bacillus was cultivated on lactose for two years, after which the organism was able to ferment the lactose.

Burri, 1910, explained the variation as due to the activation of a latent ability to secrete a specific enzyme, through the stimulation derived from the particular sugar. The capacity once activated is transmitted to its progeny.

Bronfenbrenner and Davis, 1918, had a similar conception of the variation. The variant was an indication of the "rejuvination of power" of the organism to ferment lactose by the gradual adaptation of successive generations of the bacteria.

Another theory to explain the mutabile variation was proported by Mellon, 1925, who explained it as due to chromatic rearrangements caused by isoagamous conjugation. He claimed to have obtained zygospre formation by special growth on a glycerophosphate medium. He considered them part of a complicated life cycle, and regarded the mutabile strains of E. coli difinitely associated with what he considered as sexual reorganization.

Stewart, 1926, believed that mutabile colon bacilli possessed a routine Mendelian variation with respect to fermentation of lactose, and the lactose reactions are the result of Mendelian segregation of characters. The mutabile form he regarded as the central heterozygote, the colon as the pure recessive, and the non-lactose fermenting "paracolon" as the dominant.

Many investigators have since added their opinions to one theory or another. Considerable work has been done concerning the factors influencing the mutabile variation. The studies are difficult to evaluat for several reasons. All of the work has not been done on the same type of variation as occurs with the mutabile forms. Some workers have entirely missed the existence of a variant and have not even identified the organism with which they are working as a mutabile form. Such state-

ments as the following, although they are very true, are without significance because the investigator overlooked the mutabile type of variation: "--with some strains speeding up of the lactose fermentation takes place rather readily, and with other strains it occurs exceedingly slowly"; "--after several successive transplants in lactose broth, fermentation of this sugar was markedly accelerated"; "--found Salmonella aertrycke to become a typical coli commune, by passage through litmus milk sugar agar".

An attempt is made, however, to review the literature and briefly summarize the work that has been done in this laboratory concerning the various factors that influence the variation. There is no logical way of dividing these factors, as they are very much dependent upon each other and interrelated. For convenience, however, we have divided the subject into the discussion of the following questions:

Does the variation occur suddenly?

Is the variant stable?

Can a rapid lactose fermenting variant be produced by simple selection?

Can any organism be trained to produce a mutabile type of variant?

Is lactose necessary to obtain the mutabile variation?

Is lactose utilized by the mother organism?

Does the concentration of lactose influence the speed of lactose fermentation?

Does the composition of the medium other than lactose influence the variation?

Do the metabolic products produced by the mother organism influence the variation?

Do environmental factors such as temperature of incubation and oxygen tension influence the variation?

What effect has the volume of the culture medium on the manifestation of the variant?

What is the role of the secondary colony in relation to the mutabile variation?

DOES THE VARIATION OCCUR SUDDENLY? There has been much argument as to whether the appearance of the variant is sudden or gradual. This subject has been mentioned in the review of theories of the variation phenomenon.

The greatest evidence appears to point toward a sudden change. In a study of the growth curves of E. coli mutabile, Jarl, 1935, found four types of reactions, (1.) the variant may appear early and dominate the field, (2.) it may appear on a later day and dominate the field, (3.) it may appear on a later day, never reach a high count and soon die out; or, (4.) it may not appear at all in large enough numbers to be counted at any time. These results were obtained by making repeated counts from cultures on ten cc. amounts of lactose broth. Both the variant and mother organism were counted by making dilutions and plating on Endo agar. The explanation of the first type of growth would indicate that the variant was a sudden change; the second would indicate that the variation occurred as a gradual change from mother organism to lactose fermenter; the third curve, that it was a slow incomplete change from mother organism

to variants which either died out or reverted back. The fourth curve indicates that variation did not occur at all and the mother organism utilized the sugar after being exposed to it for some time, or simply that the variant was not present in large enough numbers to be isolated on the plates, but in sufficient numbers to form acid. Subsequent work led to a more plausable explanation of the fourth growth curve as due to a variant that appear, but was unable to dominate the picture because of the metabolic products and alkalinity produced by the mother organism. The possibility of the gradual change of the mother organism thru intermediate fermenting types to variant organisms, due to the present of the sugar, and the reverse change when the sugar has all been utilized, has not been studied sufficiently, to warrant a definite conclusion whether the Variation is gradual or sudden.

IS THE VARIANT STABLE? Can reversion occur naturally or can it be induced experimentally?

Since the first study of the mutabile type of colon bacillus by Neisser and Massini, it has been generally considered that the mutabile variants of E. coli which ferment lactose rapidly do not revert back to the mother type, failing to ferment lactose readily and giving off the variant form.

Burk, 1908, over a period of 5 months observation found no evidence of reversion of the rapid lactose fermenting variants to the non-lactose or slow lactose form.

Nungester, 1931, described a number of variants of a coli like organism isolated from a case of empyema of the gall bladder.

Apparently reversion was obtained from the lactose fermenting to the non-lactose fermenting types. The report makes no mention of secondary colony formation. The article is inconclusive and little information is added to the problem of the mechanism of variation and reversion.

Penfold, 1911, described a typhoid bacillus which did not ferment dulcitol, but which gave rise to secondary colonies on dulcitol containing medium, that contained organisms fermenting the sugar rapidly. The dulcite fermenters, however, tended to revert to the original form on repeated subculture.

Lentz, 1912, also reported a variant of the typhoid bacillus that was able to ferment rhamnose. It reverted to the mother non-rhamnose fermenting form, in part, after 5 consecutive passages through mice, also after exposure for 14 days to human bile and 2 passages on ascitic agar. After either of these treatments the organism was again able to produce secondary colonies containing the variation. Lentz considered this to be an atavistic phenomenon.

Bernhardt and Markoff, 1912, worked with the mutabile forms of E. coli and also with several dysentery strains showing a similar mutabile type of variation towards maltose; that is, secondary colonies on maltose which gave rapid maltose fermentation. The dysentery bacilli were isolated from stools of Rhesus monkeys, during an epizootic of dysentery among them. This strain agglutinated with stock Flexner serum to titer. It also agglutinated with the serum of one of the monkeys in a dilution of 1-100. Both the mother strain and the variant agglutinated alike. The variant organism was fed to a healthy

monkey which began having the typical dysentery symptoms in 5 days. A pure culture of the organism was obtained from the stools. These organisms, however, were not the variant, but the mother form that in turn gave rise to the same type of organism that was originally fed the animal. Mutabile variants of E. coli were then inoculated into rabbits and mice in large enough doses to cause death. On several occasions, the mother non-lactose fermenting strain was isolated from blood taken from the hearts of these animals. This apparent reversion of the variant strain led the authors to term the variation a "modification", rather than a "mutation", as the variation was not permanent. The E. coli mutabile strain of Neisser and Massini was used in similar experiments, but no reversion could be obtained with its variant. This work has not been substantially confirmed. It is significant and warrants further study.

There has been a great deal of interest recently in "transmutation" of types, particularly among the dysentery bacilli. Barber, 1913, and Calalb, 1925, studied the artificially produced and naturally occurring variations among this group. Calalb was able to cause a "reversion" of the abberrant, or variant cultures to one of the well recognized types, by intraperitoneal passages through rabbits.

Ledingham, 1918, described what might be called a "reversion" phenomenon in bacterial fermentation. Two strains of dysentery bacilli that agglutinated with the stock Flexner Y serum, were found to produce secondary colonies on isodulcite-containing medium. The mother organism was already able to ferment

this sugar, but the white daughter colony when picked off into this sugar was unable to attack it at all. This "reversion" type of variant seems to be analogous to the mutabile type of variation, except that a fermentative quality is lost instead of gained.

There is as yet no convincing evidence in the literature that the lactose variants of E. coli mutabile ever revert back to mother type. There is considerable doubt whether the types of variants that have been reported to revert, are of the same nature as the mutabile variation. It is conceded that the mutabile type of variation is an inherent property of the particular colon bacillus strain. It must be kept in mind that there are other variations of a different nature. Those characters which are due to immediate environmental influence, (e.g. temperature, nature and concentration of substances in the medium) are of a definitely temporary nature.

In the past six years we have never discovered a reversion among the variants in pure culture, even though they have not been in contact with lactose during that length of time, and have been under many different environmental influences. The possibility of a variant reverting to the mother organism in the animal body is, however, not excluded from being possible or even probable. When a flask of lactose broth is inoculated with E. coli mutabile, the variant can be found, the culture passes through an acid stage and then reverts back to alkalinity. Upon testing the culture after 2 to 3 weeks, no variant forms are found. Whether the variant has died off or

whether it has reverted back has not been fully determined. The consensus of opinion at present is that the variant of mutabile strains of Escherichia is stable.

CAN A RAPID LACTOSE FERMENTING VARIANT BE PRODUCED BY SIMPLE SELECTION? There have been many reports in the literature regarding the gradual changes of fermentation properties of intestinal bacilli, involving some selective action. Hiss, 1904, and Twort, 1907, were the first to report such selective variations. Twort was able to secure a lactose fermenting Dysentery Shiga bacillus, saccharose fermenting Dysentery Shiga-Kruse, and Flexner strains, and other peculiar modifications. He stated that the principle at the basis of these variations depends on selection of individuals inclined to use the particular carbohydrate, when the other constituents of the medium are used up.

Goodman, 1908, obtained variants showing differences in fermentative ability by a simple technic of selection. Starting with a particular strain of Coryn. diptheriae, which produced a certain degree of acidity in dextrose broth, he inoculated 15 tubes of this medium from a single colony, and determined the degree of acidity obtained after several days incubation. From the tube showing the highest acidity he inoculated 15 tubes of the same medium, and a similar number from the tube showing the lowest acidity. This process he repeated through 36 successive subcultures. At the end of this series the high-acid strain produced a titratable acidity more than twice as great as the parent strains; while the low-acid strain produced no

acid at all. As would be expected this strain had also lost its power to produce acid from maltose, but it is interesting to note that its power to produce acid from dextrine was almost unaffected. These observations would appear to afford an example of the separation of a bacterial strain into a fermenting and a non-fermenting variant, by a simple process of selection without any modification of the environmental condition.

To determine whether a rapid lactose fermenting variant could be produced by simple selection from a strain of E. coli mutabile, the following experiment was devised:

TECHNIC: A typical E. coli mutabile strain, our number 269f, was used in this experiment. It was isolated from a stool and had never been in contact with lactose at any time during its laboratory cultivation. Before working with the organism the culture was plated and replated, typical smooth colonies picked, and a fresh plain agar culture used. Incubation throughout the experiment was at 37° centigrade. Four plan agar plates were streaked, incubated 24 hours, then 100 colonies were numbered and picked off into correspondingly numbered lactose broth tubes, composed of 1% lactose in ordinary extract broth, pH 7.0, sterilized at 10 pounds pressure for 30 minutes. Five inch tubes were used, having in them a small inverted tube. The plates were placed in the refrigerator until further subcultures were made. The fermentation tubes were carefully watched to determine which gave rise to the most rapid lactose fermentation and which to the most delayed fermentation. The 2 corresponding colonies from which the "fastest" and the

"slowest" cultures had been inoculated, were then emulsified in sterile saline and each streaked to four plain agar plates. One hundred colonies were again picked from each and placed into lactose broth tubes. The subcultures from the selected colony that fermented most rapidly was labeled $269ff_1$. From the 24 hour plate culture of $269ff_1$, one hundred colonies were again labeled and inoculated into correspondingly labeled lactose fermentation tubes. The colony which gave rise to the most rapid lactose fermentation was plated out on four fresh agar plates as before, and labeled $269ff_2$, again 100 colonies were picked. This was repeated for 10 generations. Likewise, the corresponding colony that showed the greatest delay in fermentation of lactose was plated on four agar plates and labeled $269fs_1$. Selections were made in a similar manner for 10 generations. With this technic the organisms that were selected were at no time in contact with lactose. The fermentation tubes were considered acid when the entire broth content became distinctly red.

TABLE XIII

SELECTION OF FAST LACTOSE FERMENTERS 269ff

Number of tubes showing an acid reaction.

Generation	2 days	3 days	4 days	5 days	6 days	Ave. no days
1.	3	41	99	99	100	3.58
2.	5	27	86	98	100	3.86
3.	2	21	95	100	100	3.82
4.	3	35	89	99	100	3.79
5.	5	62	100	100	100	3.37
6.	8	35	89	100	100	3.68
7.	1	49	89	100	100	3.61
8.	8	49	95	100	100	3.50
9.	2	12	96	100	100	3.90
10.	5	28	98	100	100	3.71
Average	4.1	35.9	93.4	99.6	100	3.67

TABLE XIV

SELECTION OF SLOW LACTOSE FERMENTERS 269fs

Number of tubes showing acid reaction

Generation	2 days	3 days	4 days	5 days	6 days	Ave. No. days
1.	2	26	93	100	100	3.79
2.	3	21	82	98	100	3.99
3.	1	6	95	99	100	3.99
4.	3	34	96	100	100	3.67
5.	0	49	86	98	100	3.67
6.	1	8	93	100	100	3.98
7.	6	13	82	97	100	4.02
8.	3	39	92	99	100	3.67
9.	2	30	90	96	100	3.82
10.	2	22	78	100	100	3.98
Average	2.3	24.8	88.7	98.7	100	3.85

DISCUSSION No evidence of any appreciable change in the speed of fermentation resulted. Only ten generations were followed through, perhaps over a larger series some difference might have developed. It might reasonably be supposed that since no change in time of fermentation occurred in these ten generations, there sould be no change in the next ten generations.

Variation in time of fermentation was between 2 and 6 days with this particular strain. Part of this variation is undoubtedly due to experimental variations of technic, but certainly the greater part of this wide range is due to the variation in metabolism of the organism itself. There was an extremely wide variation in gas production, which is not charted. A few tubes showed gas on the fifth day, about half of them showed a small amount of gas by the 16th day, and the rest never gave gas. Nearly all of the tubes became alkaline between the 7th to 15th day. The few tubes that retained their acidity all fermented within 48 hours and produced sufficient acid to kill the bacteria. About 10,000 cultures were studied in this experiment, and many hundreds in other experiments. At no time were we ever able to find the variant from organism not in contact with lactose.

CAN ANY ORGANISM BE TRAINED TO PRODUCE A MUTABILE TYPE OF VARIATION? Winslow, Kligler, and Rothberg, 1919, have a complete bibliography of the variations found among the fermentation reactions of the intestinal bacilli. There are many reports in the literature of "training" organisms to ferment a sugar they

had not previously attacked. Twort, 1907, enumerates many examples of this.

Dawson, 1919, found that by changing the character of the medium upon which a variety of E. coli had grown for 200 generations, the chemical constitution of the organism was made to vary and with it certain biological characteristics, e.g. agglutinability and production of sugar splitting enzymes.

It is conceded that variations can be produced by change of the environment. These variations, however, usually prove to be temporary in nature, are not stable, occur gradually, and are not an inherent quality of the organism. The mutabile type of variation can not be produced in an organism that does not already possess this inherent variability. The evidence in support of this would include a long list of investigators to whom we have already referred.

IS LACTOSE NECESSARY FOR THE MUTABILE VARIATION TO OCCUR? It is quite generally agreed that lactose is necessary for the mutabile variation to occur. We have never found the variant except on lactose containing mediums. Smith, 1913, and several others, however, have suggested that variation occurs spontaneously in the absence of any specific sugar, but if the sugar is present, it acts as a selective agent, since the variant cells are benefited by it and are thus enabled to multiply more rapidly than the original type.

Stewart, 1927, concluded from his investigations that lactose or other sugars were wholly without influence except at a certain brief period in the life of a culture when its vege-

tative activity ceases and a process of segregation and autogamic conjugation occurs. The sugar if present at this time influences the segregation of the characters. The experimental evidence presented in support of this hypothesis is not convincing.

Nungester and Anderson, 1931, studied a coliform bacillus which varied in respect to lactose, and obtained lactose positive variants in one instance by plating to lactose agar from sucrose broth cultures.

Havens and Irwin, 1932, were able to isolate both sucrose positive and negative strains from plain agar cultures of a Salmonella morgani. They concluded that variation occurred spontaneously under ordinary conditions of cultivation with no apparent environmental stimulus.

Lewis, 1934, considered lactose to act as a selecting agent, but not as an inciting stimulus to specific variation. Variant cells were present in the colony on plain agar, but could not be recognized by conventional methods.

CAN THE LACTOSE VARIATION BE CORRELATED WITH ANY OTHER FORM OF VARIATION e. g. R & S. We have repeatedly found that the mutabile variation occurs on both rough and smooth cultures. The lactose fermenting papillae appear on both types of colonies. We have been unable to show any correlation of the variation from rough to smooth with the variation to lactose.

The independent variation of bacterial properties has been emphasized by Nungester, 1933, Lewis, 1934, and Hall, 1935, with particular relation to the mutabile variation as occurring independently from other forms of dissociation.

IS LACTOSE UTILIZED BY THE MOTHER ORGANISM? This question is of considerable importance because one of the theories to explain the variation is that the mother organism is itself capable of attacking lactose, and on lactose containing medium this characteristic is simply stimulated because of the beneficial effect derived from its utilization. This does not explain why all the organisms are not affected. There is not sufficient proof that the mother organism either does or does not ferment lactose, although the evidence points to the latter. There has been a great deal of argument about this point.

Jones, Orcutt, and Little, 1932, were the first to attempt to show that the mother organism, the "white" strain attacks lactose. Their lactose determinations, however, were done only after 2 day's incubation, at which time, appreciable quantities of lactose had been utilized. As shown by Jarl, 1935, the variant organisms probably appear in 7 to 10 hours after inoculation to lactose broth. Their results, therefore, do not prove that the lactose was not utilized by the variant itself.

Wedum, 1933, made daily sugar and pH determinations of cultures of slow lactose fermenting E. coli and found that the organisms grew in lactose medium several days without utilizing the sugar appreciably. Then suddenly overnight there appeared a sharp drop in the pH and in the amount of sugar remaining in the medium.

The most convincing experimental work has been performed by Deere, Dulaney, and Michelson, 1936. They made lactose determinations on a series of cultures of E. coli mutabile,

using the official gravimetric method for determining this sugar of the Association of Official Agricultural Chemists. They concluded that the mother organism, or white form, used no lactose or very slight traces of lactose (the possibility of slight traces can not be eliminated with the technic used) before the variant appeared or could be demonstrated. To demonstrate the variant, they plated the cultures on lactose agar for "white" and "red" colonies. They found that white and red colonies when grown on plain broth produced similar changes in the ammonia content and the pH of the medium. The ammonia content of the white cultures was similar whether grown on plain or lactose containing broth. But the white strain produced much more ammonia in lactose containing medium than the red strain. This fact is additional evidence that the white strain did not utilize lactose, but must make use of the nitrogenous compounds as the source of energy. They also believed that delayed appearance of acid and gas when E. coli mutabile is grown in lactose is due principally to delayed lactose fermentation rather than to production of alkali.

Jarl, 1935, added more experimental evidence to show that the mother organism does not utilize lactose. In a study of the growth curves of a typical mutabile strain of E. coli following both the mother organism and the variant, the mother organisms were found to go into decline after acidity is produced. If the mother organism would attack lactose it should produce a sharp rise due to the acquired ability to utilize the sugar.

DOES THE CONCENTRATION OF LACTOSE INFLUENCE THE SPEED OF FERMENTATION OF LACTOSE? Bronfenbrenner and Davis, 1918, studied methods of enhancing and speeding up lactose fermentation in the strains attacking that sugar "slowly". They made cultures, "slow" fermenters on broth mediums containing, 0.5%, 1.0%, 2.0%, and 3.0% lactose, and found acid and gas to appear earlier on the 3.0% lactose than on the lower concentrations. Repeated transfers on the more concentrated lactose mediums induced rapid lactose fermentation after 10 transplants. The secondary colony formation and the mutabile type of variation is not mentioned at all in his work. No indication was made whether the fermentation was produced by the mother organism or a variant.

Kriebel, 1934, studied "slow" lactose fermenting coliform bacilli on various concentrations of lactose ranging from 1 to 10%. She found that acid and gas was produced earliest in the tubes containing 5% lactose. In this paper also nothing was said of secondary colony formation or the mutabile variation, although it appears that she was working with a mutabile forms of colon bacilli.

In a search for a method of early identification of the mutabile forms of the colon bacilli, it appeared from the two above reports that the use of more concentrated lactose broth cultures might be of practical routine value. Several strains of mutabile forms of E. coli were tested on lactose broth cultures, containing Andrade's indicator. The lactose was in concentrations of 0.5%, 1%, 2%, 3%, 4%, 5%, and 10%. Controls were run with a known Salmonella paratyphi and a Salmonella

schottmulleri. The results varied somewhat as might be expected in view of the great variation in the appearance of 1% lactose broth cultures as diagrammed in Table VIII. The 5 and 10% lactose broth tubes gave a definite pink color, stronger than the lower dilutions. The Salmonella schottmulleri, however, also gave a similar pink color in the bottom of the inverted inner tube in the 10% lactose cultures. Gas appeared earlier in the 5 and 10% lactose tubes. The amount of definite pink color, however, was not great enough and the variability even in the higher concentrations of lactose, was marked. An important factor in the interpretation of the pink color in the higher concentrations of lactose, is the hydrolysis of the sugar during sterilization, and the accumulation of minute amounts of hexoses. Acid and gas are manifested earlier in the higher concentrations of lactose than in the lower; the difference, however, is small. The possibility of error in interpretation of such early evidences of lactose fermentation is great. The use of higher lactose broth concentrations as an aid in the earlier recognition of mutabile forms was not considered feasible.

DOES THE COMPOSITION OF THE MEDIUM OTHER THAN LACTOSE INFLUENCE THE VARIATION? Any substance in the medium that hastens multiplication of the bacteria hastens the appearance of the variant. From the work of Jarl, 1935, and Lewis, 1933, it can be concluded that the variant appears every few billions or trillions of organisms, in about seven hours after inoculation into lactose. There is no other substance that has been found to cause or

support the variation except lactose. Much work has been done on the inhibition of fermentative characters by chemical substances especially dyes, that phase of the subject of variation is beyond the scope of this paper. Mungester, 1931, believed that there was greater tendency to variation in broth medium than on solid agar medium. The liquid and solid mediums tested had the same concentrations of lactose. This was observed for both maltose and lactose. Lewis, 1933, worked with a synthetic medium in which lactose was the only source of carbon. He found secondary colonies to develop readily, and concluded from his investigations that non-utilizable carbohydrates and other carbon compounds have no affect on secondary colony formation or the production of the variant.

<u>IS THE VARIATION INFLUENCED BY THE METABOLIC PRODUCTS PRODUCED BY THE MOTHER ORGANISM?</u> Soule, 1928, found that the dissociation from smooth to rough forms was aided by the growing of the smooth form in the metabolic products produced by the organism.

Stewart, 1927, reported that a strain of E. coli mutabile could not be caused to form secondary colonies any sooner by preliminary cultivation in lactose broth for several hours before plating.

Very little work has been done to indicate whether the variation in respect to sugar fermentation is hastened by the presence of metabolic products of the mother organism. It was conceivable since variants appear to occur only after the

mother organism has grown for a time on lactose, the metabolic products might stimulate the variant to appear. Jarl, 1935, inoculated the variant into old lactose broth cultures of E. coli mutabile that had been killed at 60°C. She found that the accumulation of metabolic products from 24 hour's to one week's time, had no inhibiting affect upon the growth and acid production of the variant, but that the accumulated products in one month old cultures did have a slight inhibiting affect on the ability of the variant organism to produce acid. In another experiment in which the variant was inoculated into old lactose cultures in which the mother organism was growing, the results showed that more variant organisms were necessary to produce acidity when grown along with the mother organism than when grown alone in the metabolic products of the mother organisms. This may be explained on the basis that the mother organism is producing in addition to metabolic products, an alkalinity from its protein metabolism which tends to neutralize the acid as it is produced by the variant.

Sears, 1931, inoculated mutabile forms of E. coli into sterile Berkfeld filtrates made from 24, 48, and 72 hour lactose broth cultures of the same mutabile strain. The presence of the metabolic products neither accelerated nor inhibited the production of the rapid lactose fermenting variant.

DO ENVIRONMENTAL FACTORS--TEMPERATURE, OXYGEN TENSION, ETC. INFLUENCE THE VARIATION? Wilson, 1910, isolated an organism from a typhoid carrier that fermented lactose in 7 to 24 days. The organism resembled a colon anaerogenes type, which Wilson considered formed a connecting link between typhi and coli. The lactose splitting enzyme failed to appear at 37° centigrade, but consistently appeared at 22°. There was complete disappearance of lactose at 22° and little if any at 37°. On Conradi Drigalski medium the colonies remained blue at 37° and became red at 22°. The appearance of the enzyme seems to be dependent upon temperature.

The mutabile variation takes place at room temperature as well as at incubator temperature. Anything that delays the multiplication of the bacteria, however, delays the appearance of the variant, since the multiplication is slower at room temperature than at incubator temperature. Fermentation of lactose would occur later at the lower temperature. This same principle holds true for environmental factors. Whether reduced oxygen tension has an additional effect is not known. The cultures tested under reduced tension produced the variant as under normal conditions.

WHAT EFFECT HAS THE VOLUME OF THE CULTURE MEDIUM ON THE MANIFESTATION OF THE VARIANT? Penfold, 1911, found that in using large volumes of media to hasten the multiplication of organisms, the dulcite fermenting variant of B. typhosus was produced in a shorter time than when multiplication was slower in small volumes.

Kennedy and Cummings, 1932, noted that slow lactose fer-

menting coliform organisms gave evidence of rapid lactose fermentation in toxin flasks.

Dulaney and Michelson, 1935, noted similar results and performed pH determinations on various amounts of media.

Jarl, 1935, concluded from clever experimental work that large amounts of media inoculated with E. coli mutabile form acid in 24 to 33 hours. The variant appears after 10 hours of incubation at the rate of one organism per 10 cc or 100 organisms per liter of media. They occur early as a result of rapid multiplication of the mother organism under favorable conditions and a large number of organisms in which there can be greater variation from the mean. In small volumes, the variants occur in 24 hours as a matter of chance or a matter of one variant per certain number of multiplications of the mother organism.

WHAT IS THE ROLE OF THE SECONDARY COLONY IN RELATION TO THE MUTABILE VARIATION? Lewis, 1933, reviewed the literature on secondary colonies and discussed their origin and significance. It should be emphasized that papillae are frequently found in various organisms under various conditions wholly unrelated to a mutabile type of variation.

The papillae in E. coli mutabile occur only on lactose containing agar. They appear to be a very rapid growth of certain members of a colony. These members, because they have the ability to use the sugar, are beneficially enabled to continue to multiply after the mother organisms have ceased to divide.

Mutabile variations are characterized by secondary colony formation on the carbohydrate to which they are variable.

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