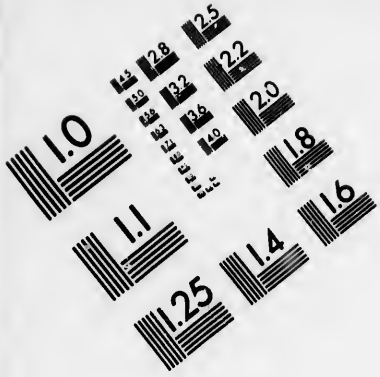

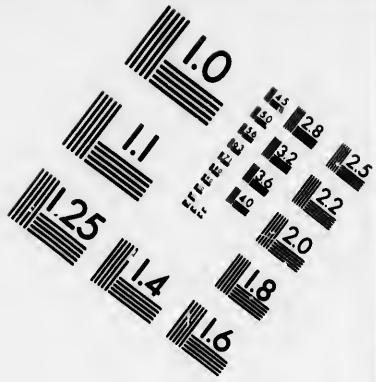

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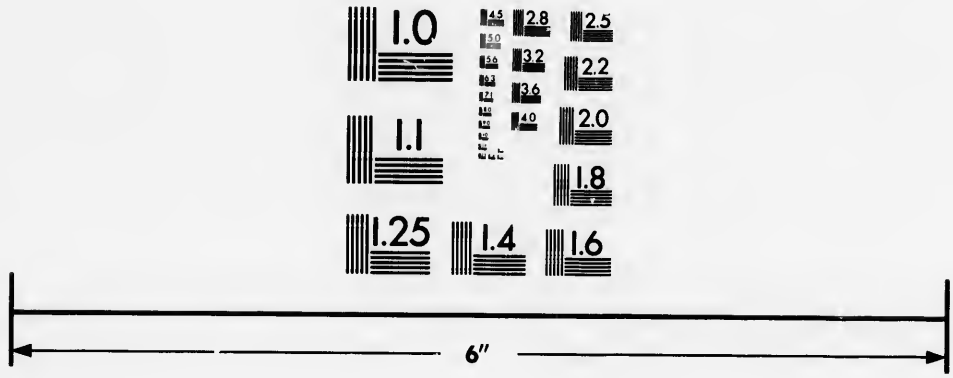

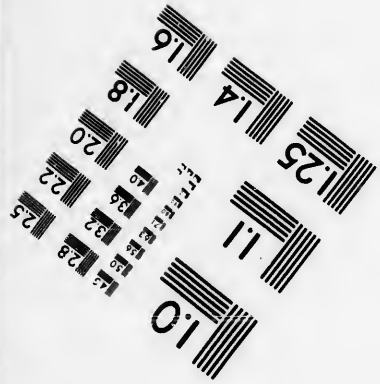

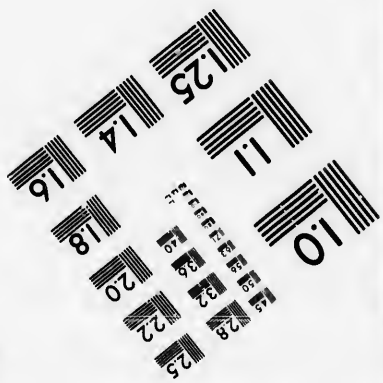

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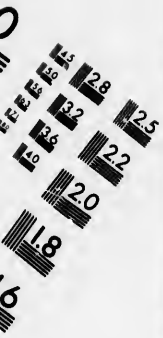

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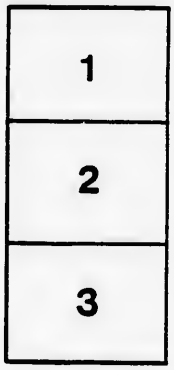

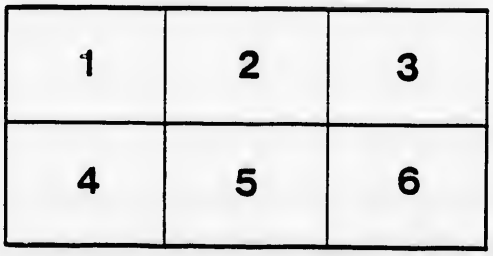

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VARIETIES OF COLON BACILLI ISOLATED FROM MAN

BY

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Fellow in Pathology, McGill University.

Reprinted from the Montreal Medical Journal, November, 1900.

Dr. F. G Finley JUN 23 '31

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VARIETIES OF COLON BACILLI ISOLATED FROM MAN.

BY

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Fellow in Pathology, McGill University.

(From the Molson Pathological Laboratory, McGill University.)

The discovery of the colon bacillus by Emmerich in 1885 in the blood, organs, and alvine discharges of cholera patients in Naples, its later isolation from normal and abnormal fæces by Weisser and its further accurate differentiation from other intestinal bacteria coupled with a minute account of its biological characters by Escherich, were the three scientific achievements which laid the foundation of our modern knowledge of the flora of the alimentary canal.

In the decade and a half which have passed since the observations of these men, a more or less universal interest has been centred in the varied reactions of this organism under artificial laboratory conditions, while the elucidation of the problems connected with its widely spread habitat in nature and the growing belief in its power as a pathogenic agent to cause serious lesions in man and animals, have become more and more the excuse for a careful study of its life history.

Since the original description of the colon bacillus, many allied forms have been isolated in normal and pathological conditions, from sources both within and without the animal body, and bacteriologists have become convinced that this organism, instead of being the simple and possibly only constant inhabitant of the lower bowel in man, should in reality be looked upon as a group of bacilli, the many members of which differ considerably from each other in their cultural features and their pathogenic action.

Within a short time after Escherich's work Booker, in an exhaustive study of the bacteriology of summer diarrhœa, isolated seven different members of the colon group related to each other in their fundamental characters, but separated by important, although minor tests. In the then state of our knowledge of this organism Booker was unable to give a classification of these forms which he considered satisfactory and contented himself with naming the different varieties and calling attention to their principle reactions.

The experience of Booker in regard to the differentiation of these colon forms has been duplicated in many laboratories since the publication of his results and the confusion which necessarily arises from the

* Read before the 28th Annual Meeting of the American Public Health Association, held in Indianapolis, Oct. 22nd, 1900.

study of the same or allied micro-organisms by observers working in widely separated institutions in different countries, has been engendered by the universal employment of obscure and indefinite terms in bacteriological protocols.

The beginning of the present movement among scientific students to adopt definite known terms of positive or negative value in the description of intestinal bacteria was made in 1895 by Theobald Smith, who employed the fermentation tube in the separation of the different members of the colon group, estimating the quantity and composition of the gases formed from the different carbohydrates, as well as the rapidity of their evolution and the temperature best suited for their development. By this means Smith was able to give accurate data concerning the reactions of the typical B. Coli Communis as compared with B. Typhosus and B. Lactis Aerogenes, and to make out a number of intermediate forms related to these organisms.

Two years later, Gordon following the work of Smith in connection with the fermentation of the sugars and employing as well as a criterion of specificity, the number of flagella the different forms possess, was able to distinguish twenty-two distinct varieties of the colon species. Meanwhile Adelaide Ward Peckham had published the results of her work on the indol-producing powers of the typhoid and the colon. By cultivating an indol-producing colon on a variety of media she could deprive it of the capacity to generate indol. Again, she could impart this function to a bacillus not ordinarily producing this substance. Going still further she found that she could cause the Bacillus Typhosus itself to give definite reactions for indol. In fact, Peckham was able to cause every non-indol-forming colon and every typhoid culture to which she had access, to assume the property of producing indol.

In her work Peckham frequently met members of that group of micro-organisms which seemed to stand midway in their cultural features between the bacillus of Escherich and the bacillus of Eberth. Such forms as B. Choleræ Suis and B. Enteriditis of Gærtner, had long been considered to form the intermediate stages between these two species. It remained for Durham, however, to call special attention to these forms and to divide the whole series into three groups consisting of :

1. The Eberth Group—including B. Typhosus and its allies.
2. The Gærtner Group—including B. Enteriditis and its allies.
3. The Escherich Grop—including B. Coli Communis and its allies.

The Gærtner group includes besides B. Enteriditis of Gærtner, B. Choleræ suis, B. Morbificans bovis of Basenau, B. Breslaviensis of von Ermenghen, the Wurstvergiftung bacillus of Fischer, B. Friedeber-

gensis of Gaffky and Paak, the Cotta Fleischverigiftung bacillus and a number of similar organisms described by various observers in epidemics of meat poisoning.

Cushing has recently made this group the subject of elaborate study in connection with the problems of fermentation, reactions of acidity and alkalinity imparted to the media, and pathogenicity. He groups with these intermediate forms the bacillus isolated by Sanarelli from yellow fever patients, a bacillus isolated by Gwynn from a cervical abscess and a bacillus which he himself has isolated from an abscess over a rib, called by him Bacillus O. or, by other workers, Paracolon Cushing.

The agglutination test so valuable in the recognition of the bacillus typhosus has proved in Cushing's hands to have an equal significance in the study of these intermediate forms. Cushing's work in this particular has recently been confirmed by McCrae working under Adami.

The term *Paracolon*, introduced originally by Gilbert to indicate the members of the colon group which differed in a few reactions from the typical colon, has found a ready acceptance among bacteriologists. Under the nomenclature of paracolibacillary organisms, Gilbert has described five different types. The first type has two members, the *opaque* variety of B. lactis aërogenes of Escherich, identified by its opaque yellow colonies on gelatine, and the *transparent* variety of the same, identical with the bacillus of endocarditis of Gilbert and Lion.

"The paricolibacillus of the second type is distinguished from the B. Coli by its inability to generate indol; that of the third type by its failure to act on lactose; that of the fourth type, by the absence of motility and of the power of generating indol; the fifth type by lack of motility, incapacity of producing indol and inactivity in respect to lactose, three of the cardinal properties of the colon."

The chief objection to such a classification is the unreliability of the reaction for indol, which as stated above has been shown to be an inconstant character of any organism.

The recent work of Fuller and Johnston, in which they classified the water bacteria found in the Ohio River, according to a large number of constant characters—using those reactions which were recommended by the Bacteriological Section of the American Public Health Association, and eliminating any tests which failed to give 100% of constancy, has suggested to the writer the advisability of adopting a somewhat similar classification in the descriptions of members of the Colon-Typhoid series. Therefore, all of the organisms which were in the laboratory were subjected to the treatment recommended by Fuller and Johnston, namely, they were grown in broth three days, in gelatine plates three days and on slant agar three days after which time the various culture media were seeded.

By such a means an organism is forced to assume a constant laboratory character, parallel in a way with the constant character it may have in its normal habitat. We thus avoid in our description of its biology that period in the life history of any bacterium when it possesses inconstant features, as is the case for instance, immediately after isolation from the intestines. The various members of this series have been classified as far as possible in accordance with these constant characters and the result of this classification is the chart which is appended to this paper. While the different types described seem to be separated from each other by only a few characters, yet each type is represented by a number of corresponding cultures and the organisms of one type never assume the properties of other types. In order to assure completeness in this table of bacteria the reactions of B. Typhosus were estimated at the same time and the reactions of B. Lactis Aerogenes and B. Cloacæ are taken directly from the tables of Fuller and Johnston. The fermenting properties of certain members of the intermediate group as well have been adopted from Cushing's paper, as some of the cultures used in Montreal died out.

The typical Bacillus Coli Communis originally described by Escherich is a motile bacillus without the property of forming spores, whose diameter is less than 1 micromillimeter. It forms a scum on broth, with production of a turbidity, is not dull or wrinkled on agar, fails to show a characteristic appearance on gelatine plates. It causes an abundant growth of potato, grows in the closed arm of the fermentation tube, grows as an anaërobe, grows at body temperature, and is affected by the range of the reaction of the media. It does not liquify gelatin casein or blood serum; it produces gas with dextrose, saccharose and lactose; it produces nitrites, indol and a fæcal odor, produces acid and coagulates milk. On agar it is not chromogenic or fluorescent, but usually prefers an agar which is slightly acid in reaction. It is pathogenic for mice in intraperitoneal inoculation of 1 c.c.m. doses of a 24-hour fluid culture.

The estimation of these reactions while apparently simple may at times be very difficult of attainment, as the colon when first isolated from the body does not show a characteristic biology. Its morphology is subject to the greatest variation. It may appear either as simple rod-shaped bacilli, as straight bacilli many times the length of the typical form, maintaining the same diameter throughout, or as a diplococcoid body, as has been pointed out by Adami. The latter form, according to Adami, is more or less an attempt on the part of the organism to form resistant bodies, and is the condition in which the organism appears in the tissues. Cultures from internal organs which on section

show no bacilli but only a profusion of coccoid and diplococcoid bodies, from secretions like ascitic fluid which show the same bodies, will invariably grow out after a lapse of 24 hours into a typical colon bacillus. The same may be said of the long straight forms which are often encountered in the fluid from the gall bladder. These, like the diplococcoid bodies, invariably produce bacilli differing in no particular from the typical colon in morphology and belonging to some pure type of this species.

The question of motility has been disputed by different observers and the colon has been said at times to possess and at other times not to possess this character. Examined in a 24-hour fluid culture, the organisms classed as colon or allied forms, have never failed to show active motility. The velocity of the colon is not as great as that of many motile bacilli but its appearance is unquestionable. Despite the fact that Theobald Smith, in determining the position of an organism which in its cultural features corresponded to the hog cholera bacillus but which lacked motility, unhesitatingly placed it with the hog cholera group, according to Fuller and Johnston permanent absence of motility must be considered as a radical departure from the pure colon type.

The production of gas in carbohydrates must be studied only with sugars which have been sterilised in the steam steriliser—for the pressure and temperature of the autoclave are sufficient to break down saccharose and lactose into the simpler dextrose and by-products. It is essential in studying the colon that these three sugars be used, as certain varieties ferment one and not the others ; only the typical colon and its near allies fermenting all three carbohydrates.

The test for indol has never yielded a satisfactory result. Only broth which has been rendered free from sugar by the previous growth in it of a fermenting bacillus can be utilized for this purpose, for it has been shown by Theobald Smith that a small amount of carbohydrate will inhibit the formation of indol. Even with this precaution the different varieties of colon seem to produce this substance in a most unreliable fashion, some forms only producing it when fresh from the intestine. Probably the only possible way of estimating this character with accuracy will be to utilize large quantities of sugar-free broth which after an incubation of 15 to 20 days may be distilled and the distillate tested for indol and by-products. In this way Dr. Bruere of the Royal Victoria Hospital is at present endeavoring to ascertain the differences between the various paracolibacillary forms.

The perception of a fæcal odor, similar to the test for indol, does not give always that reliability which is desirable in the estimation of a constant character. Like all tests which depend on the sense percep-

tion, of smell, which differs so largely in different individuals, the fæcal odor can only be considered as a doubtful reaction.

The production of nitrites may be estimated in connection with the routine tests for indol by sulphuric acid, when one uses a broth which does not itself contain traces of nitrites. A more reliable method, however, since ordinary broth or sugar-free broth contains this substance, is to utilize a Dunham's solution made from a peptone free from nitrites, or the so-called nitrate broth containing a small percentage of potassium nitrate and a little peptone. In either case the organism by its growth may directly reduce the nitrates to the nitrite condition, or, breaking up the peptone, oxidise the nascent nitrogen to a nitrate. The test may preferably be made with nitrate broth, using Dunham's solution as a control.

The inoculation of the colon bacillus and its allies on agar tubes, the reaction of which varied between an acidity of 1.5° and an alkalinity of 6.0, revealed a fairly constant law in regard to the profusion of growth. The colon grows most abundantly on agar which is slightly acid or of an acidity of 1.5°, the growth on neutral agar being as a rule slightly less. As one passes from the neutral to the alkaline agars the abundance of growth rapidly diminishes—an agar of an alkalinity of 1.5 giving a less visible growth than the neutral, 3.0 agar only a faint growth, 4.5 agar only traces, while an agar of an alkalinity of 6.0 inhibits the growth entirely.

All of the organisms belonging to the colon, typhoid or intermediate groups which we have worked with may be divided into a certain number of types which are represented on the chart. For convenience of description only, they have been arranged in certain orders, not that they necessarily have this place in nature. The different varieties of pure colon, if we may use the phrase, have been divided into, Colon A., B. ,C., D. and E.

Under Colon A., have been included all those forms which correspond to Fuller and Johnston's table of characters. These colons ferment all the sugars, produce indol, nitrites and a fæcal odor ; grow luxuriantly on potato and produce a scum on broth. They are pathogenic in all cases to mice by intraperitoneal inoculation. As a rule they grow most luxuriantly on neutral agar.

The second type, named Colon B., differs from the first in never producing a pellicle on broth. It produces indol and a fæcal odor, grows most luxuriantly on agar of an acidity of 1.5° and is pathogenic. While the characters separating this type from the previous one seem so slight as not worthy of making a separate class, yet the confusion of statements in the literature about the colon at times produc-

ing a definite scum on broth, and at other times not, and the experience in the Molson laboratory that the form of organism which produced a scum always did so, and the form not producing such a scum invariably failed to do so—has led us to formulate these two types. In fact, the majority of bacilli which were encountered in Montreal correspond to type B. rather than type A., but for uniformity of description Fuller and Johnston's colon has been considered the model form.

Between Colon B. and the next type C. may be most conveniently placed those forms which have lost their pathogenicity. This doubtless may occur with any variety of colon under unfavourable conditions of growth and cannot be said to constitute a different variety. This loss in pathogenicity was encountered in only one form, a variety obtained from Dr. Harris in Baltimore. All other varieties of colon, with one exception to be mentioned later on, were pathogenic in intraperitoneal inoculations.

If we combine loss of pathogenicity with loss of motility we have a form which is identical with B. Lactis aërogenes of Escherich, not producing indol or a fæcal odor, but agreeing in its other reactions with B. Coli Communis. As has been indicated above, these differences should suffice to make this form a distinct variety, separated as it is by constant unvarying characters.

Under Colon C., have been included those forms which agree with the typical colon in most of its reactions, but differ in the fermentation of the sugars. Dextrose and lactose are fermented, saccharose never. A scum is produced on broth, indol is formed and a fæcal odor exudes from the cultures. This variety also has been encountered a number of times.

Colon D. includes bacilli similar in all respects to the preceding, in respect of the fermentation of lactose and dextrose, but like Colon B., the corresponding first derivative from Colon A., it fails to produce a definite pellicle on broth.

A further derivative of the pure colon is that form which produces fermentation with dextrose and saccharose but not with lactose. It has been described frequently by earlier writers and is a well recognized variety of B. Coli Comm. In our case it was not pathogenic, did not produce indol, nitrites, or a fæcal odor, but otherwise was identical with the prototype Colon A.

As we pass from these varieties of paracolibacillary organisms which ferment two of the three sugars, to those which ferment but one, namely dextrose, we enter upon the intermediate group of Gærtner, or the Hog Cholera group. The first form which is encountered here, which goes by the name of paracolon, is the organism of Cushing called by

him Bacillus O. or by other authorities, Paracolon Cushing. It may well be called Paracolon A., and may serve as the introduction to the intermediate group. Isolated by Cushing from an abscess over a rib, this form possesses characters intermediate between typhoid and colon. It grows very slowly on potato, giving a visible growth only after several days incubation; it ferments dextrose, not lactose and saccharose; it does not coagulate milk or produce acidity; it produces indol after the lapse of a number of days, has a fæcal odor and is pathogenic to mice.

With the paracolon of Cushing may be grouped B., variety Hatton, of Durham and B. Morbificans Bovis of Basenau, which isolated from far different sources by different observers agree in so many details as to be grouped by Durham in this intermediate group, all the members of which are pathogenic. Included in the same group are the so-called "icteroides" isolated by Sanarelli and Reed from yellow fever patients. These bacteria differ from the preceding only in the formation of a moist luxuriant growth on potato, fermenting only one sugar—dextrose—as the other forms do. Associated with these icteroides we have placed a further variety which has been called Paracolon B. It was obtained from the liver of a healthy rabbit and was originally considered to be a simple colon derivative. It agrees in all cultural features with the organisms of Reed and Sanarelli, and without doubt belongs to this group—failing to ferment lactose and saccharose and failing to produce acidity in, or to coagulate milk.

Among the intermediate members of this group should doubtless be included paracolons isolated by Widal and Gwynn. The description given by Widal is too meagre to furnish a means of classification but the work of Gwynn and of Cushing on the paracolon isolated by the former shows clearly the place this bacillus should occupy. From Gwynn's description has been compiled the life history of this bacillus and it has in this way been included in the chart. It is actively motile, grows on bouillon as a distinct cloud, not forming a pellicle, acidifies milk faintly, without coagulating, gives a luxuriant growth on potato and does not liquify gelatine. It ferments dextrose, not lactose or saccharose and it does not produce indol.

The next member of this group is the B. Choleræ Suis, which is identical with the others in the main characters, yet liquifies gelatine and blood serum. Because of these characters it has been placed last in this group and has been associated with the B. Cloacæ which is yet more positive in liquifying gelatine, casein and blood serum. The latter produces indol, nitrites and fæcal odor, has a luxuriant growth on potato and produces a scum on broth. Naturally it does not belong

to this intermediate group and has only been mentioned here for purposes of comparison with B. Choleræ Suis.

Intermediate between colon and typhoid but approaching more to the type of the Eberth's bacillus, are a number of little known and little studied forms of paratyphoid. The only organism which we have encountered which seems to belong to this group is one we have provisionally named Paracolon C. It was obtained from the liver of a healthy rabbit and is evidently allied more to typhoid than to colon, forming a faint growth on potato but not producing indol or a fæcal odor, not coagulating or acidifying milk, nor fermenting the carbohydrates. It seems to be closely related to the form isolated by Flexner from cases of dysentery, in association with the amœba coli, agreeing with it in its fundamental characteristics. It, like Flexner's organism, should doubtless be classified as a para-typhoid. It corresponds, as far as can be told, with other varieties of intestinal bacteria described heretofore, especially the non-fermenting varieties of Widal and Gilbert.

The B. Typhosus stands next for purposes of comparison. This organism is too well known to need any further words, but its place in this chart is justified by reference to its invisible growth on potato, its failure to produce gas or to coagulate milk and its being non-pathogenic for mice under ordinary laboratory conditions.

The next organism in our series of paracolibacillary forms, which is named Paracolon D., provisionally, has been isolated in two instances, from the stomach of a healthy man in one case and, again, from a typhoid spleen. It is the furthest removed from both the colon and typhoid type and represents a variation from the colon in not fermenting any sugar, not producing nitrites, indol, or a fæcal odor, in not growing on potato, not being pathogenic, and in liquifying gelatine. In morphology it is a fine short bacillus, barely distinguishable from a micrococcus and its especial characteristic is the growth on slightly alkaline agar as a faint film which after a lapse of 48 hours seems indistinguishable from the substratum of medium. While it may be identical with other intestinal bacteria described before, yet we have not come upon any similar organisms recorded in the literature and we are therefore inclined to look upon it as a new species. It has been named provisionally Paracolon D.

From the study of the organisms given in the chart, one is convinced that while a proper biological classification of bacteria is still as far as ever from attainment, yet much may be done by this graphic method in illustrating the relations which the different intestinal bacteria bear to each other. With one or two exceptions the bacteria described here are well known forms which have already been reported. By means of

such a table of constant characters it becomes possible however, to fill in the gaps of our knowledge concerning intestinal bacteria, and adopting standard reactions in our descriptions, by means of a comparison of results in different laboratories, to arrive at a more and more complete knowledge of the colon and its allies the paracolibacillary organisms.

I wish to express my thanks to Dr. Adami, under whose supervision the work reported in this paper has been carried out, and to Dr. Harris of Baltimore, who has furnished me with a number of cultures from the Johns Hopkins Pathological Laboratory.

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Type.	Name.	Investigators.	Source.	Morphology.				Cultutral Features.									
								Broth.		Agar.		Gelatine Plate.	Potato.		Fermentation Tube.		
				Bacillus.	Diameter greater than 1 micromillimeter.	Motile.	Spores.	Semm.	Turbidity.	Dull.	Wrinkled.	Characteristic appearance.	Visible.	Luxuriant.	Growth in closed arm.	Grows at body temperature	
Colon A....	B. Coli Com	Escherich	Normal Intestine ..	+	—	+	—	+	+	—	—	—	+	+	+	+	
Colon B....	B. Coli Com	Ford	Stomach Pernicious Anæmia	+	—	+	—	—	+	—	—	—	+	+	+	+	
	B. Coli Com	Harris....	?	+	—	+	—	—	+	—	—	—	+	+	+	+	
	Lactis Aerogens...	Escherich	Normal Duodenum	+	—	—	—	+	+	—	—	—	+	+	+	+	
Colon C ..	B. Coli Com	McCrae ..	Faces	+	—	+	—	+	+	—	—	—	+	+	+	+	
Colon D....	B. Coli Com	Ford	Typhoid Spleen...	+	—	+	—	—	+	—	—	—	+	+	+	+	
Colon E....	B. Coli Com	Ford	Kidney, Rabbit...	+	—	+	—	—	+	—	—	—	+	+	+	+	
Paracolon A	Paracolon Cushing.	Cushing ..	Abcess Rib.....	+	—	+	—	—	+	—	—	—	+	—	+	+	
	Paracolon Gwynn..	Gwynn ...	Abscess of Neck...	+	—	+	—	—	+	—	—	—	+	+	+	+	
	B. Hatton	Durham ..	?	+	—	+	—	—	+	—	—	—	+	—	+	+	
	B. Morb. Bovis....	Basenau ..	?	+	—	+	—	—	+	—	—	—	—	—	+	+	
	Icteroides	Sanarelli .	Yellow Fever Patients	+	—	+	—	—	+	—	—	—	+	+	+	+	
	Icteroides	Reed	Yellow Fever Patients	+	—	+	—	—	+	—	—	—	+	+	+	+	
Paracolon B.	Paracolon B.....	Ford	Liver, Rabbit.....	+	—	+	—	—	+	—	—	—	+	+	+	+	
	B. Cholera Suis ...			+	—	+	—	—	+	—	—	+	—	—	+	+	
Cloacæ	B. Cloacæ	Jordon ...	Sewage	+	—	+	—	+	+	—	—	—	+	—	+	+	
Paracolon C.	Paracolon C.....	Ford	Liver, Rabbit.....	+	—	+	—	—	+	—	—	—	+	—	—	+	
Typhosus...	B. Typhosus.....	Eberth ...	Typhoid Lesions..	÷	—	+	—	—	+	—	—	—	—	—	+	+	
Paracolon D	Paracolon D	Ford	Stomach and Typhoid Spleen..	+	—	+	—	—	+	—	—	—	—	—	—	+	

Note.—In preparing the chart which is appended to this paper the special varieties have been ascribed to the investigator who first described them. gator" has been placed simply the name of the individual from whom the culture used was obtained. The reactions of all the media employed are estima

BIOLOGY.																														
Features.				Biochemical Features.																								Pathogenicity.	
																												Mice.	
zine te.	Potato.		Fermentation Tube.	Grows at body temperature.	Facultative anaerobe.	Affected by range of reaction.	Liquifaction.			Gas Production.			Nitritis.	Indol	Milk.		Fæcal Odor.	Agar.			Intraperitoneal Inoculation.	
pearance.	Visible.	Luxuriant.	Growth in closed arm.				Gelatine.	Casein.	Blood Serum.	Dextrose.	Saccharose.	Lactose.			Acidity.	Coagulation.		Chromogenesis.	Fluorescence	Most favorable reaction.		
	+	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	+	—	—	0	+	
	+	+	+	+	+	+	—	—	—	+	+	+	—	+	+	+	+	—	—	+ 1.5	+	
	+	+	+	+	+	+	—	—	—	+	+	+	—	+	+	+	+	—	—	+ 1.5	—	
	+	+	+	+	+	+	—	—	—	+	+	+	+	—	+	+	—	—	—	?	—	
	+	+	+	+	+	+	—	—	—	+	—	+	—	+	+	+	+	—	—	+ 1.5	+	
	+	+	+	+	+	+	—	—	—	+	—	+	—	+	+	+	+	—	—	+	—	
	+	+	+	+	+	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+ 1.5	+	
	+	—	+	+	+	+	—	—	—	+	—	—	—	+	—	—	+	—	—	+	+	
	+	+	+	+	+	+	—	?	?	+	—	—	?	+	—	—	?	—	—	?	?	
	+	—	+	+	+	+	—	—	—	+	—	—	—	+	—	+	—	—	—	+	+	
	—	—	+	+	+	+	—	—	—	+	—	—	—	+	—	+	—	—	—	0	+	
	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—	—	+	—	—	0	+	
	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—	+	—	—	—	0	+	
	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—	—	—	—	—	+ 1.5	+	
	—	—	+	+	+	+	+	—	+	+	—	—	—	—	—	+	+	—	—	0	+	
	+	—	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	—	—	?	+	
	+	—	—	+	+	+	—	—	—	—	—(?)	—(?)	—	—	—	—	—	—	—	?	?	
	—	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	
	—	—	—	+	+	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	

igator who first described them. In separating the different varieties of colon, however, this has been found impossible, hence under the division of " Investi-
all the media employed are estimated according to the neutrality to Phenol Pthalein.