STUDIES ON INTESTINAL BACTERIA

by

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PREFACE

This thesis records the results of a series of investigations concerning certain bacteria belonging to the normal or the pathological flora of the intestine; these studies were continued over several years including the period 1915-1918 during the Great War when I was engaged in bacteriological research and routine in the Mediterranean and Egyptian Expeditionary Forces.

The studies are collected here into five main parts.

The first part deals with certain normal inhabitants of the intestine -- the B. coli group -- and an account is given of a series of biological investigations of B. coli and its congeners , with special reference to their serological characters, mutations , and biological classification.

The other parts refer to specific intestinal pathogens.

Part 2 records certain observations on the group of dysentery bacilli and the bacteriology of bacillary dysentery both from the biological and pathological aspects; and in part 3 an account is given of a group of intestinal vibrios associated with choleraic and diarrhoeal conditions -- the so called paracholera vibrios. The studies concerning the paratyphoid bacilli (parts 4 and 5) were mainly with reference to the bacteriological diagnosis of enterica infections , but also

include observations of particular bacteriological and serological interest.

Special attention has also been devoted to the technique appropriate for the study of these intestinal organisms and the importance of certain methods is emphasized in the thesis.

In part I full records and protocols of experiments are included; in parts 2 and 3 it has been impossible to quote certain records in full detail as all my detailed laboratory notes on enterica, dysentery and vibrio infections were lost at sea in 1918 due to the sinking by submarine of the transport on which I was travelling from Egypt. It has been possible, however, to include the most important data and numerical records from papers published by me while in Egypt and from official reports and summaries of results at present in my possession.

A number of the observations included in the thesis have already been recorded in published papers; certain of these were dealt with in personal communications, others in joint publications. I was personally responsible for those observations incorporated in the thesis which have appeared in papers published in collaboration with other workers on the same subject.

The following are the publications referred to:

The immunity reactions of the coli group---- by Mackie; Journ. of Path. and Bact.,vol.I8,I9I3,p.I37;also in Applied Bacteriology Browning,I9I8,p.202.

Variation in agglutinability of bacteria associated with variation of cultural characters---by Mackie; Brit.Journ. of Exper. Path.,vol.I,no.4,I920,p.2I3.

The gram-negative bacilli found in pyogenic infection of the urinary system--- by Mackie;Trans. of Medico-chirurg.Soc.Glasg., vol.I2,I9I3,p.II9.

The atypical dysentery bacilli---by Mackie;Journ. of Hygiene, vol.I8,no.I,I9I9.

Clinical and laboratory researches on dysentery in Egypt with some remarks on sanitation--- by Thomson and Mackie;Journ. of R.A.M.C.,vol.28,I9I7,p.403.

A note on the modes of infection in bacillary dysentery---by Cowan and Mackie;Journ. of R.A.M.C.,vol.32,no.3,I9I9,p.209.

Report on the incidence and nature of the dysentery in the Egyptian War Zone in I9I6 with bacteriological reports---- Medical Advisory Committee,G.H.Q.,M.E.F.,I9I7.

Two vibrio species of the paracholera group associated with a cholera- like outbreak--- by Mackie and Storer;Journ. of RAMC., August,I9I8.

The brilliant-green enrichment process for the isolation of paratyphoid bacilli from faeces--- by Mackie; Journ. of RAMC., vol. 29,I9I7,p.475.

The diagnostic interpretation of the agglutination test in typhoid and paratyphoid infections occurring among typhoid inoculated troops---by Mackie and Wiltshire; Journ. of RAMC., vol.29,1917,p.276.

The diagnosis of enterica infections by bacteriological and serological methods--- by Browning,Mackie and Thornton,Applied Bacteriology,Browning,1918,p.9.

Reference to the classification of B.coli types was also made in the following papers:

The isolation of typhoid bacilli from faeces by means of brilliant green in fluid medium--- by Browning,Gilmour and Mackie; Journ. of Hygiene,vol.13,1913,p.335.

The isolation of B.typhosus from faeces by the use of potassium tellurate along with brilliant green in fluid medium----- by Browning,Mackie and Smith; Journ. of Path. and Bact.,vol.19, 1914,p.127.

References to literature bearing on the particular subjects discussed are included at the end of the respective parts under the various authors quoted in the text.

The thesis was written in 1920.

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P A R T I.

A Study of the

B. COLI GROUP

with special Reference to

the Serological Characters of

these Organisms.

Introductory discussion: the classification of " coliform " bacilli and the biological relationships of the B. coli group to other gram-negative aerobic intestinal bacilli.

In 1885 Escherich recorded the isolation of a gram-negative intestinal bacillus which he designated B. coli communis and this classical type has been long considered the prevalent intestinal bacterium. In general this organism has been described as a motile, non-sporing, aerobic, facultatively anaerobic, gram-negative bacillus, which grows at 37° C., ferments glucose, lactose and dulcite with acid and gas production, forms indol from peptone, clots milk, reduces nitrates, and grows in gelatin media without producing liquefaction.

With the advance of bacteriological knowledge and as a result of extensive investigations on the bacteriology of faeces, water, milk, etc., this original type has come to represent only one of a considerable bacterial class or group. The organisms of this group, however, are characteristic normal inhabitants of the animal intestine and are therefore conveniently designated by the generic term B. coli.

Their pathological importance has been well established in virtue of their potentially pathogenic properties, and, in the bacteriological diagnosis of intestinal infections, their precise recognition and the separation and differentiation of specific pathogenic organisms from them have necessitated

the most careful study of their biological characters. Moreover their occurrence in water supplies has led to an extensive study of the whole group from the point of view of sewage contamination and the importance of certain types as indicators of such contamination has been emphasized. Thus, the detection of these organisms constitutes an essential method in the routine examination of water supplies. This group of bacteria is also concerned in the process of souring of milk and certain of the classical types were originally isolated from this source, e.g., B. acidi lactici (Hüppe).

While the whole group of organisms biologically allied to Escherich's original type has been classified as B. coli, the question as to which varieties represented " typical " B. coli, as apart from " atypical " forms, has been studied in connection with the bacteriology of water (Houston and others). Much confusion has resulted, however, as regards the definition of a typical B. coli owing to the different identification standards set up by various observers. Some of these definitions have been too limited in their application and different degrees of importance have been attached to different characters. (Savage, Prescott and Winslow, Konrich, Reports of the English and American Committees on standard methods for the examination of water).

While there has been considerable variation in the criteria adopted by different workers for the identification of

the colon bacillus, the characters generally accepted as common to the whole group of " B. coli " may be summed up as follows:- gram-negative, aerobic, facultatively an-aerobic, non-sporing bacilli growing at 37° C., and fermenting glucose and lactose. Other characters, e.g., different fermentative reactions, motility, indol production, Voges and Proskauer reaction, simply determine the differentiation of separate types. Thus, the term " lactose-fermenter " has been frequently used as a convenient designation for the group (MacConkey).

The question arises as to whether gelatin-liquefying, gram-negative bacilli which correspond in other characters to B. coli types are to be included in this biological group. Among the lactose-fermenters classified by MacConkey certain gelatin liquefiers were represented, e.g., B. cloacae, B. oxytocus perniciosus, and other authorities (Prescott and Winslow) have accepted this reaction as one of the possible characters of the group. In my own experience of a large number of coliform strains from faeces, urine, etc., and also water (Egypt 1915-1918) I have never met with gelatin-liquefying, gram-negative, aerobic, non-sporing bacilli capable of growing at 37° C., apart from those referable to the B. proteus type.*

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* B. proteus: gram-negative, motile, aerobic, non-sporing, bacilli, growing well at 37° C., showing a tendency to " spreading " type of growth, fermenting glucose with or without gas, not fermenting lactose, dulcite or mannite, varying in fermentation of saccharose and in indol formation, liquefying rapidly gelatin or solidified serum.

While the characteristic B. coli has the property of fermenting lactose, it is doubtful (as will be shown later, p. 43) if lactose fermentation can be regarded as a specific character of a particular group of intestinal bacilli and studies on the variation of these organisms (v.p. 55) have shown how certain types only acquire this property by mutation on culture medium.

Thus non-gelatin-liquefying, glucose-fermenting, non-lactose-fermenting bacilli of intestinal origin have to be considered in any system of classification of the gram-negative intestinal bacilli.

From our present knowledge of the aerobic intestinal bacilli it would appear more rational from the purely biological standpoint to recognise a large class of intestinal bacilli having the common characters: aerobic, gram-negative, non-sporing, growing at 37° C., fermenting glucose with or without gas production, not liquefying gelatin; comprising certain specific pathogenic organisms specially designated, and identified by cultural and serological characters, e.g., B. typhosus, etc., and a large number of saprophytic, though potentially pathogenic, varieties which can be generally classified into different types according to their cultural characters. The term B. coli (if it is to be used at all) in its widest application would thus become referable to those members of the class which do not possess <u>specific</u> pathogenic

properties irrespective of certain cultural characters including lactose fermentation. The term " typical B. coli " would be used, if necessary, to designate those types (fermenting lactose, clotting milk, and producing indol) which are most prevalent in the intestine and therefore undoubted indicators in water of recent sewage pollution. The only justification for recognising a special group of " lactose-fermenters " depends on the statistical fact that these types are most prevalent in fresh animal excreta but it is questionable if the statistical basis can be used for a biological classification.

The further question arises as to the significance of gelatin liquefaction and whether a wider definition of the class is required, i.e., to include B. proteus. It must be admitted that with the exception of gelatin liquefaction, types of B. proteus may correspond closely to certain non-liquefying intestinal bacilli.

Organisms of the B. faecalis alkaligenes type have also to be considered in regard to the classification of the gram-negative intestinal bacilli.* B. faecalis alkaligenes has been recognised as differing from the motile organisms of the coli-typhoid class in the " terminal " position of its

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* In Jensen's classification (v. infra) B. faecalis alkaligenes is grouped along with the other gram-negative intestinal bacilli.

flagella as opposed to the " lateral " arrangement in the case of B. coli and B. typhosus. (Berghaus, Klimenko). While this is true for my own observations of these organisms, non-motile, gram-negative, aerobic bacilli which do not ferment glucose or any other sugar have been frequently noted in dysenteric stools, and strains of this type have been found which, on first isolation, did not ferment glucose and only gained this property after a period of growth in a glucose medium. This might appear to link up organisms of the B. faecalis alkaligenes type with the sugar fermenting intestinal bacilli.

The " lactose-fermenters " have been the subject of much careful study: originally different classical types had been separately described, e.g., B. coli communis, B. neapolitanus (Emmerich), B. acidi lactici (Hüppe), B. lactis aerogenes (Escherich), etc., and these names may still be retained for organisms which accurately correspond to such classical strains.

The work of MacConkey on the lactose-fermenting coliform bacilli represented the first attempt in the direction of a rational system of classification of these organisms.

A number of cultural tests had been commonly employed which were shown by him to be of little differential value and he further established the importance of a selected though comprehensive series of biochemical reactions as type criteria.

MacConkey (1905) in his first paper dealing with this subject arbitrarily divided the lactose fermenters into four sub-groups according as they did or did not decompose saccharose and dulcite. The first group represented by the classical B. acidi lactici included those which fermented neither dulcite nor saccharose; the second included those which fermented dulcite but not saccharose, e.g., B. coli communis; the third comprised types such as B. neapolitanus which fermented both dulcite and saccharose; and the fourth consisted of strains which fermented saccharose but not dulcite.

This classification was of course entirely arbitrary and incomplete.

In 1909 MacConkey reviewed the whole subject and indicated that if, in addition to the fermentation of dulcite and saccharose, further tests were added, - effect on adonite and inulin, presence of motility, indol production, the Voges and Proskauer reaction,-theoretically 128 possible varieties could be differentiated. At that time he had examined 497 strains from human and animal faeces, water, etc., and of the 128 possible types had met with 36 varieties differentiated according to their action on (1) gelatin, (2) dulcite, (3) saccharose, (4) adonite, (5) inulin, in some instances, (6) inosite, and by (7) the presence or absence of motility, (8) indol production and (9) the Voges and Proskauer reaction.

MacConkey had at the same time tested a number of other fermentable substances which had been commonly employed, but indicated that no further information was to be obtained by the use of sugars, etc., such as galactose and laevulose on which the various sub-groups had all the same effect; and in the case of quercite and erythrite found that practically none of his strains had any fermentative action. Out of 497 strains examined, 178 were from human faeces and of the various types noted the most prevalent were type No. 71, B. coli communis, and B. vesiculosus (see table I).

MacConkey's system of classification has been supported and adopted by Bergey and Deeham, Clemesha and others, and modified by Jackson who employed the reactions in mannite and raffinose as further differential characters.

Howe, on the other hand, claimed that motility, indol formation, mannite and dulcite fermentation were of little value for classification owing to the fact that, from the statistical point of view, these reactions showed no correlation with one another or with other criteria. Prescott and Winslow have also urged the value of the statistical basis for a biological classification and that the characters of these organisms should be considered not independently but in relationship to one another.

Kligler emphasized the importance of salicin fermentation for differential purposes and elaborated a comprehensive system

of classification of dextrose-fermenting intestinal bacilli; he subdivided these organisms first according to their action on lactose and the further classification depended mainly on the reactions in dulcite, saccharose and salicin; he included among the various sub-groups B. proteus and B. cloacae (gelatin liquefiers).

An older system of classification which is of interest to consider briefly is that of Jensen who on the basis of certain simple fermentative reactions arbitrarily divided the whole coli-typhoid group into several main sub-groups; the criteria were the reactions in glucose, lactose, saccharose and maltose, and in this way the following organisms were differentiated: B. faecalis alkaligenes, B. " metacoli," B. typhosus, B. paracolon, B. coli anaerogenes, B. coli (2 types) and B. " pseudocoli;" this system was later amplified, for the differentiation of coliform bacilli, by Wulff who employed certain additional tests: galactose, glycerin, adonite, mannite, dulcite and xylose fermentation.

This system of classification cannot be considered as sufficiently complete; thus no cognisance was taken of indol production and the fermentation of inosite. As will be shown later (v.p. 41) these reactions are important and characteristic features of certain B. coli sub-groups.

Houston in connection with the bacteriological examination of water has classified coliform bacilli into

" typical " and " atypical " varieties (v. supra) and further divided the typical organisms according to the fermentation of dulcite and saccharose.

While different systems of classification have thus been adopted which in the hands of various workers have served a practical purpose in enabling them to recognise typical varieties as apart from those which are less characteristic, it can hardly be claimed that these organisms have yet been completely classified nor that the significance of different characters has been accurately assessed.

GENERAL OUTLINE OF THE INVESTIGATION.

In the observations to be recorded, a further study has been made of the " B. coli " group and the characters of a large number of strains of gram-negative, aerobic, non-sporing, glucose-fermenting, non-liquefying bacilli (excluding the specific pathogenic organisms of this class) have been investigated.

The question of the biological classification of these organisms has also been studied from the serological aspect.

In addition certain observations have been made with regard to variations among the coliform bacilli and their biological significance.

The various strains were isolated from pathological specimens of urine, faeces, appendix abscesses, etc.

CRITERIA EMPLOYED FOR DIFFERENTIATION OF TYPES.

In classifying coliform bacilli into particular types, MacConkey's criteria were for the most part adopted and for all practical purposes proved sufficiently complete. Raffinose and salicin fermentations were, however, found to yield further differentiation and these tests were, therefore, included among the criteria adopted.

Certain other fermentable substances which have been employed by different workers proved of little or no differential value; thus maltose was found to be fermented by all the lactose-fermenting strains; glucose-fermenting, non-lactose-fermenting strains, however, vary in their action on this sugar. The same was found to be true for mannite. Laevulose and galactose were with few exceptions fermented by all the glucose-fermenting strains, and glycerin by all the lactose-fermenters. Of course, as is well known all lactose fermenters are also glucose fermenters. The behaviour of these bacilli in raffinose in the majority of cases corresponded to their effect on saccharose (as shown also by Winslow and Walker); but a certain proportion exhibited differences in their effects on these sugars.

The action of the lactose fermenters on dextrine differs from the fermentation produced in other carbohydrates: in the case of simple peptone water media containing dextrine and

neutral red (as indicator) it was found that after 24 hours incubation the neutral red became of a bright yellow colour and there was some degree of gas production; the gas formed was analysed and found to consist mainly of hydrogen. The medium remained neutral. This effect was common to all the lactose-fermenting types and the test afforded no information as regards differentiation of various strains.

With one exception all the strains that fermented inosite also fermented adonite. In all cases the Voges and Proskauer reaction was only given by inosite-fermenting strains.

It was found on repeated testing of various strains that the presence (or absence) of motility was a definite and constant character.

Similarly the presence (or absence) of indol in peptone water cultures (after 10 days at 37° C.) proved to be a stable property of these organisms.

It was concluded therefore that of the various tests used by different workers, the most complete set of criteria for the differentiation of B. coli types were:-

(1) presence or absence of motility, (2) production of indol, (3) the Voges and Proskauer reaction, the fermentation of (4) glucose, (5) lactose, (6) dulcite, (7) saccharose, (8) adonite, (9) inulin, (10) inosite, (11) raffinose, (12) salicin.

The majority of the strains to be described were retested after about two or three months and apart from the variations noted (p. 56-57) their characters proved markedly stable.

METHODS.

Motility: in determining the presence or absence of this character a hanging-drop preparation from a 4-6 hours bouillon culture, or the " condensation " fluid of a sloped-agar culture of the same age, was examined. Tested in this way the presence (or absence of motility) proved to be a definite character.

Liquefaction of Gelatin: this was tested for by making stab inoculations in ordinary nutrient gelatin and incubating the tubes at 22° C. for two weeks.

Indol production: the presence or absence of this property was determined by testing a ten days peptone water culture of the strain in question with Ehrlich's reagents: paradimethylamidobenzaldehyde and persulphate of potassium, according to the usual procedure.

Voges and Proskauer reaction: for this test a 2% peptone water solution containing 1% glucose was inoculated, and incubated at 37° C. for 3 days, when a solution of potassium hydrate was added and the tube allowed to stand at room temperature for several hours. A positive reaction was indicated by the development of a red fluorescence.

Fermentation Tests: the basis of the medium used for testing sugar fermentations was a 2% peptone solution with 0.5% sodium chloride. To avoid the possible decomposition of the sugar in the medium by overheating in the process of sterilization, the different fermentable substances were added to the already sterilized medium in the form of sterile watery solutions. The proportion of sugar in the medium was 1%; neutral red (0.25% of a 1% watery solution) was added as an indicator of acid production. The medium was distributed in Durham's tubes (for the observation of gas production) and placed at 100° C. in the Koch's sterilizer for 10 minutes on two successive days. This short final sterilization while not acting deleteriously on the sugar was sufficient to ensure complete sterility.

I have observed that for the proper appreciation of gas production, fluid media must be used, as shake or stab cultures in solid agar media are open to fallacy. With certain specimens of peptone-water-agar (without meat extract), it was found that gas production might result on inoculation with B. coli, i.e., even in the absence of sugar, and this was especially marked if the agar was not freshly prepared.

Thus, a shake culture of B. coli communis made in peptone water agar immediately after preparation showed no gas production in the medium; the same medium a week later was again inoculated and on this occasion a considerable number of gas bubbles appeared in the medium after 24 hours incubation;

a fortnight later inoculation resulted in an abundant production of gas throughout the medium. No acid formation was however noted.

Litmus has been frequently used as an indicator of acid formation, but with this agent, especially when incorporated in nutrient medium, difficulties have frequently arisen owing to the many intermediate degrees of colour between unequivocal alkalinity on the one hand and undoubted acidity on the other. It has the further disadvantage, as I have noted, of being decolorized rapidly by many organisms of the coli group. In my own experience neutral red is a more valuable indicator as it is not subject to these disadvantages.

SYSTEM OF CLASSIFICATION AND DESIGNATION ADOPTED.

Among the coliform bacilli studied, it seemed possible to classify into separate sub-groups types of organisms having well defined common characters.

Thus the different types which were characterized by the absence of gas production in the case of all the sugars fermented even after repeated subculture (coli anaerogenes) were classified in a separate sub-group. Serological

investigations (v. infra) in which the group action of the complement-deviating antibody of immune sera to certain more typical B. coli varieties showed that the coli anaerogenes organisms were not closely related to the gas producing types of B. coli.

It was also noted that certain types of coliform bacilli which produced on culture medium unusually large, thick, opaque, slimy and sometimes " viscid " colonies were all characterized by possessing the power of fermenting inosite, while the usual B. coli varieties had not this property. These organisms were, therefore, grouped together and classified apart from the more typical coliform bacilli. All these varieties proved to be non-motile, all fermented lactose, saccharose, raffinose and salicin, and with one exception adonite; in this sub-group, therefore, several characters were definitely correlated. The separation of these organisms from the typical coliform bacilli was also found to be justified by serological tests as in the case of the anaerogenes sub-group.

It was further concluded from the serological studies referred to above and dealt with in detail later that a sub-group comprising all the " typical B. coli " could be recognised whose common characters were: gas-producing, indol-forming, non-inosite-fermenting.

After defining these three sub-groups, there still remained the organisms having the common characters: gas-producing, non-inosite-fermenting, non-indol-forming. Serological observations showed that this category did not represent a serologically homogeneous sub-group, but for purposes of a preliminary classification I have grouped all the organisms of the category together.

Thus, four main sub-groups of the gram-negative, glucose-fermenting, non-gelatin-liquefying, intestinal bacilli may be recognised and this system of classification has been used in the following records. In addition, the various types belonging to these sub-groups have been designated numerically (i.e., 1, 2, 3, 4, etc.) in order of their prevalence in the series investigated. In the B category the lactose-fermenters have been distinguished from the non-lactose-fermenters by a different series of numbers, 1 to 11 and 101 to 107. The latter class includes certain " paracolon " types which in the serological tests appeared to be differentiated from organisms fermenting lactose in primary culture.

246 Strains from urine (pathological specimens), faeces and other sources have been carefully studied and classified in this way (tables 2, 3, 4, 5). In table 6, for comparison, those strains which correspond to MacConkey's types are classified according to his system.

TABLE 1. (quoted from MacConkey 1909).

Type (MacConkey's Classification)	Number of Strains	Motility	Lactose	Dulcite	Saccharose	Adonite	Inulin	Inosite	Indol	Voges and Proskauer Reaction	Gelatin
No.1	9	+	+	—	—	+	—	—	+	—	—
B. acidi lactici (Hüppe), No.2	1	—	+	—	—	+	—	—	+	—	—
B. Grünthal, No.4	8	+	+	—	—	—	—	—	+	—	—
B. vesiculosus, No.5	33	—	+	—	—	—	—	—	+	—	—
No.7	1	+	+	—	—	—	—	—	—	—	—
B. coli mutabilis, (Massini), No.8	0	—	+	—	—	—	—	—	—	—	—
No.33	-	+	+	+	—	+	—	—	+	—	—
B. coli communis, No.34	37	+	+	+	—	—	—	—	+	—	—
B. Schafferi, No.35	11	—	+	+	—	—	—	—	+	—	—
No.67	1	—	+	+	+	+	—	+	—	+	—
No.71	42	+	+	+	+	—	—	—	+	—	—
B. neapolitanus, No.72	15	—	+	+	+	—	—	—	+	—	—
No.74	1	+	+	+	+	—	—	—	—	—	—
No.75	-	—	+	+	+	—	—	+	—	+	—
No.99	-	—	+	—	+	+	+	+	—	—	—
No.101	1	—	+	—	+	+	—	+	+	—	—
B. lactis aerogenes, No.103	8	—	+	—	+	+	—	+	—	+	—
No.106	2	+	+	—	+	—	—	—	+	—	—
B. coscoroba, No.107	1	—	+	—	+	—	—	—	+	—	—

In this and subsequent tables) + = acid and gas, liquefaction of gelatin, motile, indol production as the case may be;) ⊥ = acid without gas; — = no acid or gas production, etc.

TABLE 2.

Sub-group A: Gas producing - Indol forming - Non-Inosite-fermenting - Non-liquefying. Type	Motility	Glucose	Lactose	Dulcite	Saccharose	Adonite	Inulin	Raffinose	Salicin	Proskauer reaction	Mannite	Maltose	Faeces	Urine	Appendix Abscesses	Other Sources	Total
1. McConkey No.71	+	+	+	+	+	−	−	+	+	−			10	15	3		28
2. B. coli communis	+	+	+	+	−	−	−	−	+	−			14	5	1	1 (Blood culture)	21
3. B. vesiculosus	−	+	+	−	−	−	−	−	−	−			4	5	4		13
4. B. Grünthal	+	+	+	−	−	−	−	−	+	−			4	6	2		12
5. B. Schafferi	−	+	+	+	−	−	−	−	+	−			4	6	1		11
6. B. neapolitanus	−	+	+	+	+	−	−	+	+	−			2	3		1 (Puerperal Fever)	6
7. B. Schafferi type	−	+	+	+	−	−	−	−	−	−			1	3	2		6
8. McC. No.71 type	+	+	+	+	+	−	−	+	−	−			2	1	1	1 (Conjunctivitis)	5
9. McConkey 106	+	+	+	−	+	−	−	+	+	−			1	4			5
10. Neapolitanus type	−	+	+	+	+	−	−	+	−	−			1	2	1		4
11. McConkey No.1	+	+	+	−	−	+	−	−	+	−			4				4
12.	−	+	−	+	−	−	−	−	−	−	+	+	4				4
13. B. coscoroba	−	+	+	−	+	−	−	+	+	−			2		1		3
14. B. vesiculosus type	−	+	+	−	−	−	−	−	+	−				2	1		3
15.	+	+	+	+	+	+	−	+	+	−			1	1		1 (Otitis)	3
16. B. coli comm. type	+	+	+	+	−	−	−	+	+	−			1	2			3
17. B. coli comm. type	+	+	+	+	−	−	−	−	−	−			1	2			3
18. B. Schafferi type	−	+	+	+	−	−	−	+	+	−			1	1			2
19.	−	+	−	+	+	−	−	+	−	−	+	+	1	1			2
20. B. McC. 106 type	+	+	+	−	+	−	−	−	+	−			1	1			2
21. Neapolitanus type	−	+	+	+	+	−	−	−	+	−			1	1			2
22. McC. No.71 type	+	+	+	+	+	−	−	−	+	−						1 (Conjunctivitis)	1
23. B. vesiculosus type	−	+	+	−	−	−	−	+	−	−			1				1
24. B. coli comm. type	+	+	+	+	−	−	−	+	−	−			1				1
25. B. Grünthal type	+	+	+	−	−	−	−	+	+	−				1			1
26. B. Grünthal type	+	+	+	−	−	−	−	−	−	−			1				1
27. McC. No.1 type	+	+	+	−	−	+	−	−	−	−				1			1
28. B. acidi lactici	−	+	+	−	−	+	−	−	−	−			1				1
29.	+	+	−	+	−	−	−	−	−	−	+	+	1				1
30.	−	+	+	+	+	+	−	+	+	−			1				1
31. McConkey No.33	+	+	+	+	−	+	−	−	+	−			1				1
32.	−	+	+	−	+	−	+	+	+	−					1		1
33.	−	+	+	−	+	+	−	+	−	−			1				1
34.	−	+	−	−	+	−	−	−	+	−	+	+		1			1
35.	−	+	−	+	+	−	−	−	+	−	+	+		1			1
36.	+	+	−	−	−	−	−			−	−	−		1			1
												Total	68	66	18	5	157

TABLE 3.

Sub-group E: Gas producing + - Non-indol-forming - Non-inosite-fermenting - Non-liquefying

	Type	Motility	Glucose	Lactose	Dulcite	Saccharose	Adonite	Inulin	Raffinose	Salicin	Proskauer reaction	Mannite	Maltose	Faeces	Urine	Appendix Abscesses	Other Sources	Total
1.	B. McConkey No.74	+	+	+	+	+	−	−	+	+	−			4	8		1 (Cholecystitis)	13
2.		−	+	+	+	+	−	−	+	+	−			2	4			6
3.		−	+	+	+	−	−	−	−	+	−			1	1			2
4.	B. McConkey No.8	−	+	+	−	−	−	−	−	−	−			1		1		2
5.	B. McConkey No.8	−	+	+	−	−	−	−	−	+	−			1	1			2
6.		+	+	+	−	+	−	−	+	+	−				1			1
7.		−	+	+	−	+	−	−	+	+	−				1			1
8.		−	+	+	+	−	−	−	−	−	−				1			1
9.		+	+	+	−	+	−	−	+	−	−				1			1
10.		−	+	+	+	+	+	−	+	+	−				1			1
11.	B. McConkey No.7	+	+	+	−	−	−	−	−	−	−			1				1
101.	B. paracolon type	+	+	−	−	−	−	−			−	+	+	2	4			6
102.		+	+	−	+	−	−	−	−	+	−	+	+	5				5
103.	B. paracolon type	−	+	−	−	−	−	−			−	+	+	2	3			5
104.		+	+	−	−	+	−	−	+	+	−	+	+	2				2
105.		+	+	−	−	+	−	−	+	−	−	+	+	1				1
106.		−	+	−	−	+	−	−	+	−	−	+	+		1			1
107.		−	+	−	+	+	−	−	+	+	−	+	+		1			1
													Total	22	28	1	1	52

TABLE 4.

Sub-group C: Gas producing - Inosite-fermenting - Non-liquefying

	Type	Motility	Glucose	Lactose	Dulcite	Saccharose	Adonite	Inulin	Raffinose	Salicin	Proskauer reaction	Indol	Faeces	Urine	Appendix Abscesses	Other Sources	Total
1.	B. MacConkey No.67	—	+	+	+	+	+	—	+	+	+	—	3	2			5
2.		—	+	+	+	+	+	+	+	+	+	+	3				3
3.		—	+	+	+	+	+	+	+	+	—	+	3				3
4.		—	+	+	+	+	+	—	+	+	—	+	3				3
5.	B. lactis aerogenes	—	+	+	—	+	+	—	+	+	+	—	2			1 (Skin ulcer)	3
6.	B. MacConkey No.99	—	+	+	—	+	+	+	+	+	—	—	1	1			2
7.		—	+	+	—	+	+	+	+	+	+	+	1	1			2
8.		—	+	+	+	+	+	—	+	+	+	+	1		1		2
9.	B. MacConkey No.101	—	+	+	—	+	+	—	+	+	—	+	1	1			2
10.		—	+	+	—	+	+	—	+	+	—	—	1				1
11.	B. MacConkey No.75	—	+	+	+	+	—	—	+	+	+	—	1				1
												Total	20	5	1	1	27

TABLE 5.

Sub-group D: Non-gas-producing in all sugars fermented - Non-liquefying

Type	Motility	Glucose	Lactose	Dulcite	Saccharose	Adonite	Inulin	Inosite	Raffinose	Salicin	Proskauer reaction	Indol	Mannite	Maltose	Faeces	Urine	Other Sources	Total
1.	–	⊥	–	–	–	–	–	–			–	–	–	–		2	1 (skin ulcer)	3
2.	–	⊥	⊥	⊥	–	–	–	–			–	+	⊥	⊥		1		1
3.	+	⊥	⊥	–	⊥	–	–	–			–	+	⊥	⊥	1			1
4.	–	⊥	⊥	–	⊥	–	–	–			–	+	⊥	⊥		1		1
5.	+	⊥	⊥	⊥	⊥	–	–	–			–	+	⊥	⊥		1		1
6.	–	⊥	⊥	⊥	⊥	–	–	–			–	–	⊥	⊥		1		1
7.	–	⊥	–	⊥	–	–	–	–	–		–	+	⊥	⊥		1		1
8.	–	⊥	–	–	⊥	⊥	–	–	–	⊥	–	–	⊥	⊥		1		1
														Total	1	8	1	10

TABLE 6.

MacConkey's types	Faeces	Urine	Appendix Abscesses	Other Sources	Total
No.1	4	1			5
No.2 B. acidi lactici	1				1
No.4 B. Grünthal	5	7	2		14
No.5 B. vesiculosus	5	7	5		17
No.7	1				1
No.8	2	1	1		4
No.33	1				1
No.34 B. coli communis	17	9	1	1	28
No.35 B. Schafferi	6	10	3		19
No.67	3	2			5
No.71	12	16	4	2	34
No.72	4	6	1	1	12
No.74	4	8		1	13
No.75	1				1
No.99	1	1			2
No.101	1	1			2
No.103 B. lactis aerogenes	2			1	3
No.106	2	5			7
No.107 B. coscoroba	2		1		3
				Total	172

Note: of 246 strains 172 corresponded to types described by MacConkey.

Types of coliform bacilli found in pyogenic infection of the urinary system.

As shown by MacConkey, certain coliform types tend to be more prevalent than others in human and animal faeces; thus the type designated by him No. 71 is of commonest occurrence. As is well known gram-negative bacilli are the most frequent causative agents in pyogenic infections of the urinary tract and are found either alone or associated with the pyogenic cocci. In order to ascertain what types were characteristic of these pathological conditions a number of coliform bacilli isolated from urinary cases were carefully investigated.

The specimens of urine were from cases of cystitis and pyelitis. The samples were centrifugalized in sterile tubes and from the sediment, plates of Endo-agar or MacConkey's bile-salt-neutral-red-lactose-agar were inoculated by successive strokes of the platinum loop. Separate colonies were thus obtained, and to ensure the purity of the ultimate culture investigated, further successive strokes were generally made (from single colonies) on another plate and the final culture made from a single colony on the second plate. In some cases agar slope cultures were made from single colonies on the primary plate; this culture was then replated and the final culture obtained by subinoculating a single colony.

107 strains of coliform bacilli from 90 cases of urinary sepsis were examined. In the majority of instances, pure cultures of individual types were isolated. It might be expected in dealing with a bacterial group such as B. coli that separate colonies similar in appearance might on examination prove to belong to different types. I have on several occasions selected two or three similar colonies at random from the same plate and found on examination that they represented the same cultural type. Not infrequently, however, mixed cultures are met with but it is remarkable that in such instances one notices some difference in the colonies, e.g., difference in size, thickness, depth of colour on Endo or MacConkey's agar, or complete absence of pink coloration. Of course, non-lactose-fermenters produce " pale " colonies on these differential media, but in primary culture the absence of red coloration is no proof that the organism is a non-lactose fermenter. The fact that mixed cultures of different coliform species may occur in urinary sepsis is of special and obvious interest as regards the preparation of autogenous vaccines.

As in the case of faecal strains there is a distinct tendency towards the prevalence of certain types in urinary conditions and the commonest intestinal species are also the most frequent types met with in pathological urines.

Type 1, Sub-group A, corresponds to B. MacConkey No. 71 and is the commonest coliform species met with (v. tables 1, 2, 6). It proved the most frequent type (15 strains) in urinary sepsis. The other prevalent varieties were types A2 (B. coli communis), A3 (B. vesiculosus), A4 (B. Grünthal), A5 (B. Schafferi), B1 (B. MacConkey No. 74).

Of the 107 strains

66 belonged to the sub-group A
28 " " " B
5 " " " C
8 " " " D.

Thus the majority are referable to the A sub-group which comprises all the " typical " B. coli varieties. Strains of non-lactose-fermenters were also isolated, A19, A34, A35 and A36; those of types 19, 34 and 35, however, developed lactose-fermenting variants in lactose medium (v.p.56-57).

Type A36 corresponded in cultural reactions to B. Morgan No. 1; it was isolated in pure culture from a case of pyelitis and cystitis.

In sub-group B, type 1 was of commonest occurrence corresponding to MacConkey's type No. 74. The relative prevalence of other varieties is shown in table 3. Among the organisms of sub-group B certain non-lactose-fermenters are also represented including 7 strains of " paracolon " types, B.101 and B 103.

Patrick reported organisms of these types in cases of bacilluria occurring in the course of typhoid fever. They were present in such large numbers as to render the urine turbid. In primary culture they did not exhibit any gas production and, therefore, simulated B. typhosus in cultural reactions. I had the opportunity of examining his strains and found them to be typical " paracolon " bacilli with the reactions shown (B 101 and 103) and similar to strains I had isolated from cases of urinary sepsis.

Certain non-lactose-fermenters of the B sub-group were also noted which developed lactose-fermenting variants spontaneously in lactose media (B 106 and 107) (v.p. 56-57).

5 inosite-fermenters (sub-group C) were also isolated from urine and 8 strains referable to sub-group D. 4 of the latter were non-lactose-fermenters; 2 of these fermented only glucose and, being non-motile, were, therefore, similar in cultural reactions to B. dysenteriae Shiga. They were not, however, agglutinated by an anti-Shiga serum. One of these strains developed a lactose fermenting variant. The characters of these strains are shown on table 5.

Among the gram-negative bacilli found in these cases of urinary sepsis, B. proteus (Urobacillus liquefaciens septicus Krogius) was not infrequently noted, usually associated with B. coli or pyogenic cocci; gram-negative bacilli characterized by absence of carbohydrate fermentation have also been noted both in mixed and pure culture: 4 strains of this type were isolated; 2 were motile (corresponding to the typical B. faecalis alkaligenes) and 2 non-motile.

Types isolated from faeces, appendix abscesses and other sources.

111 Strains from specimens of faeces were analysed in the same way; these were derived partly from normal specimens and also specimens submitted for bacteriological examination, e.g., for detection of B. typhosus. Their grouping was as follows:-

Sub-group A - 68
" B - 22
" C - 20
" D - 1.

As in the case of the urinary strains most belonged to the sub-group A; only one anaerogenes type was noted (D 3).

The majority of the strains classified in this series were isolated from plates made directly from faeces, but 4 of sub-group B and 10 of C were obtained from cultures in brilliant green peptone water employed for the enrichment of B. typhosus (Browning, Gilmour and Mackie). As indicated later it was found that types of B and C sub-groups were more resistant to brilliant green than the A types; hence the relatively large proportion of C types in the series.

Among these strains were 4 non-lactose-fermenters of the A 12 type and 1 of the A 29 type; these two varieties differ from one another in motility. 1 non-lactose-fermenter of the

A 19 type was also isolated. This strain like the similar type found in urine developed a lactose-fermenting variant (p.56-57).

5 non-lactose fermenters of the B 102 type were isolated from faeces, but all of these developed lactose fermenting mutants. The other non-lactose fermenters noted were B 101 (2 strains), B 103 (2 strains), B 104 (2 strains), and B 105. Single strains of the B 103 and B 105 types developed lactose-fermenting variants (v.p. 56-57).

20 Strains from appendix abscesses were also investigated; these were grouped as follows:-

Sub-group A - 18, of which the A 3 and A 1 types were most prevalent.

All were lactose fermenters (v. table 2).

Sub-group B - 1

Sub-group C - 1.

A few strains from other sources were examined: 2 from cases of conjunctivitis both belonging to the A sub-group, 1 from a suppurative otitis (A 15), 1 from a case of puerperal sepsis (A 6), 2 from skin ulcers (C 5 and D 1), 1 from a case of cholecystitis (B 1), 1 from blood culture after an abdominal operation (A 2).

The total number of strains examined from different sources could therefore be classified as follows:-

Sub-group A -- 157

(A 1 most prevalent type - 28 strains;
10 primarily non-lactose fermenters).

Sub-group B - 52

(B 1 most prevalent - 13 strains;

21 primarily non-lactose fermenters).

Sub-group C - 27

(C 1 most prevalent type - 5 strains).

Sub-group D - 10

(D 1 most prevalent type - 3 strains).

Considering the various strains collectively in these series which correspond to types isolated and classified by MacConkey, the commonest is the No. 71 type (34 strains) (table 6). Of the series isolated from urine, this type was also the most prevalent, though among the faecal strains the B. coli communis was the commonest. Collectively, however, the most prevalent types in my series were B. MacConkey No. 71 and B. coli communis.

With comparatively few exceptions, the gram-negative bacilli found in urinary sepsis, as shown, are normal inhabitants of the intestine and this parallelism between the faecal and urinary types is a further confirmation of the generally accepted view that these infections are auto-infections from the bowel.

As indicated above the A sub-group comprises the most frequently occurring types of coliform bacilli, the so called typical varieties. This group also appears to embrace the largest number of different varieties or types (36). While

organisms of the sub-groups B and C are less frequently met with in excremental material, they nevertheless represent characteristic faecal organisms. Organisms of the anaerogenes type are of comparatively rare occurrence, but as shown, this type may be associated with certain cases of urinary sepsis.

THE SEROLOGICAL INVESTIGATION OF THE B. COLI GROUP.

The following serological studies were originally carried out with a view to throwing further light on the biological relationships of the various types of B. coli and its congeners. The agglutination and complement deviation reactions of immune sera to certain of the commoner varieties were studied and the results, apart from their bearing on this particular question, represent observations of considerable interest from the purely immunological standpoint.

AGGLUTINATION REACTIONS.

While in the case of B. typhosus, precise species specificity is characteristic of an agglutinating antiserum, among the coliform bacilli a much more restricted degree of specificity was observed - specificity for the individual strain.

An immune serum to a particular strain of B. typhosus will agglutinate most B. typhosus strains with little variation in degree; immune sera to certain B. coli types,

on the other hand, have been found to exert little or no action on other strains identical as regards cultural reactions to that used for immunization.

Several authors have drawn attention to the high degree of specificity of B. coli agglutinins (Van Everen, Pfaundler, Cany, Wolf, Amiradzibi) but the agglutination reactions of these organisms have not been accurately studied in correlation with their classification into different types qua cultural reactions; thus Amiradzibi immunized guinea pigs against five strains which all possessed certain common characters - power of fermenting glucose, lactose, clotting milk and producing indol; he found that the strain used for immunization was the only one agglutinated. These common characters are, however, as shown above, referable to a considerable variety of types or species. In the experiments to be recorded it is shown, that among the commoner types of B. coli (A sub-group) even after these organisms have been classified and separated into different species by cultural tests, it is still impossible to demonstrate any species specificity of an agglutinating antiserum, and that agglutinating sera are specific only for the individual homologous strain.

Antisera were obtained to certain strains which represented common coliform types (1) Type A 1 (B. MacConkey No. 71), (2) Type A 4 (B. Grünthal), (3) Type A 3 (B. vesiculosus), (4) Type A 2 (B. coli communis) and a number

of other strains corresponding in all their characters with these types were tested with the immune sera.

Immune Sera: Rabbits were immunized against the particular organisms by repeated intravenous injection of increasing amounts of bacillary emulsions sterilized at a temperature of 65° C. for ½ hour. For this purpose 24 hours agar slope cultures were emulsified in convenient quantities of 0.85% sodium chloride solution. The series of doses were as follows:- $\frac{1}{10}$, $\frac{1}{5}$, $\frac{1}{2}$, 1 and 2 emulsified agar slope cultures, given at intervals of 7 to 10 days. Ten days after the last injection the sera were tested with the strains used for immunization and if found of suitable value, i.e., agglutinating in a dilution of 1:2000 or in higher dilutions, the animal was bled and the serum after separation stored in sealed tubes. In the original experiments sterility of the serum was ensured by heating at 57° C. for 1 hour on ~~two~~ three successive days. In some cases it was noted that there was a marked depreciation of the agglutinating value of the serum by heating, due apparently to the varying thermostability of the agglutinin. To obviate this the measures adopted for bleeding and collecting the serum were carried out with the utmost precautions to exclude contamination and the serum was heated at 57° C. for only ½ hour on 2 successive days.

Method of carrying out the agglutination tests: A 24 hours agar slope culture was emulsified in 5 C.C. of 0.85% salt solution,

and the emulsion allowed to stand in the incubator for about I hour to allow the larger clumps and fragments of agar to deposit. The supernatant fluid was then decanted and made up to I0 C.C.. Varying dilutions of the antiserum were mixed with equal volumes (0.5 C.C.) of bacillary emulsion and the mixtures placed in narrow tubes in which the agglutination could be observed by the naked eye. As a control 0.5 C.C. of the bacillary emulsion was mixed with an equal volume of salt solution and included in the test series; this eliminated any fallacy due to autoagglutination. It is to be noted, however, that autoagglutination was rarely seen among these bacilli. The tubes were placed in the incubator for I½ hours and then at room temperature for ½ hour; they were again placed in the incubator for 2 hours, when readings were taken of the results.Ultimately they were allowed to stand at room temperature till next day when further readings, if necessary, were made. It was usually noted that the variations in temperature produced in this way set up convection currents in the fluid and this hastened agglutination. The degree of agglutination was determined by the amount of sediment in the various tubes as compared with the control, or by the clarity or turbidity of the supernatant fluid as compared with the fluid in the control tube.

Complete agglutination is signified in the tables by + + + +, and lesser degrees by + + +, + +, and +.

Results observed with antisera to A types 1, 2, 3 and 4.

Marked specificity for the individual strain on the part of these immune sera was observed. Tables 7, 8, 9 and 10 show that the only strains agglutinated to any extent by the corresponding antiserum were the particular strains used for immunization. Thus, the antiserum to strain 1 type 1 agglutinated strain 1 in dilutions up to 1:50,000 ; 14 other strains of the same type were tested with the antiserum but none showed any agglutination by dilutions higher than 1:500 and 8 were not even agglutinated by a dilution of 1:100. A strain of type 3 also exhibited little reaction with the type 1 antiserum (table 7). Similar results were obtained with antisera to types 2, 3 and 4.

While this restricted specificity was found to be the general rule with antisera to these common types of B. coli, an exception has been noted; an antiserum to a type 2 (B. coli communis) was found to agglutinate a particular strain of type 1 (B. MacConkey No. 71) in a four times higher dilution than in the case of the homologous strain (table 11), and this type 1 strain was not found to be specially susceptible to other B. coli agglutinins, (tables 9 and 10) i.e., it was not itself susceptible to other agglutinating sera nor did it show any

TABLE 7.

Antiserum to Strain 1, B. coli, sub-group A type 1.

(B. MacConkey No.71)

Dilution	1:100	1:500	1:1000	1:5000	1:10000	1:20000	1:30000	1:50000
Strain 1, A 1	++++	++++	++++	++++	++++	++++	+++	+

Other strains having same characters i.e. of type A 1.

Strains 4, 6, 7, 10, 11, 12, 13, 15.	0	0	0	0	0	0	0	0
Strains 3, 9.	++	+	0	0	0	0	0	0
Strains 2, 8.	+	0	0	0	0	0	0	0
Strains 5, 14	+++	++	0	0	0	0	0	0
Strain 1, A 3	++	0	0	0	0	0	0	0

Controls showed no agglutination.

TABLE 8.

Antiserum to Strain 1, B. coli type A 2
(B. coli communis).

Dilution	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:8000	1:10,000
Strain 1, A 2	++++	++++	++++	++++	++++	+++	++	+
Other strains having same characters i.e. of type A 2.								
Strains 2, 3, 4, 5, 6, 7, 8, 9, 10.	0	0	0	0	0	0	0	0
Strain 11	+	0	0	0	0	0	0	0
Strain 1, A 4	0	0	0	0	0	0	0	0

Controls showed no agglutination.

TABLE 9.

Antiserum to Strain 1, B. coli type A 3 (B. vesiculosus).

Dilution	1:60	1:120	1:240	1:480	1:960	1:2000
Strain 1, A 3	++++	++++	++++	+++	+++	++

Other strains having same characters i.e. of type A 3.

Strains 3, 4, 5, 6, 7.	0	0	0	0	0	0
Strains 2	+	0	0	0	0	0
Strain 8	++	+	0	0	0	0
Strain 1, A 1	+	0	0	0	0	0
Strain 1, A 4	+	0	0	0	0	0

Controls showed no agglutination.

TABLE 10.

Antiserum to Strain 1, B. coli type A 4

(B. Grünthal)

Dilution	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:8000	1:10000
Strain 1, A 4	++++	++++	++++	++++	++++	++++	++++	+++	+

Other strains having same characters i.e. of type A 4.

Strains 3, 4, 6, 8.	0	0	0	0	0	0	0	0	0
Strains 2, 5, 9.	+	+	0	0	0	0	0	0	0
Strains 7, 10.	+	0	0	0	0	0	0	0	0
Strain 1, A 1	+++	+	0	0	0	0	0	0	0
Strain 1, A 3	++	0	0	0	0	0	0	0	0

Controls showed no agglutination.

tendency to autoagglutination. Moreover, this agglutinin had no effect on a number of other strains of type 1 (table 11).

To ascertain whether this peculiarity was a function of the immune animal, another antiserum to the same type 2 strain was obtained and the serum behaved in practically the same manner, indicating that this property of " paragglutination " was dependent on the particular strain (table 11).

It is noteworthy that this instance of paragglutination is characterized by the more powerful effect of the paragglutinin than the primary agglutinin.

As is well known the agglutinin has marked affinities for the homologous bacillus and is absorbed or used up by the organisms during the process of agglutination. Moreover, a bacillary emulsion is capable of absorbing much more agglutinin than is required for complete agglutination of the organisms. It was found that if these agglutinating sera were absorbed, (1) by the homologous strain, (2) another strain of the same type and (3) another strain of a different type, and then tested with the homologous strain, the agglutinin could be almost completely absorbed by the homologous strain, but that the absorptive effect of the other organisms was relatively weak and equal in degree (tables 12 and 13).

For this purpose concentrated emulsions were prepared and a certain dilution, 1:500 or 1:1000, of the antiserum was treated with the different organisms at 37° C. for 2 hours;

TABLE 11.

First antiserum to Strain 1, B. coli type A 2 (B. coli communis).

Dilution	1:100	1:1000	1:8000	1:10,000	1:20,000	1:30,000	1:40,000
Strain 1, A 2	++++	++++	++	+	0	0	0
Strain 1, A 1 (B. MacConkey No.71)	++++	+++	+++	+++	+++	+++	++

Ten other strains having same characters as Strain 1, A 1 showed no agglutination with antiserum diluted 1:500

Second antiserum to Strain 1, A 2.

Dilution	1:200	1:400	1:1000	1:2000	1:4000	1:8000	1:16000
Strain 1, A 2	++++	+++	+++	++	0	0	0
Strain 1, A 1	+++	+++	+++	+++	++	+	0

Controls showed no agglutination.

TABLE 12.

Antiserum to Strain I, B. coli type A 1 absorbed at 1:500 by large excess of emulsion. (v.table 7)

of (1) Strain I, A 1.

(2) " 2, A 1.

(3) " 1, A 2.

Treated Sera.

Dilution	1:600	1:1200	1:2400	1:4800	1:9600	1:12,000
	tested with Strain I , A 1.					
(1)	++	+	0	0	0	0
(2)	++++	++++	++++	+++	+++	+
(3)	++++	++++	++++	+++	+++	+

Controls showed no agglutination.

TABLE 13.

Antiserum to Strain 1, A 4 (B. Grünthal) absorbed at 1:1000 by excess of emulsion (v. table IO)

of (1) Strain 1, A 4

(2) " 2, A 4

(3) " 1, A 3

Treated Sera.

Dilution	1:1000	1:2000	1:4000	1:8000	1:10,000
	tested with Strain 1, A 4.				
(1)	0	0	0	0	0
(2)	++++	++++	++++	++	0
(3)	++++	++++	++++	+	0

Controls showed no agglutination.

the mixtures were centrifugalized till the supernatant fluids were quite clear; these were pipetted off and tested each with the homologous strain in various further dilutions.

Absorption tests were also carried out with the antiserum which showed the paragglutination phenomenon. It was found that while treatment of the serum by the strain used for immunization removed the agglutinin both for this strain and the heterologous strain, the heterologous strain was only capable of absorbing its own agglutinin (table 14). This is true for coagglutination effects generally (Castellani), and showed that the two strains were not serologically identical.

It was thus quite impossible to establish any differentiation by agglutination tests between different types of these coliform bacilli, and no species specificity was displayed by their agglutinins; the specificity is restricted to the individual strain and, by the ordinary agglutination reaction, and also by absorption tests, it is quite impossible to demonstrate that organisms of the same species (determined by cultural reactions) as the homologous strain, are more closely related to it than representatives of other types. Thus, the individuality of the bacterial strain is most strikingly elicited. In this group strains are constantly assuming new characters (v.p. 55) and becoming highly specialized. Hence we must assume that a high degree of individuality is attained by each strain and that

TABLE 14.

(1) Antiserum to Strain 1, A 2 (B. coli communis) absorbed by Strain I, A 1 (v. Table 11.) at 1:1000 dilution.

(2) Same antiserum absorbed by Strain 1, A 2 at 1:1000 dilution.

	Treated Serum (1)					
Dilution	1:1000	1:2000	1:3000	1:4000	1:6000	1:8000
tested with						
Strain 1, A 2	++++	++++	++++	++++	+++	++
Strain I, A 1	++	+	0	0	0	0

	Treated Serum (2)					
tested with						
Strain 1, A 2	++	+	0	0	0	0
Strain I, A 1	0	0	0	0	0	0

Controls showed no agglutination.

differences of cultural characters within certain limits are of little significance.

Results observed with antisera to B types 1 and 2.

In the foregoing observations regarding the action of B. coli agglutinins, the commoner types, B. MacConkey No. 71, B. coli communis, B. Grünthal, B. vesiculosus belonging to the indol forming sub-group (A) of coliform bacilli were studied; further experiments were then carried out with agglutinating sera to certain of the B sub-group which are of less frequent occurrence.

Antisera were obtained to strains of types B 1 (B. MacConkey No. 74) and B 2 whose characters are shown in table 3. A number of other strains identical in their characters to these types were tested with the respective immune sera. The results with an antiserum to strain 1, type B 1 are shown in table 15. It was noted that while the strain used for immunization was agglutinated by a 1:8000 dilution of the serum, 3 other identical strains were only agglutinated by a lower dilution (1:100, 1:1000) and strains of sub-group A type 1 and B 2 also were not agglutinated except by low dilutions. Strains 3 and 6 were agglutinated, however, to the same degree as the strain used for immunization.

An antiserum to strain 3 was also obtained and tested with the other strains (including No. 1). The corresponding effect was found to occur, i.e., marked agglutination of

TABLE 15.

Antiserum to Strain 1, B. coli type B 1 (B. MacConkey No.74)

Dilution	1:100	1:500	1:1000	1:2000	1:4000	1:6000	1:8000	1:10,000
Strain 1, B 1	++++	++++	++++	++++	++++	+++	++	0
Strain 2, B 1	++++	+++	++	0	0	0	0	0
Strain 3, B 1	++++	++++	++++	++++	++++	+++	++	0
Strain 4, B 1	++	0	0	0	0	0	0	0
Strain 5, B 1	++++	0	0	0	0	0	0	0
Strain 6, B 1	++++	++++	++++	++++	++++	+++	+	0
Strain 1, A 1	+++	++	0	0	0	0	0	0
Strain 1, B 2	+++	+	0	0	0	0	0	0

Controls showed no agglutination.

strain 3 and also of strains 1 and 6 while the other strains were not affected to any extent (table 16). In this type therefore the specificity was not so restricted and the homologous strain was not the only strain which showed marked agglutinability by the antiserum. Certain strains of the same type were, however, not more agglutinable than a heterologous strain belonging to an entirely different sub-group. Thus no species differentiation could be elicited by means of these antisera.

In the case of the antiserum to a strain of B 2, two other corresponding strains were also tested. The strain used for immunization was agglutinated by dilutions up to 1:10,000 (table 17); strain 2 was agglutinated by dilutions up to 1:3200, but strain 3 showed a less degree of agglutinability (end-titre 1:800).

Among these B types there is a relative specificity of the agglutinin for the individual strain as in the case of the indol forming types, but the results indicate that the specificity is much less restricted. In the case of agglutinating sera to A types 1, 2, 3, and 4, the strain used for immunization showed marked agglutination, while other strains of the same types respectively were practically inagglutinable except by low dilutions of the serum. In the case of antisera to type B 1 strains, other strains of the same type showed an almost equal agglutinability, and as

TABLE 16.

Antiserum to Strain 3, B. coli type B 1.

Dilution	1:50	1:100	1:800	1:1600	1:3200	1:6400
Strain 1, B 1	++++	++++	++++	++++	+++	+
Strain 2, B 1	++++	+++	0	0	0	0
Strain 3, B 1	++++	++++	++++	++++	++	0
Strain 4, B 1	++++	+++	+	0	0	0
Strain 5, B 1	++	0	0	0	0	0
Strain 6, B 1	++++	++++	++++	+++	++	+
Strain 1, A 1	+++	+	0	0	0	0

Controls showed no agglutination.

TABLE 17.

Antiserum to Strain 1, B. coli type B 2.

Dilution	1:50	1:100	1:400	1:800	1:1600	1:3200	1:6400	1:10,000
Strain 1, B 2	++++	++++	++++	++++	++++	++++	+++	+
Strain 2, B 2	++++	++++	++++	++++	+++	++	0	0
Strain 3, B 2	++++	++++	+++	++	0	0	0	0
Strain 1, A 1	+++	0	0	0	0	0	0	0

Controls showed no agglutination.

regards the antiserum to strain 1, B 2, of the two other corresponding strains, one was agglutinated by relatively high dilutions though not quite equal in agglutinability to the strain used for immunization.

Results observed with antisera to B types 101 and 103 (paracolon bacilli).

Agglutinating sera for two types of the so called paracolon bacilli, i.e., types which ferment glucose and mannite with gas production and do not ferment lactose or saccharose, were prepared. In this case specificity for the individual strain was completely absent and exact species specificity was observed.

The immune serum to strain 1, B 101 agglutinated this strain in dilutions as high as 1 in 8,000,000 (an unusually powerful agglutinin) and 3 other similar strains were agglutinated by equally high dilutions (table 18).

It is of interest to note that strain 4, B 101 underwent spontaneous variation in saccharose medium (fluid); so that a new strain was developed differing from the original strain in fermenting saccharose within 24 hours growth (v.p. 57). Both the original and variant strain were equally agglutinable by the antiserum. The immune serum to strain 1, B 103 agglutinated 3 other similar strains to the same degree as the strain used for immunization (table 19).

TABLE 18.

Antiserum to Strain 1, B 101 (B. paracolon type)

Dilution	1:1000000	1;2000000	1:4000000	1:6000000	1:8000000	1:10000000
Strain 1, B 101	++++	+++	++	++	+	0
Strain 2, B 101	++++	++++	++++	+++	++	0
Strain 3, B 101	++++	++++	++++	+++	++	0
Strain 4, B 101	++++	++++	++++	+++	++	0
Strain 1, A 1	0	0	0	0	0	0
Strain 1, B 1	0	0	0	0	0	0
Strain 1, B 103	+++	0	0	0	0	0

(Note the very high degree of potency attained by this particular agglutinating serum).

TABLE 19.

Antiserum to Strain 1, B 103 (B. paracolon type)

Dilution	1:500	1:1000	1:2000	1:4000	1:8000	1:16000	1:32000
Strain 1, B 103	++++	++++	++++	++++	+++	+++	+
Strain 2, B 103	++++	++++	++++	++++	+++	+++	+
Strain 3, B 103	++++	++++	++++	++++	+++	++	+
Strain 4, B 103	++++	++++	++++	++++	+++	++	++
Strain 1, B 101	++	0	0	0	0	0	0

Controls showed no agglutination.

Thus among the coliform bacilli investigated serologically, different grades of specificity on the part of agglutinating sera have been noted:

(1) in the case of the commoner types, sub-group A types 1, 2, 3 and 4 (gas producing, indol +, inosite —, lactose +) marked specificity for the individual strain was observed.

(2) in the case of certain less common types, sub-group B types 1 and 2 (gas producing, indol —, inosite —, lactose +) absolute specificity for individual strains was not observed but there was not complete specificity for the species or type as determined by cultural tests.

(3) in the case of certain paracolon types (B 101 and 103) (gas producing, indol —, inosite —, lactose —) precise specificity for the cultural type was observed.

COMPLEMENT DEVIATION REACTIONS.

As in the case of agglutination by immune sera to organisms of the A sub-group, the specificity of the complement deviating immune body is found to be related not to the homologous species but to the strain used for immunization. This specificity for the individual strain was not so pronounced as in the case of the agglutinin and was only relative. While " group " agglutination among the different

B. coli species was slight and often inappreciable, the complement deviating antibody displayed marked " group " action within certain well defined limits and in the experiments to be recorded some indication of the biological relationships of different B. coli types has been elicited from a study of these group reactions.

Complement deviation methods.

Antigen: Emulsions of the bacilli in 0.85% salt solution were generally used as antigen; these were prepared by mixing an 18 to 24 hours agar slope culture of the particular organism with a given quantity (10 C.C.) of salt solution. The whole agar surface had been inoculated abundantly so that a continuous growth was obtained, and by using tubes with agar surfaces of approximately equal size, the emulsions of different organisms generally exhibited an approximately equal degree of turbidity.* The emulsions were sterilized in a vaccine bath at 65° C. for ½ hour; this is usually sufficient to ensure the killing of organisms of the coli group and does not affect the antigenic value of the emulsions. These antigens generally exhibited a more or less degree of anticomplementary action by themselves; this effect appeared to depend mainly on two factors (1) the particular specimen of complement and (2) the presence in the emulsion of

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* Table 20 shows how closely the antigenic properties of these emulsions correspond; compare the deviation by the A 4 antiserum + strains 2 A 4, 1 A 3 and 1 A 1.

fragments of agar which after heating of the emulsion render it extremely viscous. It was generally found better to employ complement-serum 18 to 24 hours after its withdrawal as fresh complement tends to be more deviable by anticomplementary agents.* Apart from this, individual animals yield complements which may display varying degrees of deviability; some are extremely deviable, while others are little affected by the usual inhibitory agents and it is thus impossible to predicate how a certain specimen of complement-serum will behave. As regards the other complicating factor it was found essential before heating to centrifugalize the emulsions for 1 or 2 minutes. This deposited any agar fragments and the supernatant emulsion was then pipetted off.

It was thus possible to obtain bacillary emulsions which, with suitable complements, showed little anticomplementary effect in the quantities used.

Bacillary extracts have been extensively used in place of simple emulsions. These were originally employed by Wassermann and Bruck and various workers have preferred them on the ground that they are less anticomplementary than emulsions. I have prepared extracts by Dean's method of alternately freezing and thawing emulsions and then removing the bacteria by centrifugalization. These extracts were found no less inhibitory in certain amounts with various specimens of complement than the ordinary emulsions. Throughout the

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* This is well known as regards the Wassermann reaction.

experiments emulsions have been used as antigen; they are easily prepared and with suitable complements exhibit little inhibitory effect.

The immune sera used were those already referred to in connection with the agglutination experiments.

Guinea pig's serum was used as complement and ox blood corpuscles sensitized with 5 doses of a haemolytic immune body (from the rabbit) was employed as the haemolytic system.

The method of carrying out the tests, where careful comparisons were made of the deviation of an antiserum along with different bacillary strains, was as follows: varying quantities of the serum were added to a fixed quantity of the antigen and then a quantitative estimation of the amount of complement deviated by these mixtures was made by adding varying amounts of complement from 3 M.H.D. up to 20 M.H.D. (for 0.5 C.C. of the test blood suspension), incubating the mixtures for 1¼ hours at 37° C., and then adding 0.5C.C. of the blood suspension. After a further hour's incubation the results were read.

Control tests were also carried out to determine the number of doses of complement absorbed by the antigen and immune serum respectively. At the same time the dose of complement after incubation for 1½ hours was ascertained by adding suitable amounts of the complement-serum to tubes containing a volume of salt solution equal to that of the

antigen used in the tests, incubating along with the other tubes and then adding the test corpuscles.

The number of doses of complement deviated was taken as one less than that represented by the smallest amount in the series which produced complete lysis (complete lysis occurring when one dose is left free). The number of doses deviated by the serum and emulsion separately was deducted from the number of doses absorbed by the serum and antigen in combination, and the result represented the exact degree of deviation produced apart from the inhibitory effects of serum and bacillary emulsion. Thus any inequality in the anticomplementary action of different antigens was allowed for.

By this method in which the amount of complement deviated by varying amounts of antiserum along with a fixed quantity of bacillary antigen, is tested, it is possible to precisely differentiate B. typhosus from B. paratyphosus A and B. In one of my own experiments 0.0002 C,C. of an antityphoid serum along with a strain of B. typhosus (not that used for immunization) deviated over 17 doses of complement while with B. paratyphosus A 0.025 C.C. of the serum was required to produce a deviation of 10 doses and 0.0002 C.C. produced practically no deviation. Thus by careful quantitative comparisons, it is possible to establish a precise species differentiation between certain allied organisms. In the case of B. typhosus the relative specificity is for the species.

Results observed with antisera to A types 4, 1 and 3.

In the case of the antiserum to strain 1, A 4 (B. Grünthal) over 15 doses of complement were absorbed by the combination of 0.001 C.C. of antiserum and the strain used for immunization while with another strain of the same type (No. 2) it was necessary to employ 0.025 C.C. of antiserum to obtain this degree of complement absorption, and on diminishing the amount of antiserum there was a rapid falling off in the amount of complement deviated (table 20). With representatives of other typical B. coli varieties, the amounts of complement deviated by different quantities of antiserum were practically the same as those deviated with the No. 2 strain of type B. 4. Thus, even by varying the amount of antiserum no demarcation could be demonstrated in the A sub-group between, for example, types 1 and 3 on the one hand and type 4 on the other. This was found to be true also in the case of antisera to types 1 and 3 of sub-group A (tables 21 and 22).

Experiments were also carried out in which the amount of antiserum was maintained constant and the quantity of antigen varied; with the antiserum to strain 1 of type 1 no differentiation could be established between another type 1 strain and a strain of a different type (type 2) (table 23).

GROUP REACTIONS.

The group reaction was then studied to ascertain its significance as regards the biological relationships between

TABLE 20.

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain 1, A 4 (B. Grünthal).	Bacillary Emulsion.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.
0.025 c.c.	0.4 c.c. Strain 1, A 4	0	0	0	0	0	Trace	Just com.
0.01 c.c.		0	0	0	0	0	...	...
0.005 c.c.		0	0	0	0	0	...	...
0.001 c.c.		0	0	0	0	Com.	...	...
0.0005 c.c.		0	0	Al. Com.	Com.	...	...	...
0.0001 c.c.		0	Dist.	Com.	...	...	...	...
0.025 c.c.	0.4 c.c. Strain 2, A 4	0	0	0	0	0	Just com.	Com.
0.01 c.c.		Dist.	Mkd.	Al. Com.	Com.	...	...	...
0.05 c.c.		Mkd.	Al. Com.	Com.	...	...	...	...
0.001 c.c.		Al. Com.	Com.	...	...	...	...	...
0.025 c.c.	0.4 c.c. Strain 1, A 3	0	0	0	0	0	V.mkd.	Com.
0.01 c.c.		Dist.	Dist.	Mkd.	Com.	...	...	...
0.005 c.c.		Dist.	Mkd.	Com.	...	...	...	...
0.001 c.c.		Mkd.	Just com.	...	...	...	...	...

Table continued on next page; meaning of contractions given also on next page.

TABLE 20. (Contd.)

Lysis of 0.5 c.c. 5% suspension ox blood + 5" doses immune body.

Antiserum to Strain 1, A 4 (B. Grünthal).	Bacillary Emulsion.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.
0.025 c.c.	0.4 c.c. Strain 1, A 1	0	0	0	0	0	Mkd.	Al. com.
0.01 c.c.		0	Dist. Trace	V.mkd.	Com.	...	...	...
0.005 c.c.		0	Dist. Trace	Al. com.	...	...	...	...
0.001 c.c.		Trace	Mkd.	Com.	...	...	...	...

0.025 c.c. Antiserum alone deviated 2 D of Complement.

0.01 " " " " no "

In this and in subsequent tables:

C. or Com.--complete lysis
Al.C. or Al.Com.--almost complete lysis
V.mk. or V.mkd. --very marked lysis
Mk. or Mkd.--marked lysis
Dt. or Dist.--distinct lysis
Tr. or Trace --trace of lysis
F.Tr. or F.Trace --faint trace of lysis
0 -- no lysis

TABLE 21.

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain I, A 1 (B. No.71 McC)	Bacillary Emulsion 0.4 c.c.	Doses of Complement					Complement deviated by Emulsion Alone.	
		2 D.	4 D.	7 D.	12 D.	20 D.	2 D.	4 D.
0.01 c.c.	Strain I, A 1. (B. No. 71).	0	0	0	0	0	...	...
0.005 c.c.		0	0	0	0	0	...	...
0.001 c.c.		0	0	0	0	0	Mkd.	Just com.
0.0005 c.c.		0	0	0	0	0	...	...
0.0001 c.c.		0	Dist.	Just com.	...	...	...	...
0.01 c.c.	Strain 2, A 1.	0	0	0	0	0	...	...
0.005 c.c.		0	0	0	Trace	Com.	Mkd.	Just com.
0.001 c.c.		0	Dist.	Mkd.	Al. com.	Com.	...	...
0.01 c.c.	Strain 1, A 6 (B.neapolitanus)	0	0	0	0	Trace	...	...
0.005 c.c.		0	0	0	Trace	V.mkd.	V mkd.	Just com.
0.001 c.c.		0	Mkd.	Mkd.	V.mkd.	Com.	...	...

Table continued on next page

TABLE 21. (Contd.)

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain I, A 1 (B. No.71 McC.)	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		2 D.	4 D.	7 D.	12 D.	20 D.	2 D.	4 D.
0.01 c.c.	Strain 1, A 2 (B. coli Communis)	0	0	0	0	Trace	...	...
0.005 c.c.		0	0	0	Trace	Mkd.	Mkd.	Just com.
0.001 c.c.		0	Trace	Mkd.	V.Mkd.	Com.	...	...
0.01 c.c.	Strain 1, A 4 B.Grün-thal.	0	0	Dist.	Mkd.	Al. com.	Com.	Com.
0.005 c.c.		0	Dist.	Mkd.	V.Mkd.	Al. com.	...	...
0.001 c.c.		Dist.	V.Mkd.	Al. com.	Com.	Com.	...	...

0.025 c.c. Antiserum alone deviated 2 D of Complement.

0.01 " " " " no "

TABLE 22.

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0.025 c.c.	Strain 1, A 3 (B. vesiculosus)	0	0	0	0	0	...	...	...
0.01 c.c.		0	0	0	0	0	...	...	...
0.005 c.c.		0	0	0	0	0	V.mk.	Al. com.	Com.
0.001 c.c.		0	0	0	0	0	...	...	...
0.0005 c.c.		0	0	Mkd.	Al. com.	Com.	...	...	...
0.0001 c.c.		Dist.	Mkd.	Com.	...	...	...	...	...
0.025 c.c.	Strain 2, A 3	0	0	0	0	Dist.	...	...	...
0.01 c.c.		0	0	Dist.	V.mkd.	Com.	Dist.	Al. com.	Com.
0.005 c.c.		0	Trace	Mkd.	Com.	Com.	...	...	...
0.001 c.c.		Trace	Mkd.	Com.	Com.	Com.	...	...	...
0.025 c.c.	Strain 1, A 4 (B. Grünthal)	0	0	0	Ftr.	Dist.	...	...	...
0.01 c.c.		0	0	Dist.	Al. com.	Com.	Dist.	Al. com.	Com.
0.005 c.c.		0	Trace	V.mkd.	Com.	Com.	...	...	...
0.001 c.c.		Trace	Dist.	Com.	Com.	Com.	...	...	...

Table continued on next page

TABLE 22. (Contd.)

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain 1, A 3, B. Vesiculosus.	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0.025 c.c.	Strain 1, A 9 (B.No. 106 Mc:Conkey)	0	0	0	Ftr.	Mkd.	...	...	...
0.01 c.c.		0	0	Trace	V.mkd.	Com.	Dist.	Al. com.	Com.
0.005 c.c.		0	0	Dist.	Com.	Com.	...	...	...
0.001 c.c.		Trace	Dist.	Com.	Com.	Com.	...	...	...

0.025 c.c. Antiserum alone deviated 1 D of Complement.

0.01 " " " " no "

TABLE 23.

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

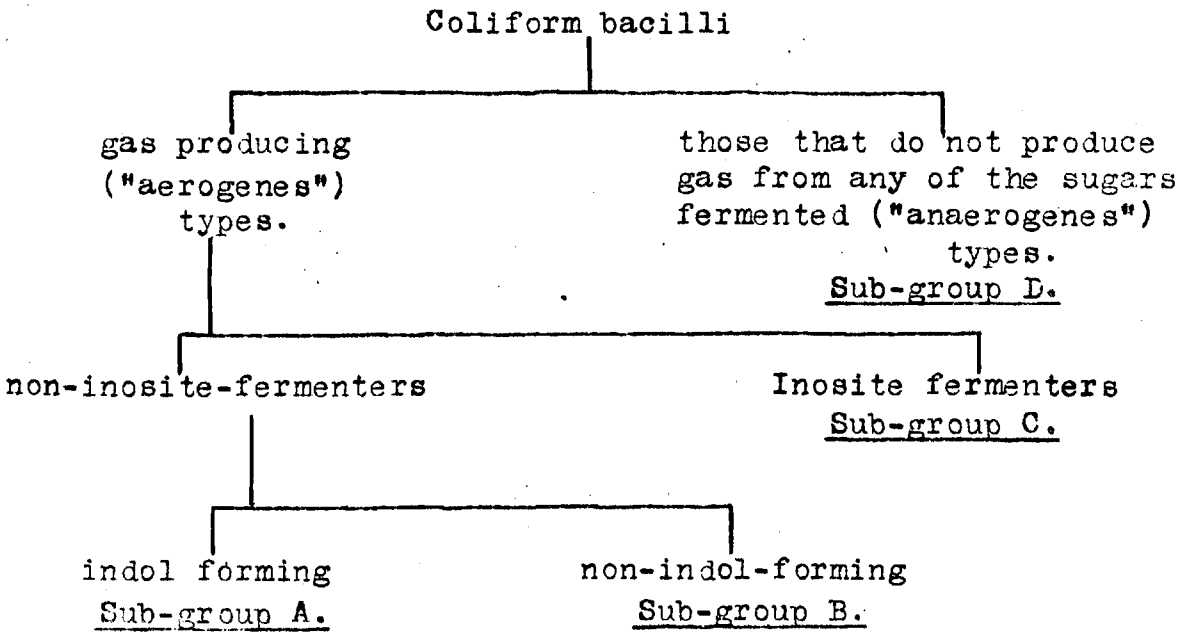
Bacillary Emulsion.	Antiserum to Strain I, A 1. (B.No.71 McConkey).	Doses of Complement.					Complement deviated by Emulsion Alone.	
	0.025 c.c.	2 D.	4 D.	7 D.	12 D.	20 D.	2 D.	4 D.
Strain I, A 1.								
0.2 c.c.		0	0	0	0	Ftr.	Com.	Com.
0.12 c.c.		0	0	Ftr.	Ftr.	Dist.	Com.	Com.
0.04 c.c.		0	Trace	Dist.	Dist.	Mkd.	Com.	Com.
0.02 c.c.		0	Dist.	Mkd.	Mkd.	Com.	Com.	Com.
Strain 1, A 2.								
0.2 c.c.		0	0	0	0	0	Com.	Com.
0.12 c.c.		0	0	0	Trace	V.Mkd.	Com.	Com.
0.04 c.c.		0	Ftr.	Mkd.	Com.	Com.	Com.	Com.
0.02 c.c.		Trace	Dist.	V.mkd.	Com.	Com.	Com.	Com.
Strain 2, A 1.								
0.2 c.c.		0	0	0	0	0	Com.	Com.
0.12 c.c.		0	0	0	Mkd.	Mkd.	Com.	Com.
0.04 c.c.		0	Ftr.	Trace	Mkd.	Com.	Com.	Com.
0.02 c.c.		0	Trace	Al. com.	Com.	Com.	Com.	Com.

0.025 c.c. Antiserum alone deviated 1 D of Complement.

different types, and the classification or grouping of these organisms.

Group action by complement deviating sera has been studied in other bacterial classes: thus Sacquépée found that the food-poisoning group could be classified into two sub-groups (Enteritis I and II). An immune serum to an organism of sub-group I reacted with other sub-group I types but not with organisms of sub-group II: similarly an antiserum to a sub-group II type reacted only with other organisms of the same sub-group.

On testing the antiserum to strain 1 type 4 (B. Grünthal) along with a large number of different coliform strains (typical and atypical) it was found that the group reaction was limited to a certain class. If these coliform bacilli are divided up as follows, as in the original classification given above (p. 15):



then sub-group A will comprise all the typical varieties, e.g., B. MacConkey No. 71, B. coli communis, etc. It was found that the group effect with the A 4 antiserum was limited to these types, i.e., to sub-group A; for example with representatives of this sub-group over 15 doses of complement were deviated in the presence of 0.025 C.C. of the antiserum while with representatives of the other sub-groups B, C and D, in the presence of the same amount of antiserum not more than 5 doses were deviated and with some no deviation was observed (table 24). Some of the strains were tested both with the A 4 antiserum and also with the A 3 antiserum and the results were found to correspond (table 24). It is noteworthy that certain representatives of sub-group A whose fermentative reactions did not correspond to those of the accepted B. coli types, deviated in the presence of 0.025 C.C. of antiserum over 15 doses of complement and even by varying the amount of antiserum one could not differentiate them from an A 4 strain other than strain 1, i.e., the strain used for immunization (table 25). Included among these were two strains which according to the usual criteria would not have been classed as typical B. coli, viz., two non-lactose-fermenters, and a strain which only fermented lactose after mutation; these strains all produced indol. The fact that such gram-negative bacilli behave in complement deviation experiments with an antiserum to a strain of typical B. coli similarly to other typical organisms

TABLE 24.

Lysis of 0.5 c.c. 5% suspension ox blood + 5 doses immune body.

Antiserum to Strain 1, A 4(B. Grünthal) — 0.025 c.c.

Different Strains	of Type	1	1	2	1	1	2	2	3	4	3	5	4	5	6	7	6	30	11	10	2	3	9	101	34	19	19	15	12	22	9	1	
Bacillary Emulsion 0.4 c.c.	Sub-group	A	C	C	Coli anaerogenes D	B	B	A	B	B	A	B	A	A	A	A	B	A	A	B	Coli anaerogenes D	C	B	B Paracolon	A	A	A	A	B. paratyph. B	A, conjunctiva	A	A	B. proteus
Doses of Complement.	3	D	O	J.C.	V.mk.	Al.C.	Mk.	O	O	Al.C.	Dt.	O	J.C.	O	O	O	O	O	O	O	Al.C.	O	C	C	C	O	O	O	O	O	C	Mk.	Tr.	C	C
	5	D	O	C	C	C	Al.C.	Mk.	O	C	mk.	O	C	O	O	O	O	Tr.	O	O	C	Tr.	C	C	C	O	O	O	O	O	C	Al.C.	Dt.	C	C
	10	D	O	C	C	C	C	C	O	C	C	O	C	O	O	O	O	Mk.	O	O	C	C	C	C	C	O	O	O	O	O	C	C	Al.C.	C	C
	15	D	O	C	C	C	C	C	O	C	C	O	C	O	O	O	O	C	O	OO	C	C	C	C	C	O	O	O	O	O	C	C	C	C	C
	20	D	O	C	C	C	C	C	Tr.	C	C	Tr.	C	O	Tr.	O	O	C	Tr.	Tr.	C	C	C	C	C	O	O	O	O	O	C	C	C	C	C

Antiserum to Strain 1, A 3(B, Vesiculosus) — 0.025 c.c.

Doses of Complement.	3	D		Dt.	V.mk.	Dt.	Dt.		Tr.	O	J.C.	O	O	O	O	O													Tr.	C	Mk.	Tr.	C	
	5	D		Al.C.	C	V.mk.	V.mk.		Dt.	O	J.C.	O	O	O	O	Tr.													Tr.	C	C	Dt.	C	
	10	D		C	C	C	C		C	O	J.C.	O	O	O	O	Mk.													Tr.	C	C	Al.C.	C	
	15	D		C	C	C	C		C	O	J.C.	O	Tr.	O	O	C.													Mk.	C	C	C	C	
	20	D		C	C	C	C		C	Tr.	J.C.	Mk.	Dt.	Dt.	O	C													V.mk.	C	C	C	C	
Complement deviated by emulsion alone.			8D	2D	3D	2D	4D	5D	4D	5D	4D	3D	2D	4D	5D	4D -6D	3D	7D	3D	4D	4D	6D	2D	2D	2D	4D	4D	6D	4D	4D	3D	4D	4D	3D	4D

TABLE 25.

Antiserum to Strain 1, A 4 (B. Grünthal).	Bacillary Emulsion.	Doses of Complement.					Complement deviated by Emulsion Alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0.025 c.c.	0.4 c.c. Strain 2, A 4. Lactose + Indol +	0	0	0	0	0	Mkd.	Com.	...
0.01 c.c.		0	0	Trace	Dist.	Al. Com.	...	...	...
0.005 c.c.		0	0	V.mkd.	Com.	...	...	...	...
0.001 c.c.		Trace	Dist.	Al. com.	...	...	...	...	...
0.025 c.c.	0.4 c.c. Strain A 12. Lactose — Adonite — Inosite — Indol +	0	0	0	0	0	Mkd.	Com.	...
0.01 c.c.		0	0	0	Dist.	V.mkd.	...	...	...
0.005 c.c.		0	Trace	Al. com.	Com.	...	...	...	...
0.001 c.c.		Trace	Dist.	Al. com.	...	...	...	...	...
0.025 c.c.	0.4 c.c. Strain A 19. Lactose + after muta- tion. Adonite — Inosite — Indol +	0	0	0	0	0	...	Mkd.	Com.
0.01 c.c.		0	0	0	Trace	Mkd.	...	...	...
0.005 c.c.		0	0	Dist.	Mkd.	Com.	...	...	...
0.001 c.c.		0	Dist.	V.mkd.	Com.	...	...	...	...

Table continued on next page

TABLE 25. (Contd.)

Antiserum to Strain 1, A 4 (B. Grünthal).	Bacillary Emulsion.	Doses of Complement.					Complement deviated by Emulsion Alone.		
		2 D.	5 D.	10 D.	15 D.	20 D.	2 D.	5 D.	7 D.
0.025 c.c.	0.4 c.c. Strain A 15. Lactose+ Adonite+ Inosite− Indol +	0	0	0	0	0	Mkd.	Com.	...
0.01 c.c.		0	0	0	0	Trace	...	...	...
0.005 c.c.		0	0	Dist.	Com.	...	...	...	...
0.001 c.c.		0	Trace	Com.	...	...	...	...	...
0.025 c.c.	0.4 c.c. Strain A 34. Lactose− Indol + Inosite−	0	0	0	0	0	Mk.	Com.	...
0.01 c.c.		0	0	0	Trace	Com.	...	...	...
0.005 c.c.		0	Dist.	V.Mk.	Al. Com.	Com.	...	...	...
0.001 c.c.		Trace	Dist.	Com.	Com.	Com.	...	...	...
0.025 c.c.	0.4 c.c. Strain A 6. Lactose+ Indol + Inosite −	0	0	0	0	0	...	...	...
0.01 c.c.		0	0	0	0	0	Dist.	Al. Com.	Com.
0.005 c.c.		0	0	Trace	Al. Com.	Com.	...	...	...
0.001 c.c.		0	Dist.	Com.	Com.	Com.	...	...	...

strongly suggests that lactose fermentation which has always been considered one of the most important characters of B. coli can hardly be taken as of more importance biologically than other sugar reactions.

Among the 31 strains of sub-groups A, B, C and D examined in this way, three A types (No. 1, $\frac{9}{5}$ and 22) did not react characteristically with the A 4 and A 3 antisera, i.e., the group reaction was absent in the case of the No. 1 and 22 strains and not well marked in the case of the No. $\frac{9}{5}$ strain (table 24). The No. 22 strain was isolated from a case of conjunctivitis in which it was present in pure culture; the No. 1 and $\frac{9}{5}$ strains were of direct faecal origin. On the other hand no representatives of the other sub-groups B, C and D exhibited any reaction with the A 4 and A 3 antisera.

Results observed with an antiserum to a No.1 type sub-group B.

The specificity and group action of an antiserum to a strain belonging to one of the common types of sub-group B was also investigated. The antiserum to strain $\frac{3}{1}$, B 1 (B. MacConkey No. 74) whose agglutinating properties have already been referred to was employed for this purpose.

It was found that the serum displayed no specificity either for the individual strain or the type to which it belonged. In fact certain strains of other different types within the sub-group B exhibited as much affinity (as determined by complement deviation tests) for the serum as the strain used for immunization (table 26). The group reaction was strictly

TABLE 26.

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1. (B. No. 74 MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.005 c.c.	Strain 3, B 1. (B. No. 74, MacConkey)	0	0	0	0	0	...	...
0.001 c.c.		0	0	Dist.	Al. Com.	Com.	Mk.	Com.
0.0005 c.c.		0	Dist.	Mk.	Com.	Com.	...	...
0.0001 c.c.		Trace	V.mk.	Al. Com.	Com.	Com.	...	...
0.025 c.c.	Strain 2, B 1 (B. No. 74, McConkey)	0	0	0	0	0	...	...
0.01 c.c.		0	0	0	Trace	Al. Com.	Mk.	Com.
0.005 c.c.		0	0	Trace	Al. Com.	Com.	...	...
0.005 c.c.	Strain 1, B 2.	0	0	0	Trace	Dist.	...	...
0.001 c.c.		0	Trace	Dist.	Mk.	V.mk.	V.mk.	Just com.
0.0005 c.c.		0	Trace	Mk.	Al. Com.	Com.	...	...
0.0001 c.c.		Trace	Mk.	V.mk.	Just com.	Com.	...	...

Table continued on next page

TABLE 26. (Contd.)

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1. (B. No. 74 MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.005 c.c.	Strain 2, B 2.	0	0	0	0	Trace	...	...
0.001 c.c.		0	Trace	Dist.	Mk.	Al. Com.	Mk.	Com.
0.0005 c.c.		0	Trace	Mk.	Just com.	Com.	...	...
0.0001 c.c.		Trace	Mk.	Al. Com.	Just com.	Com.	...	...

0.025 c.c. Antiserum alone deviated 1 D of Complement.

limited to types belonging to the B sub-group. Thus with a number of strains of sub-group A, C and D practically no deviation was obtained (table 27).

In the case of sub-group A it was found that by deviation tests non-lactose-fermenters and organisms which only fermented lactose after mutation could be classed along with the typical lactose fermenting types. In sub-group B non-lactose-fermenters (B. paracolon types) and strains which developed lactose-fermenting mutants (B. coli mutabilis types) could not be identified with the lactose fermenting types, i.e., the group action of an antiserum to a lactose fermenter was limited to those types which fermented lactose in primary culture.

As a result of these serological findings some indication has been elicited of the biological grouping of the different cultural types of coliform bacilli.

The results may be summarized as follows:-

In sub-group A (gas-forming, indol +, inosite —, gelatin —) as determined by tests with antisera to types 1, 2, 3 and 4 there is a high degree of specificity of the agglutinin for the individual strain used for immunization, but no evidence of specificity for the type, or group action towards other organisms of the sub-group; there is a relative

TABLE 27.

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1 (B. No. 74 MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.05 c.c.	Strain 2, B 1 (B. No. 74 (McConkey)	0	0	0	0	Trace	...	...
0.025 c.c.		0	0	Etr.	Dist.	Mk.	Mk.	Com.
0.01 c.c.		0	0	Dist.	V.mk.	Com.	...	....
0.005 c.c.		0	Trace	Mk.	Com.	Com.	...	...
0.05 c.c.	Strain of B 3.	0	0	0	0	Dist.	...	...
0.025 c.c.		0	0	Trace	Dist.	Mk.	V.mk.	Com.
0.01 c.c.		0	Trace	Dist.	Mk.	Com.	...	...
0.005 c.c.		0	Dist.	V.mk.	Com.	Com.	...	...
0.05 c.c.	Strain of B 10.	0	0	0	0	0	...	...
0.025 c.c.		0	0	0	Trace	Dist.	Dist.	Just Com.
0.01 c.c.		0	0	Trace	Dist.	Al. Com.	...	...
0.005 c.c.		0	Trace	Dist.	Al. Com.	Com.	...	...

Table 27 continued on next 4 pages

TABLE 27. (Contd.)

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1 (B. No. 74 (MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.05 c,c.	Strain of B 10.	0	0	0	0	0	...	...
0.025 c.c.		0	0	0	0	Dist.	Dist.	Just Com.
0.01 c.c.		0	0	Dist.	Mk.	V.mk.	...	...
0.005 c.c.		0	Trace	Dist.	V.mk.	Com.	...	...
0.05 c.c.	Strain of B 9.	0	0	0	0	0	Mk.	Com.
0.05 c.c.	Strain of B 4.	0	0	0	0	0	Mk.	Com.
0.05 c.c.	Strain A 1, B. No. 71 McConkey)	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0.05 c.c.	Strain of A 6 neapoli-tanus.	V.mk.	Com.	Com.	Com.	Com.	V.mk.	Com.

TABLE 27. (Contd.).

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1 (B. No. 74 (MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.05 c.c.	Strain of A3 of B. coli communis	Al. Com.	Com.	Com.	Com.	Com.	Al. Com.	Com.
0.05 c.c.	Strain of C 1.	Mk.	Com.	Com.	Com.	Com.	Mk.	Just Com.
0.05 c.c.	Strain of C 2.	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0.05 c.c.	Strain of D 6 Coli Anaero-genes.	Mk.	Com.	Com.	Com.	Com.	Mk.	Com.
0.05 c.c.	B. para-typhosus	Dist.	Mk.	Com.	Com.	Com.	Mk.	Com.
0.025 c.c.	B.	Mk.	Al. Com.	Com.	Com.	Com.	...	...
0.01 c.c.		V.mk.	Com.	...	...	...	...	...

TABLE 27. (Contd.)

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B 1 (B. No. 74 MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.05 c.c.	Strain of para-colon bacillus Lactose – Indol – Inosite – Glucose + B 101.	Dist.	Mk.	Al. Com.	Com.	Com.	...	...
0.025 c.c.		Dist.	V.mk.	Com.	Com.	Com.	...	Just Com.
0.01 c.c.		Mk.	V.mk.	Com.	Com.	Com.	...	...
0.005 c.c.		Mk.	Com.	Com.	Com.	Com.	...	...
0.05 c.c.	Strain of para-colon bacillus B 103.	Trace	Dist.	Al. Com.	Com.	Com.	...	...
0.025 c.c.		Dist.	Mk.	Just Com.	Com.	Com.	...	Com.
0.01 c.c.		Dist.	V.mk.	Com.	Com.	Com.	...	...
0.005 c.c.		Dist.	Com.	Com.	Com.	Com.	...	....
0.05 c.c.	Strain of B. coli mutabilis B 103 (before mutation)	Mk.	Al. Com.	Com.	Com.	Com.	...	...
0.025 c.c.		V.mk.	Com.	Com.	Com.	Com.	Just Com.	Com.
0.01 c.c.		V.mk.	Com.	Com.	Com.	Com.	...	...
0.005 c.c.		Just Com.	Com.	Com.	Com.	Com.	...	...

TABLE 27. (Contd.)

Lysis of 0.5 c.c. 5% ox blood suspension + 5 doses immune body.

Antiserum to Strain 3, B. 1 (B. No. 74 MacConkey).	Bacillary Emulsion 0.4 c.c.	Doses of Complement.					Complement deviated by Emulsion Alone.	
		3 D.	5 D.	10 D.	15 D.	20 D.	3 D.	5 D.
0.05 c.c.	Strain of B 105 Lactose-(+ after mutation). Inosite — Indol -	Mk.	Com.	Com.	Com.	Com.	Just Com.	Com.
0.05 c.c.	Strain of B. Lactose-(+ after mutation) Inosite — Indol - B. 102.	V.mk.	Com.	Com.	Com.	Com.	Just Com.	Com.
0.05 c.c.	B.proteus	Mk.	Com.	Com.	Com.	Com.	Just Com.	Com.

0.05 c.c. of Antiserum alone deviated 1 D of complement.

specificity of the complement deviating immune body, as determined with antisera to types 1, 3 and 4, for the individual strain but not for the cultural type; there is, however, a well marked group reaction limited to strains of the sub-group irrespective of other cultural reactions (e.g., lactose, dulcite, saccharose, inulin fermentation, motility) and not extending to the B, C or D sub-groups, B. paratyphosus B, or B. proteus.

In sub-group B as determined by observations with antisera to types 1 and 2, ~~there is a more limited degree of~~ the specificity of agglutinating antisera ~~for the individual strain~~ is less restricted, but there is not complete specificity for the cultural type; there is absence of relative specificity of the complement deviating antibody (antiserum to type 1) for the individual strain and the group reaction, as far as my observations go in the case of the types tested, is limited to the lactose-fermenting types and does not extend to the A, C or D sub-groups, the non-lactose fermenting types of the B sub-group, the types which only fermented lactose after mutation, B. paratyphosus B or B. proteus.

While among the indol +, inosite —, gas + types lactose fermentation appeared of no more significance than certain other reactions, in the case of the indol — inosite — gas + types, the lactose fermenting types seemed

to be separately grouped as apart from the non-lactose-fermenters and those which only fermented lactose after mutation.

Also in the case of two types of non-lactose-fermenters of the B sub-group exact specificity of the agglutinin for the type or species was noted and this also differentiated these organisms from the lactose fermenting types.

The comparative resistance to brilliant green of different types of coliform bacilli with reference to the classification of these organisms.

In the course of certain observations on the enrichment of B. typhosus by culture from faeces in fluid media containing brilliant green (Browning, Gilmour and Mackie) it was noted that different types of B. coli exhibited different degrees of susceptibility to this chemical. The " typical " varieties (sub-group A types 1, 2, 3, etc.) were completely inhibited in their growth on culture medium by concentrations of brilliant green which had no effect on B. typhosus, but it was noted that the inosite fermenters (sub-group C) on the contrary exhibited a resistance to the dye greater even than that of the typhoid bacillus.

As this appeared to be a striking difference between two B. coli sub-groups already classified separately on an entirely different basis, there seemed some likelihood that the study of

the behaviour of different coliform types towards this dye might throw some further light on the biological relationships of the various cultural types.

For this purpose a series of representative strains from the four subgroups were tested as regards the inhibition pf their growth on peptone-water-agar by different quantities of brilliant green incorporated in the medium.

The concentrations of the dye tested were 0.I6, 0.22, 0.32, 0.42 C.C. of a I:I0,000 watery solution in I0 C.C. of the medium.

Emulsions of the various organisms were prepared in sterile salt solution, of such density that the fluid showed a mere trace of turbidity to the eye, and cultures were made by taking one loopful of the emulsion and spreading it on the medium in stroke form(one stroke only). On ordinary medium this inoculation produced an abundant line of growth along the needle tract. One plate was, of course, used to accommodate several strokes from different organisms.

The plates were incubated for 48 hours and readings were made after 24 and 48 hours.

With two exceptions, all the sub-group A organisms tested proved relatively susceptible (as compared with B. typhosus) to the dye , including non-lactose-fermenters (table 28), the exceptions were (I) a strain of type 9 and (2) a strain of type 22 (isolated from a case of conjunctivitis);

TABLE 28.

Brilliant Green 1:10,000 watery solution per 10 c.c. peptone-water-agar.

Observations after 24 hours incubation.

Strains of	1	2	3	4
	0,16 c.c.	0.22 c.c.	0.32 c.c.	0.42 c.c.
Sub-group A.				
Type 1 (B.MacConkey No.71)	++	-	-	-
" 3 (B.vesiculosus)	+	+	-	-
" 3	++	-	-	-
" 5 (B.Schafferi)	+	-	-	-
" 1	++	-	-	-
" 6 (Neapolitanus)	+	-	-	-
" 4 (B. Grünthal)	+	-	-	-
" 3	+	+	-	-
" 6	+	-	-	-
" 15	+	-	-	-
" 30	+	-	-	-
" 2	++	-	-	-
" 35 (Lactose -)	-	-	-	-
" 2	+	-	-	-
" 9	+	+	-	-
" 13	-	-	-	-

(Table 28 continued on next 3 pages)

TABLE 28. (Contd.)

Strains of	1 0.16 c.c.	2 0.22 c.c.	3 0.32 c.c.	4 0.42 c.c.
Sub-group A.				
Type 28	+	-	-	-
" 33	+	-	-	-
" 12	++	-	-	-
" 9	+++	+++	++	-
" 2	++	-	-	-
" 4	+	-	-	-
" 3	+	-	-	-
" 1	++	-	-	-
" 22	++++	+++	++	-
B.typhosus	++++	++++	++	-
B. Proteus	+	-	-	-
Sub-group B.				
Type 1	++++	++++	++	++
" 9	++++	++++	++	+
" 10	++++	++++	++	++
" 2	++++	++++	++	-
" 2	+++	+++	++	-
" 10	+++	+++	+	-
" 1	++++	+++	++	+

TABLE 28. (Contd.)

Strains of	1	2	3	4
	0.16 c.c.	0.22 c.c.	0.32 c.c.	0.42 c.c.
Sub-group B.				
Type 101 (paracolon)	-	-	-	-
" 104	-	-	-	-
" 103 (paracolon)	-	-	-	-
" 106	-	-	-	-
" 103 developed lactose fermenting mutant	++++	++++	++++	++++
" 102 developed lactose fermenting mutant	++++	++++	++++	++++
" 105 lactose fermenting mutant	++++	++++	++++	++++
" 102 Lactose fermenting mutant	++++	++++	++++	++++
" 103 lactose fermenting mutant	++++	++++	++++	++++
Sub-group C.				
Type 1	++++	++++	++++	++++
" 2	++++	++++	++++	++++
" 5	++++	++++	++++	++++
" 1	++++	++++	++++	++++

TABLE 28. (Contd.)

Strains of Sub-group C	1 0·16 c.c.	2 0·22 c.c.	3 0·32 c.c.	4 0·42 c.c.
Type 2	++++	++++	++++	++++
" 4	++++	++++	++++	++++
" 3	++++	++++	++++	++++
" 9	++++	++++	++++	++++
Sub-group D.				
Type 1	++++	++++	+++	+++
" 3	-	-	-	-
" 5	-	-	-	-
" 7	++	-	-	-
" 4	++++	+	-	-
" 2	+	-	-	-

The amount of the resulting growth is denoted by: ++++, +++, ++, +; — signifies no growth.

another strain of type 9, however, corresponded in its behaviour to the other A types. It is noteworthy that these two strains also differed in the complement deviation experiments from other A types.

The strains of sub-group B showed some variation in their resistance to brilliant green as might have been expected from the biological differences elicited by the serological tests.

Those belonging to the series 1-11 (fermenting lactose in primary culture) all showed a higher degree of resistance to the dye than the A types and equal to or slightly greater than that of B. typhosus. Those types which developed lactose-fermenting mutants exhibited a high degree of resistance, i.e., much greater than that of series 1-11 or B. typhosus, while the paracolon types were apparently less resistant even than the A types.

In the B sub-group, therefore, the various types could be classified into 3 categories according to their resistance to brilliant green correlated with certain cultural characters and reactions.

In the C sub-group all the strains tested exhibited a high resistance to brilliant green, equal to that shown by the mutating strains of sub-group B.

In the D sub-group there was some variation in the susceptibility of different types, but the number of strains

available for testing was too limited to draw any inferences from the results.

These findings correlated with the serological observations are of considerable interest; from the serological study, it was concluded that the gas +, indol +, inosite — types could be grouped together apart from the other organisms of the B. coli group. The tests carried out with these organisms growing on brilliant green media also show the striking distinction between organisms of the sub-group A on the one hand and the C types and also certain of the B types on the other.

While the A types (with few exceptions) are all more or less similar in their behaviour and the same is also true for the C types, various B and D types behave differently.

In the B sub-group the lactose-fermenters were differentiated serologically from the paracolon varieties and those which only fermented lactose after mutation; in the brilliant green resistance tests a corresponding difference was established.

These experiments, therefore, apart from the practical bearing they had in connection with the brilliant green enrichment process for the isolation of B. typhosus were of considerable interest in correlation with the previous work on the classification of the B. coli group.

VARIATION AMONG THE COLIFORM BACILLI

Variation in Gas Production.

Among these organisms certain anomalies have been noted as regards this property (Mair, Wilson and others); thus, strains when first isolated may show complete absence of gas production, but on repeated subculture develop this property. Reference has already been made to strains of paracolon bacilli (p. 21) which in primary culture simulated B. typhosus in their cultural reactions. The possibility of this variation must be considered, therefore, in the practical identification of intestinal bacilli. A B. paratyphosus A in the first cultures made after isolation may show complete absence of gas production and if it only ferments dulcite slowly as is often the case, it may thus simulate B. typhosus in cultural characters. B. dysenteriae Shiga may also be simulated by a non-motile organism which after repeated subculture ferments glucose with gas production, though in primary culture it produces no gas (of glucose, lactose, dulcite, saccharose, mannite, maltose, only glucose fermented).

My attention was first drawn to this variation by the occurrence of a strain of B. coli (B 1) which, when tested shortly after isolation produced gas from dulcite only and when retested after a month's culture was found to have attained the power of producing gas from lactose and saccharose though still

non-gas-producing in glucose. Later it also acquired the power of fermenting glucose with gas formation.

Though the absence of gas production in the case of B. typhosus and B. dysenteriae is known to be a stable character of these organisms, the question arose as to whether " anaerogenes " types of coliform bacilli simply represented variant strains of aerogenes types.

While certain strains have been noted which immediately after isolation produced no gas from any of the sugars fermented (e.g. B 101), it was found that in many cases gas production was only absent in certain of the sugar tests (e.g. strain of B 1 quoted above). Also, as a general rule, such organisms after a few subcultures quickly developed the property of gas production, i.e., the character seemed to be only in abeyance. On the other hand the various strains classified as " anaerogenes " (sub-group D) even after repeated subculture and after being kept in artificial growth for long periods, still remained non-gas-producing.

It has been shown by Penfold that by growing B. coli on monochloracetic acid agar a variant strain could be selected out which differed from the original in the absence of gas formation in certain sugars. With a view to determining the possibility of transmuting an aerogenes type into an anaerogenes variety, certain B. coli strains were submitted to Penfold's procedure.

Method: the monochloracetic acid was made up in a 10% watery solution and after having been made slightly alkaline to litmus by adding sodium carbonate, was sterilized by filtration through a Maassen filter. The solution was then incorporated in a 2% peptone-water-agar in measured proportions. The percentages indicated below are expressed in terms of the amount of the acid in the quantity of medium used for plating.

In the first experiment a series of plates each of 10 C.C. of monochloracetic-acid-agar, the acid being in the following proportions:-

1	2	3	4
0.05%	0.1%	0.5%	1.0%

were inoculated with a typical B. coli communis strain. On plate 1 a normal amount of growth was obtained but the colonies varied considerably in size. On plate 2 the difference in the size of the colonies was more marked and many of the larger colonies showed papillae as described by Penfold. On plates 3 and 4 no growth appeared. A subcultivation on ordinary agar was made from a large colony on plate 2, and from this, plates containing the following concentrations of monochloracetic acid were inoculated as before.

1	2	3	4	5
0.2%	0.3%	0.4%	0.5%	0.7%

On all these plates abundant growths were obtained and all the colonies were of the large type. A subcultivation was again made on ordinary agar from plate 5 and from this, plates containing the acid in still higher proportions were inoculated:

1	2	3
0.5%	1%	2%

Growth was abundant on plates 1 and 2; in the case of plate 3 the growth was slower in appearing, but ultimately a few large colonies developed. Thus a monochloracetic acid resistant strain was selected out and subcultures on ordinary medium when tested were found to produce:

acid only from glucose;
acid and considerably reduced amount of gas from lactose;
Ditto. from galactose;
acid and gas (in a slightly reduced amount) from dulcite;
Ditto. from mannite.

A similar test was carried out with a sub-group A type 1 strain; the selected strain capable of growing on 2.5% monochloracetic acid agar showed also absence of gas production in glucose, considerably reduced gas production in lactose and galactose, and slightly reduced gas production from dulcite and mannite. Other strains of typical B. coli and a B. proteus were tested with similar result, as regards the particular sugars of the above series fermented.

The results differed from those of Penfold * in that the only " sugar " (of 2 monosaccharides, a disaccharide and 2 hexahydric alcohols) from which these variants completely failed to produce gas, was glucose, though in the case of lactose and galactose there was some depression of the gas producing property.

All these variants maintained their stability as regards the new character even after several months subculture on ordinary agar, but by subculturing every day in glucose peptone water for a week, a reversion of the strain was noted and the power of producing gas was regained.

Harden and Penfold found that, from the biochemical standpoint, the change was more a quantitative than a qualitative one. Thus from my observations it was only possible to completely abolish the gas production of B. coli in the case of glucose, and it was also shown that the original character could be easily regained under certain conditions. It was in no way possible to select from an aerogenes B. coli type a corresponding anaerogenes variety.

The absence of gas production after several subcultures may, therefore, be regarded as a fundamental character of certain coliform types.

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* Penfold's variant strains showed absence of gas production in lactose as well as glucose.

Variation in biochemical characters among the coliform bacilli.

Such variations have been observed occurring spontaneously in culture medium and afford some indication of the process of evolution going on among these organisms under natural conditions. The marked diversity of types in this group as regards cultural reactions has been well shown by the work of all those who have studied these organisms and in the serological observations already recorded the highly specialized characters of individual strains have been alluded to; it may be assumed, therefore, that new types or species are constantly being developed from pre-existing varieties and that these bacilli tend to acquire new characters which are not spontaneously lost, i.e., that the tendency is a progressive one and represents the origin of new species.

In 1907 Massini* described a non-lactose-fermenting gram-negative bacillus (B. coli mutabilis) which on Endo-agar developed lactose fermenting mutants represented by red papillae on the pale colonies of the original strain and after further subculture as red colonies. This was corroborated by Burk and later by Müller in the case of the fermentation of other carbohydrates.

In studying the fermentative reactions of coliform bacilli it has been noted that certain strains may not show

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* Reported also by Neisser (1906).

any obvious change in a particular sugar, e.g. lactose, until the lapse of several days; organisms of this type were investigated by Penfold who showed that these slowly fermenting strains were primarily non-lactose-fermenters which in fluid media threw off lactose fermenting variants, the variant producing rapid fermentation of the sugar. Thus the obvious difference between the variant and the original strain lay in the rate of fermentation of lactose. The strains studied were characterized by the formation on differential medium (such as MacConkey's neutral red lactose agar or Endo-agar) of pale colonies which developed after a few days red papillae as in the case of Massini's strain.

A number of strains were met with in the course of my own study of the B. coli group which though primarily non-lactose-fermenters, in fluid medium containing lactose apparently threw off lactose fermenting variants. All these organisms formed pale colonies on MacConkey's agar; certain of them corresponded to the type described by Penfold in developing red papillae. By subculturing the papillae on another plate of MacConkey's medium, red colonies were grown representing a variant which differed from the original strain in producing rapid fermentation of lactose in fluid medium, whereas the sugar was only fermented after several days (and often without gas production) by the original strain.

Some of these organisms, on the other hand, showed no evidence of mutation on solid media containing lactose (e.g. MacConkey's agar) i.e. red papillae were not observed, but on subinoculating from the fluid lactose medium on MacConkey's agar a mixture of pale and red colonies was obtained; the pale colonies corresponded to the primary strain, the red colonies to the variant.

Similarly strains were noted which mutated as regards the fer-mentation of other sugars, e.g. dulcite, saccharose, adonite.

The variations exhibited by these strains and the differences between the original and the variant strain are shown in table 29.

It is noteworthy that the majority of these mutating strains were of the B subgroup.

While most of these variations were in the fermentation of lactose, it has been shown how similar mutations may occur in the fermentation of dulcite, saccharose and adonite.

It may also be noted here that no variations have been met with as regards the fermentation of inosite. Similarly the presence (or absence) of indol formation has been found to represent a stable character. Of course, quantitative variations in indol production have been observed and it has been shown how the amount of indol produced may be increased

TABLE 29.

In this table: A = acid; G = gas, the amount of gas production being indicated by G +, G ++, G +++; C = clot (from milk).

Strain of type.	Days incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
B 102	1	—				—	Colonies on MacConkey's agar developed red papillae after 3 days.
	2	—				A	
	5	A				AC	
Variant	1	AG +++				AC	
B 104	1	—				A	Colonies on MacConkey's agar developed red papillae after 4 days.
	2	—				A	
	5	AG +				AC	
Variant	1	AG +				AC	
D 1	1	—				—	Absence of papilla formation.
	2	—				—	
	5	—				—	
	10	A				A	
Variant	1	A				A	
	2	A				AC	

Table 29 continued on next 5 pages

TABLE 29. (Contd.)

Strain of type.	Days incu-bation.	Lact-ose.	Dul-cite.	Saccha-rose.	Ado-nite.	Milk.	
B 107	1	—				—	Absence of papilla formation.
	2	A				A	
	5	AG +				A	
Variant	1	AG +++				A	
	2	AG +++				AC	
A 19	1	—				A	Absence of papilla formation.
	2	—				A	
	5	A				AC	
	10	AG +				AC	
Variant	1	AG ++				AC	
A 19	1	—				—	Absence of papilla formation.
	2	—				A	
	5	AG +				A	
Variant	1	AG ++				AC	
B 103	1	—				—	Absence of papilla formation.
	2	—				—	
	5	—				—	
	10	A				A	
Variant	1	AG ++				AC	

TABLE 29. (Contd.)

Strain of type.	Days incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
B 8	1		—				Absence of papillae from colonies on neutral red dulcite agar.
	2		—				
	5		—				
	10		A				
Variant	1		AG ++				
B 10 (after mutation)	1				—		Absence of papillae from colonies on neutral red adonite agar.
	2				—		
	5				—		
	10				AG +		
Variant	1				AG ++		
B 106	1	—				—	Absence of papilla formation on MacConkey's medium.
	2	—				—	
	5	—				A	
	10	A				A	
Variant	1	AG +				AC	
A 28	1			—			Colonies on saccharose neutral red agar developed red papillae after 4 days.
	2			—			
	5			—			
	10			AG +			
Variant	1			AG ++			

TABLE 29. (Contd.)

Strain of type.	Days incubation.	Lactose.	Dulcite.	Saccharose.	Adonite.	Milk.	
B 8	1			—			Absence of papillae from colonies on saccharose neutral red agar
	2			—			
	5			A			
Variant	1			AG +			
B 105	1	—				—	Colonies on MacConkey's agar developed red papillae after 3 days.
	2	—				A	
	5	A				A	
	10	A				AC	
Variant	1	AG +				AC	
B 103	1	—				—	Colonies on MacConkey's agar developed red papillae after 3 days.
	2	—				—	
	5	A				A	
	10	A				AC	
Variant	1	AG +				AC	
A 34	1	—				—	Absence of papillae from colonies on MacConkey's agar
	2	—				—	
	5	—				A	
	10	A				A	
Variant	1	AG +				A	
	2	AG ++				AC	

TABLE 29. (Contd.)

Strain of type.	Days incu-bation.	Lact-ose.	Dul-cite.	Saccha-rose.	Ado-nite.	Milk.	
A 35	1	—				—	Absence of papillae from colonies on MacConkey's agar.
	2	—				—	
	5	A				A	
	10	A				A	
Variant	1	AG ++				AC	
B 101	1			—			Absence of papillae from colonies on saccharose agar.
	2			—			
	5			—			
	10			A			
Variant	1			AG			

Note: In all, 5 strains were noted corresponding in cultural reactions to B 102 and also developing lactose fermenting variants (represented by red papillae on the colonies on MacConkey's agar).

or diminished under certain conditions (Peckham, Horrocks). A considerable proportion of all the coliform strains examined were retested after 2-3 months from the time of their original classification and in all cases indol formation or the absence of this property proved stable.

Variation in cultural characters associated with variation in agglutinability.

It has been shown that variation in biochemical reactions is not associated with any change in the serological characters of the strain (Penfold) and this was confirmed in the case of one of the mutating strains described above.

Recently Fletcher (3) has described atypical forms of B. paratyphosus B and B. aertryck occurring under natural conditions, which in artificial culture exhibited variation both in cultural and serological characters and reverted to the typical form; the original strains were capsulated bacilli, produced slimy or " mucoid " colonies and were much less agglutinable than the classical strains; after subculture, however, typical bacilli developed from the mucoid strain and these reacted to full titre with a specific agglutinating serum. Fletcher has also described the same variation in the case of strains of dysentery bacilli.

In the course of my observations on the B. coli group a similar variation was noted; it was found that a B. vesiculosus (MacConkey) strain which produced on agar plates large, raised,

thick, opaque, slimy or " viscous " colonies and grew in the form of a thick, white, glistening, viscous growth on agar slope cultures, when grown on agar containing brilliant green in such amounts as to produce slight inhibition of growth, developed variants from the original type as regards colony characters; it was also noted that these variations were associated with differences in agglutinability by a specific serum to the original strain.

The general characters of the original strain were as follows: gram-negative non-motile bacillus; not liquefying gelatin; fermenting with gas production glucose and lactose but not dulcite, saccharose, adonite, inulin, inosite; producing indol from peptone; not yielding a Voges and Proskauer reaction. The colony characters have already been referred to. When plated out on nutrient agar containing 0.06 C.C. of a 1:10,000 watery solution of brilliant green to 5 C.C. agar, it developed two different types of colony; thick viscous colonies corresponding to those of the original strain, and thin, transparent, non-viscous colonies.

Subcultures from the thin colonies reproduced on ordinary culture medium similar thin, non-viscous colonies and thus from the original growth a variant strain could be selected out differing in cultural characters from the original strain and remaining true to type even after repeated

subculture. In all the other general characters the new strain corresponded to the original. The original culture did not show variation in colony characters when plated on ordinary medium. It had been used as a type in certain serological investigations, and there could be no doubt as to its purity since the strain had been originally obtained after three successive platings and subculture from single colonies.

An agglutinating serum had been prepared by immunization of a rabbit.

Subcultures from a thick viscous colony on the brilliant green agar plate, replated on the same medium again yielded different types of colony:

Type A - thin non-viscous colonies as in the first experiment;

Type B - small thick opaque markedly viscous colonies;

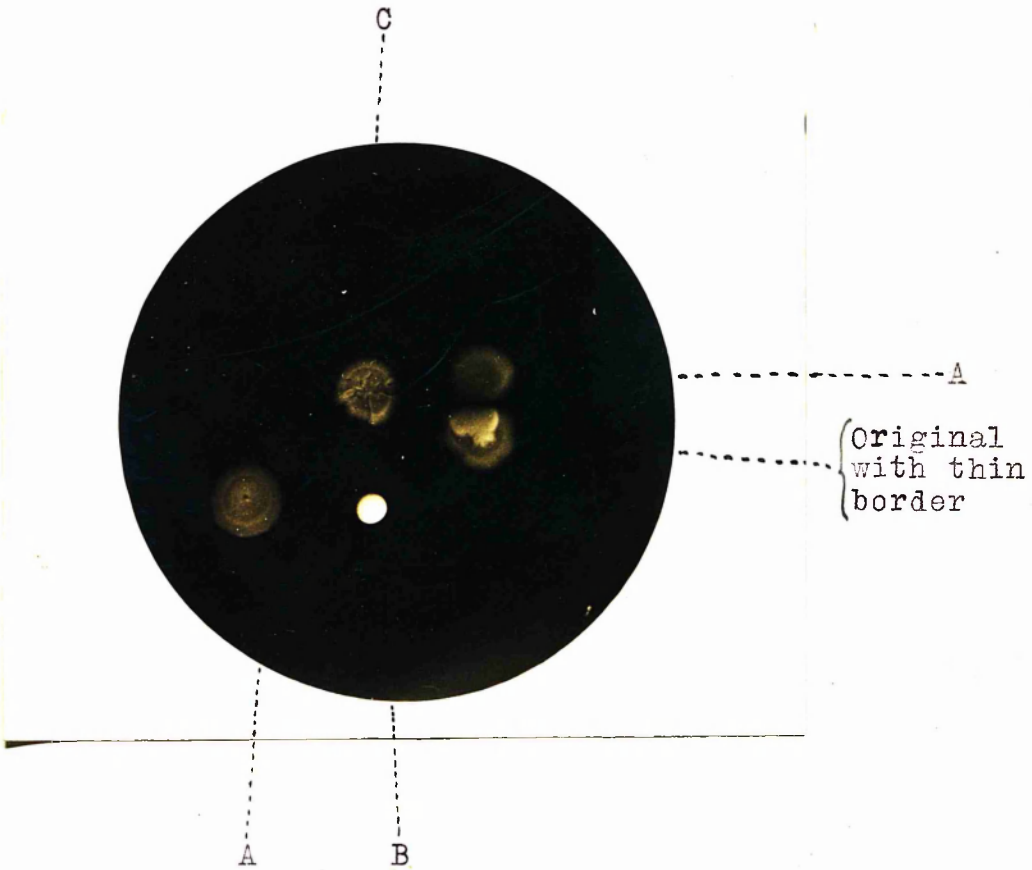
Type C - large thick opaque non-viscous colonies;

and also large opaque viscous colonies corresponding to those of the original strain.

The different appearances of these colonies are shown in the photograph.

Primary subcultures from colonies A, B and C reproduced the respective colony characters; repeated subcultures remained true to type and there was no tendency to reversion on subculture.

Photograph showing appearances of different colonies referred to in text



Colonies on agar plate after 4 days at 37°C.

On microscopic examination types B and C were more coccoid in shape, while A represented typical bacillary forms. By overstaining with Leishman's stain (10 minutes with the pure stain and 30 minutes with a 1:2 dilution in distilled water) a thick somewhat irregular capsule was demonstrated in the case of B, while A and C were apparently non-capsulate. Hiss's method failed to show a distinct capsule. It was found that, after 3 or 4 days incubation, from some of the large thick viscous colonies representing the original strain, thin transparent borders were developed and appeared to correspond to the thin colony variant; subcultures from these borders reproduced thin colonies of type A which remained true to type.

It was found later that subcultures from the large thick viscous colonies on the original brilliant green agar plate, replated on ordinary medium also developed colonies from which the type A variant grew out in the form of a thin border.

Thus from the original strain three different variants had been developed, as regards growth characters on solid culture medium; the biochemical reactions and other cultural characters of the original strain were reproduced in the new types, and it was shown how the variant A actually developed from the original type of colony.

Agglutination tests were carried out with the antiserum to the original strain, and striking differences in agglutinability were noted between the different types.

The end-titre of the reaction with the original strain was 1:1600; type A was agglutinated by the same serum in dilutions up to 1:10,000; C displayed a lesser agglutinability than the original strain, the end-titre being 1:1000; in the case of B the end-titre was 1:3000 (see table 30).

These results are of special interest as demonstrating variation in agglutinability associated with variation of other characters. On retesting these variants after several subcultures the agglutination results were found to be constant. Similar observations were made with another B. vesiculosus strain to which an agglutinating serum had been prepared.

The original strain grew in the form of large, white, opaque, non-viscous colonies. On brilliant green agar two types of colony were observed; those which represented apparently the original strain and thin transparent colonies resembling variant A of the first experiments; subcultures from these remained true to type as regards the new character but did not differ in other general characters from the original strain.

Agglutination tests yielded results which corresponded to those found in the first experiment; thus the thin colony variant again proved much more agglutinable than the original strain (see table 31). Similar mutations as regards colony characters were observed with two other B. coli strains and the variants remained true to type even after repeated subculture.

TABLE 30.

Agglutinating serum to B. vesiculosus strain H -

Serum dilutions

	1:400	1:800	1:1000	1:1200	1:1600	1:2000	1:3000	1:4000	1:6000	1:10,000	1:20,000
Original strain	++++	+++	+++	++	++	-	-	-	-	-	-
Variant A.	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	-
" C.	+++	+++	+	-	-	-	-	-	-	-	-
" B.	++++	++++	++++	++++	+++	++	+	-	-	-	-

Reading after 4 hours at 37°C.

Emulsions all of equal density.

TABLE 31.

Agglutinating serum to B. vesiculosus strain L -

Serum dilutions

	1:800	1,1000	1:2000	1,3000	1:4000	1:6000	1:8000	1:10,000	1:12000	1:15000	1:20,000
Original Strain	+++	++	++	++	+	+	-	-	-	-	-
Variant	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	-

Reading after 4 hours at 37° C.

Emulsions of equal density.

It is to be noted that many B. coli strains grown on medium containing a slightly inhibitory concentrati on of brilliant green may develop thin colonies, but on subculture the thin colonies revert to the original type.

The question, of course, arises as to whether variations in agglutinability might be dependent on morphological differences, e.g. the presence of a glutinous capsule. In the first experiments the most agglutinable variant was the A type (large thin non-viscous colonies), the least agglutinable the C type (large thick non-viscous colonies) while the original strain (large thick viscous colonies) and the B type (small thick viscous colonies) represented intermediate degrees of agglutinability. No conclusions can be drawn to show that agglutinability is influenced by the presence of a glutinous capsule. These observations are, however, of special interest in showing how the serological characters of a particular organism, interpreted by the usual methods, may vary under certain conditions.

It can be understood how a group of bacteria which are constantly developing mutants with new biochemical characters has come to represent in the course of time a considerable number of different cultural types as seen in the B. coli group; in the same way constant variation in serological characters, without change in biochemical reactions, would explain the highly specialized serological characters of the

individual strain and the absence of specificity of an antiserum for the cultural type.

The process of evolution and origin of new types going on among these gram-negative bacilli is not only of the greatest biological interest, but the study of these variations elicits some explanation of the great diversity of types and also the highly specialized serological characters among the coliform bacilli.

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P A R T 2.

OBSERVATIONS

ON THE

BACTERIOLOGY

OF

BACILLARY DYSENTERY.

INTRODUCTION.

It is a well established fact that dysentery, apart from amoebic infections, may be produced by different bacterial types, and certain classical organisms, biologically allied though specifically different, have been generally accepted as undoubted causal agents of this disease e.g. B. dysenteriae Shiga. B. dysenteriae Flexner. During the late war, dysentery was extremely prevalent in the Mediterranean and Egyptian Campaigns and the pathology of the disease attracted considerable attention both in the Military laboratories with the Expeditionary Forces and also in the home laboratories. In 1916 and 1917 bacillary dysentery attained a high incidence in the Egyptian Force and the large number of cases constantly being admitted to the Base Hospitals in Alexandria provided abundant material for a systematic bacteriological study of this condition.

Investigations of non-amoebic dysentery both in the acute and subacute stages of the disease revealed the presence of a varied bacterial flora in the intestinal discharges, comprising many unusual types foreign to the healthy intestine apart altogether from those organisms which corresponded biologically to the classical dysentery-producing bacilli.

In a not inconsiderable proportion of such cases examined in the acute stage i.e. while blood and mucus were present in

the stools, typical dysentery bacilli could not be detected. Thus in the Egyptian Expeditionary Force (1916) the percentage of acute cases* in which typical organisms (Shiga, Flexner, Y - Hiss and Russell, types) were present was 35.2 at the Base Laboratory, Alexandria,** 36.3 at the laboratory of the Australian General Hospital, Cairo and 35.2 at the State Institute of Hygiene, Cairo. (Official report of the Sanitary Advisory Committee, M.E.F., 1917). There was little variation therefore in the proportion of cases in which these organisms could be demonstrated by different observers in the same theatre of war working under different conditions and often applying different technical methods. Moreover there was no reason to suppose that these cases collectively represented amoebic infections in which amoebae were not demonstrated though as recognised by Wenyon and O'Connor microscopic examination of the faeces, unless repeated, may fail to reveal amoebae in certain cases of amoebic dysentery.

Apart from dysentery in the army it was shown by Fraser in 1916 working in the Kuala Lumpur Laboratory (Malaya) that in 50% of the cases of non-amoebic dysentery typical dysentery bacilli could not be isolated from the stools.

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*The number of cases in which typical organisms were isolated stated as a percentage of the acute cases in the total series examined.

**" Central Laboratory, Alexandria " of M.E.F. and later " The Military Bacteriological Laboratory, Alexandria " of the E.E.F.

While, among the dysentery cases that occurred in the Egyptian Force, the classical types, B. dysenteriae, Shiga and Flexner-Y, were recognised as important aetiological agents, it was apparent that other gram-negative bacilli not generally present in the normal intestinal flora, were frequently associated with non-amoebic dysentery. Certain of these organisms were closely related biologically to the classical varieties but differed in biochemical or serological characters; they were found in cases from which the classical varieties were absent and came to be spoken of generally as " atypical B. dysenteriae." Others bore no resemblance to organisms of the dysentery group though they could not be regarded as normal inhabitants of the bowel; these organisms were noted frequently as concomitants of the typical and " atypical " dysentery bacilli but in many cases they were the only unusual bacteria present in the intestinal discharges.

The question therefore arose in the investigation of bacillary dysentery in Egypt as to the exact aetiological and diagnostic significance, of these two classes of organisms, particularly the so called atypical dysentery bacilli.

It had been customary to identify the typical dysentery bacilli by certain biochemical and serological tests (v. table 2A) and organisms corresponding in these respects to the Shiga, Flexner and Y types have been accepted as undoubted dysentery bacilli. When strains were first encountered

resembling these organisms in some of their characters but atypical in biochemical reactions or inagglutinable by antisera to the classical types, the tendency was to disregard their occurrence and only the typical organisms were accepted for diagnostic purposes.

Comparatively little, however, was known at that time regarding the group of organisms biologically allied to the classical B. dysenteriae.

In 1900 Strong described a " dysentery bacillus " which differed in certain biochemical characters and in its serological reactions from the previously recognised types, though resembling them closely in other characters; this organism differed from the Flexner and Y types in fermenting saccharose and in being inagglutinable by a Flexner or Y antiserum.

Ohno among his 15 types of dysentery bacilli described organisms which differed from the classical types in the fermentation of lactose and saccharose.

Lactose-fermenters were also noted by Kruse among the types of " pseudo-dysentery " bacilli (i.e. strains other than B. dysenteriae Shiga) classified by him.

Ruffer and Willmore recorded in 1909 the finding of a dysentery bacillus which was apparently responsible for the largest proportion of cases among the Moslem pilgrims at El Tor on the Red Sea; it exhibited many of the characters of

the classical Flexner type but the reactions in saccharose and dulcite varied in the case of different strains; the serological characters also differentiated it from the Flexner bacillus.

It was apparent that a group of gram-negative intestinal bacilli could be recognised with the following common characters: absence of motility, absence of liquefaction of gelatin, fermentation of glucose without gas production and absence of gas production in the case of all sugars fermented. This group therefore comprised the typical dysentery types and also the " atypical " varieties.*

PRELIMINARY OBSERVATIONS.

There can be no doubt that epidemics of dysentery in different parts of the world have been mainly due to definite types e.g. the Shiga and Flexner bacilli, but in Egypt it appeared that the dysentery cases were by no means so uniform from the aetiological standpoint; this was indicated by a preliminary investigation carried out early in 1916 by Thomson and Mackie.** 30 cases of dysentery were carefully

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*It is to be noted that this group so defined would include certain non-motile varieties of the D subgroup of " coliform " bacilli (v. Part 1. p. 21), such as have been noted in urinary infections.

**I was personally responsible for the bacteriological data in this paper.

investigated both clinically and in the laboratory. Recently passed stools were examined frequently for protozoa and as regards the cellular exudate present, and were also immediately cultured for the detection of unusual bacteria.* A record was kept also of temperature, treatment, etc.

At that time among the non-amoebic cases no Shiga types were noted; 7 were apparently due to organisms which corresponded in character to the Flexner-Y type and reacted with an anti-Y agglutinating serum (Lister Institute, v. infra); in 8 cases organisms were observed which resembled the classical dysentery bacilli in certain of their characters but differed in biochemical reactions and in being inagglutinable by a Shiga, Flexner or Y serum.

The following types were recorded:-

Case 1. - a non-motile, non-liquefying, gram-negative bacillus which fermented glucose and maltose without gas production, produced indol, but had no action on lactose, dulcite, saccharose, or mannite, and did not agglutinate with a Shiga or Y antiserum. (No amoebae were found even after repeated examination; stools - fluid with mucus but no blood).

Case 2. - a non-motile, non-liquefying, gram-negative bacillus which fermented glucose without gas production, did not produce indol, had no action on glucose, lactose, dulcite, saccharose, mannite or maltose and did not react to a Shiga

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*Methods employed are detailed below (p. 11.).

serum though otherwise the organism was identical with B. dysenteriae Shiga in cultural and biochemical characters. (No amoebae were found after repeated examination; stools were almost entirely blood and mucus; copious cellular exudate present in stool consisting of degenerate polymorph leucocytes and macrophage cells (v.p. 12).

Case 6. - a non-motile. non-liquefying, gram-negative bacillus which fermented glucose, mannite and maltose without gas, and after mutation lactose (v.p. 27.), produced indol but had no action on dulcite or saccharose and did not react to a Y agglutinating serum. (No amoebae found; stools contained mucus but no blood: cellular exudate present).

Case 7. - a non-motile, non-liquefying, gram-negative bacillus which fermented glucose, dulcite, saccharose, mannite and maltose without gas, produced indol but had no action on lactose and did not agglutinate with a Y serum. (No amoebae found after repeated examination; stools contained mucus but no blood at time of examination; cellular exudate consisting of pus cells present).

In 2 cases Entamoeba histolytica was present.

In 5 cases the only unusual organism found in the stools was a B. Morgan No. 1 type (v. table 3). In one of these cases recorded, this organism was isolated at a relatively late stage of the illness but was present in large numbers as judged by the number of colonies in plate culture. The stool

at this time contained mucus and some blood; no amoebae were found on repeated examination but the cellular exudate was abundant.

In 2 cases large numbers of colonies of B. faecalis alkaligenes were noted in plate culture and no other unusual organisms; the stools were fluid with blood and mucus present on microscopic examination; pus cells were noted but no amoebae.

In 2 non-amoebic cases the only unusual organisms present were B. paracolon types (v. table 3) and in 1 case large numbers of B. proteus colonies developed in plate culture but no other unusual bacteria nor amoebae were detected.

Cultures from the stools in 1 case contained very large numbers of enterococci; a few colonies of B. faecalis alkaligenes were present in plate culture but no other unusual bacteria were noted and no amoebae were detected after repeated examination; there was a copious cellular exudate as in the typical bacillary dysentery; the stools were fluid, with mucus but no blood. When first examined the motions numbered 30 to 40 a day; there was a leucocytosis of 30,000 p..c.m.m.. The case was an extremely acute one but though the excreta were examined bacteriologically on three occasions, and first on the 5th day of the illness, no dysentery bacilli were isolated; on each occasion large

numbers of enterococcus colonies were present on the plates.

In 2 cases no unusual organisms were found.

In this series of cases therefore typical dysentery bacilli were found in 25% and organisms of the so called " atypical " class in 26%.

These preliminary observations raised certain important questions with regard to the aetiology and bacteriology of non-amoebic dysentery as it occurred at that time in Egypt and clearly showed that the diagnosis of the cause could only be made with certainty by laboratory examination.

The occurrence in acute cases of (1) " atypical " bacilli allied to the classical dysentery types and (2) such organisms as B. Morgan No. 1, B. faecalis alkaligenes, B. paracolon, B. proteus, enterococci (especially when present in the faeces in large numbers) suggested their pathogenic significance and indicated the necessity for the further investigation of non-amoebic dysentery.

The question has also been raised as to whether certain of these organisms might be present normally in small numbers in the intestine, held in check by the normal flora but still under certain conditions capable of replacing the common coliform types (Browning, Mackie and Thornton). It has been shown for example how the presence of a selective antiseptic such as brilliant green in faeces cultures may lead to the

appearance of unusual organisms not frequently found in the normal flora (Browning, Mackie and Thornton - 2). Also in typhoid and paratyphoid cases unusual intestinal organisms have been noted in large numbers in cultures from faeces though not so frequently as in non-amoebic dysentery e.g. B. Morgan No. 1 and similar types, B. faecalis alkaligenes B. paracolon types, enterococci (v. table 4); in no case of enterica however were atypical organisms of the dysentery group found in faeces culture.

It was noted that in amoebic dysentery unusual bacteria were only infrequently met with and it was one of the features of amoebic dysentery that plate cultures from the stools often revealed a growth of only typical coliform bacilli.

It has also been suggested that some of these strains are organisms to which the natives of Egypt may be more or less immune but to which Europeans are susceptible (Browning, Mackie and Thornton - 3); thus it was well known that few soldiers shortly after coming to Egypt escaped an acute non-dysenteric diarrhoeal attack lasting 2 to 5 days with fever sometimes reaching 103° F. Several of these cases were examined and the organisms found (usually in large numbers) in the stools were B. Morgan No. 1, B. paracolon, B. faecalis alkaligenes, or enterococci.

Morgan's investigations in epidemic infantile diarrhoea in Great Britain have also indicated that organisms of the B. Morgan No. 1 type are true pathogenic entities.

It has also been clearly shown that B. faecalis alkaligenes is capable of exerting pathogenic properties, and of invading the blood stream and producing an irregular or intermittent pyrexia with more or less toxaemia. (Shearman and Moorhead, Thomson and Hirst). The possibility of its producing a local pathogenic effect in the intestine can hardly be disregarded.

FURTHER OBSERVATIONS ON BACILLARY DYSENTERY.

With a view to the further investigation of non-amoebic dysentery, a large number of cases of obvious dysentery and also " simple " diarrhoea were systematically studied.

METHODS.

Fresh stools were examined (1) microscopically - for the detection of amoebae and other protozoa and also the recognition of the cellular exudate present; (2) bacteriologically - cultures being made on plates of MacConkey's bile-salt-neutral-red-lactose-agar.

For the preliminary microscopic examination the following preparations were made: towards one end of a microscopic slide a large drop of normal saline was placed and at the other a similar drop of Lugol's iodine solution;

a loopful of the stool or a portion of mucus (if present) was mixed in the saline and another loopful in the iodine solution; these preparations were covered with No. 1 cover-glasses and examined with a $\frac{1}{6}$" or oil-immersion lens.

As the investigation proceeded it became apparent that the preliminary recognition of a bacillary infection could be made by careful microscopic examination of the intestinal discharges; in bacillary cases, generally, microscopic examination revealed the presence of an abundant and characteristic cellular exudate and the absence of pathogenic amoebae. The cells present were mostly pus cells, often extremely degenerate, with a varying number of red corpuscles and in the early stages epithelial cells; in addition to these, large phagocytic cells (macrophages) were often a conspicuous feature; they were immobile even in recently passed stools and were differentiated from amoebae by the character of the nucleus which was relatively large and not of the ring-like type characteristic of amoebae; this was well seen in the iodine preparations. They somewhat resembled large endothelial cells. Wenyon and O'Connor (2) have drawn attention to these macrophage cells and have indicated how they have been mistaken for amoebae by inexperienced observers.

In the stool of amoebic infections there were few body cells unless the case was a mixed infection; leucocytes were scanty and not so degenerate as in bacillary dysentery.

This preliminary observation made it possible to report at once that a particular case was a bacillary dysentery so that specific therapy might be applied without delay.

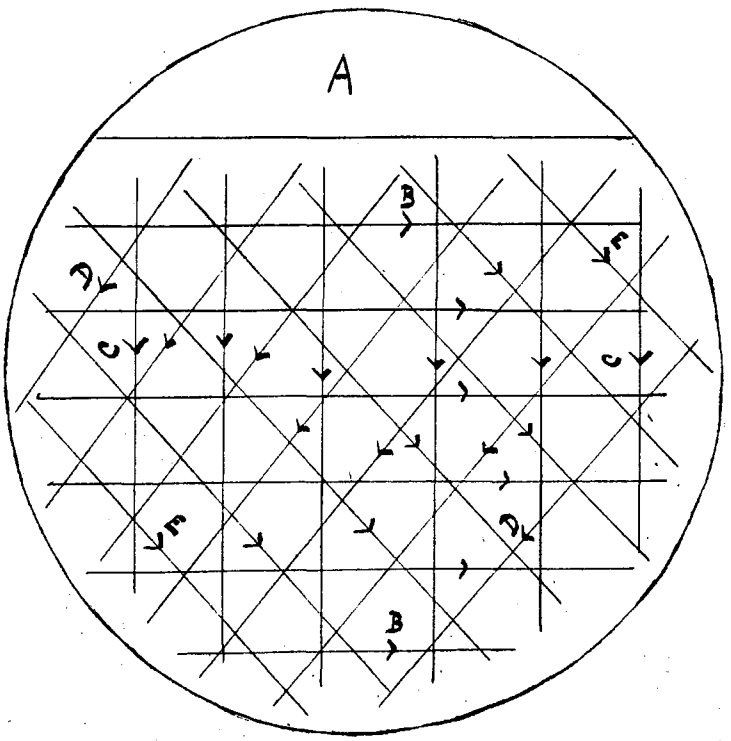
Bacteriological Examination.

The medium found most convenient for culture was MacConkey's bile-salt-neutral-red-lactose-agar made according to the original formula. It was simple in composition, easily prepared and stable as compared with other differential media for the intestinal organisms. It also proved sufficiently inhibitory towards the common aerial organisms to facilitate manipulation of plates in drying and inoculating. It always yielded well developed colonies of the dysentery bacilli when present and there was no evidence that it failed to develop sufficiently abundant growths of these organisms. The surfaces of the plates were always rendered as dry as possible before inoculating. (If even a small amount of condensation water is present, a confluent growth results instead of isolated colonies).

The Method of Inoculating Plates from Faeces.

If the stool contained mucus, a portion of the mucus was picked up on the platinum loop and used for inoculation; if the specimen was simply a fluid faecal stool, a large loopful was used. The contents of the loop were first smeared over a section of the plate (A) (v. diagram), and then the loop was sterilized in the flame. It was recharged by rubbing it over

Diagram to show method of inoculating plates



the area A and then the plate was inoculated by successive strokes, first the series B and then the series C at right angles to B. The loop was again charged from A and further successive strokes were made, D and E.

This method was found to yield more uniformly satisfactory results than other methods of inoculating plates. Of course, results depend on the concentration of organisms present in the specimen and the size of plate used. Plates of about 5½ inches diameter were used or when this size was not available the inoculation was distributed over two plates of 3½ or 4 inches, by the same method. (It is essential that the plates be abundantly inoculated but if the specimen contains an excess of organisms, it may be difficult to obtain satisfactory separation of the colonies. This is more likely to happen if the plate is inoculated with a spreader e.g. a glass rod bent at a right angle.) The method described allowed a heavy inoculation to be made with the resulting colonies well separated (unless in area A).

The plates were incubated over night; after 15-24 hours the colonies were usually sufficiently grown for picking off those that were considered likely to belong to the " dysentery group." If no suspicious colonies were noted after 24 hours, the plates were re-incubated but only in exceptional cases did this prove of additional value. As regards the colonies of the dysentery bacilli, there were apparently no morphological

characters which would enable one to differentiate them from colonies of certain other non-lactose-fermenting bacilli e.g. B. Morgan No. 1, B. faecalis alkaligenes, B. typhosus and the paratyphoid bacilli. Their colonies also tended to vary somewhat in size, opacity and type of border. On MacConkey's medium the colonies were generally small," pale," film-like, semitransparent discs with a circular or wavy margin and of course were easily distinguished from B. coli colonies which were larger, more opaque and generally coloured " red." In this investigation, as complete a bacteriological examination as possible was made and all the " abnormal " organisms present in the stools were studied; these were for the most part non-lactose-fermenters and their colonies were of the pale variety on MacConkey's medium; certain " atypical " varieties of the dysentery group were found to ferment lactose in fluid medium but in the primary cultures these organisms usually presented pale colonies after 24 hours.

Subcultures were made from each type of pale colony on the plate and as it was found that colonies which appeared to be similar might represent entirely different organisms, at least 4 colonies were subcultured.

For picking off colonies a straight platinum wire with its end shaped like a lance-head was used; this enabled one easily to pick off a small colony without touching an adjacent one.

In subculturing pale colonies from MacConkey's medium, not infrequently an enterococcus colony was subcultured; in general these were smaller, of more regular outline, and distinctly more opaque than the colonies of B. dysenteriae but sometimes they were difficult to differentiate. It was found therefore advisable to make films from the agar slopes and stain them by Gram's method before proceeding with further tests.

Sufficient growth on agar slopes was usually obtained within 8 hours at 37°C to proceed with the examination of the cultures; the following media were inoculated from each culture.

1. Tube of peptone water.
2. Durham's tube of glucose peptone water (with neutral red as indicator).
3. " " lactose " " "
4. " " saccharose " " "
5. " " mannite " " "

and these were incubated for 12 hours. The peptone water culture was examined for motility of the organisms after 6-7 hours.

In this way it was possible to ascertain to what group the various strains belonged and whether two or more represented the same type (in which case only one was investigated further).

If a culture exhibited the following characters:- gram-negative non-sporing bacillus, motility —, glucose ⊥*, lactose —, saccharose —, mannite — or ⊥ i.e. corresponding to the Shiga or Flexner-Y type (see table 2A), the final identification was made by tests with specific agglutinating sera (anti-Shiga and anti-Y). The agglutinating sera were those supplied to the Military Laboratories by the Lister Institute, London. The Y serum was prepared by immunizing animals with the original Y strain of Hiss and Russell and it was shown by Chick that antisera to this organism usually agglutinated strains of the Flexner bacillus to a degree commensurate with the homologous strain but that sera prepared with B. dysenteriae Flexner were more specific (v.p. 22).

Those strains which agglutinated with Shiga or Y antisera were classified as typical B. dysenteriae Shiga and Flexner-Y respectively.

For the agglutination test the bacillary emulsions were obtained from the original agar slope cultures and the tests were carried out by the technique described in Part 1 (p. 27). It was found that the agglutination of B. dysenteriae develops much more slowly than in the case of B. typhosus or V. cholerae and the clumps tend to be smaller and less flocculent. Before reading the results the agglutination tubes were allowed to stand in the incubator at 37° C. for 3 hours and then for 1 hour at room temperature.

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*⊥ = acid no gas; — = no acid or gas.

The cultures, whether a specific agglutination reaction was obtained or not were then studied further; dulcite and maltose tubes were inoculated and the peptone water was tested for indol after 48 hours and if negative, again after 7-10 days. The fermentation tubes were also kept at 37°C. for 7 days before final readings were made. A gelatin stab culture was also made to test for liquefaction of this medium.

If the strain still appeared to have the characteristic reactions of a typical dysentery bacillus, the agglutination test was repeated after the strain had been subcultured several times and a small proportion of strains inagglutinable after isolation rapidly became agglutinable on subculture.

If the strain remained inagglutinable, it was classified with the " atypical " varieties. If, in the preliminary tests, the sugar reactions were not those of the classical types but if the organism was a non-motile, non-sporing, gram-negative bacillus which did not produce gas from the sugars it fermented, it was classified with the atypical dysentery bacilli and its cultural and biochemical reactions were investigated thoroughly as in the case of the inagglutinable strains referred to above (v. table 2B). A large number of these organisms were also tested with anti-Shiga, -Y, and - Flexner sera but were invariably found inagglutinable. Animal virulence tests (v.p. 29) were also carried out with representatives of the various cultural types of these organisms.

GENERAL RESULTS.

During the period April to September, 1916, 1530 cases of acute and chronic dysentery, " simple diarrhoea," and cases recovered from dysenteric or diarrhoeal illnesses were examined in the course of the usual laboratory routine. Many of these cases at the time of examination showed no evidence of dysentery as judged by the condition of the stools and the bacteriological results were negative; some of the specimens examined were fluid diarrhoeal stools without obvious blood and mucus. The general results of the investigation are shown in Table 1*. To indicate the proportion of the total number of these cases which presented at the time of examination obvious pathological signs of dysentery, the number of cases with mucus or blood and mucus in the specimen examined is given.

The percentage of the different dysenteric infections reckoned in terms of the acute cases was as follows:-

Entamoeba histolytica**	—	11.2%	
B. dysenteriae Shiga	—	9.)	
		)-28.1	
B. dysenteriae Flexner-Y	—	19.1)	)
			)-47.2
" B. dysenteriae, atypical "	—	19.1	)

*It is impossible to give fuller details as the detailed laboratory records were lost at sea in 1918 due to the sinking by enemy action of the transport on which I was travelling from Egypt.

**It is not intended to deal here with the strictly protozoological aspects of dysentery; the protozoological findings are only inserted for comparison and to complete the data in the series referred to.

TABLE 1.

Total number of cases examined	1530.
Cases with mucus or blood and mucus in stool	443.
Cases in which Entamoeba histolytica (free or encysted) found	50.
Cases in which B. dysenteriae Flexner-Y found	85.
" " " " Shiga "	40.
" " " "atypical dysentery bacilli" found	85.
Mixed infections (amoebic and bacillary - 1 with B. dys. Shiga and 1 with B. dys. Flexner-Y)	2.
Cases in which no amoebae or dysentery bacilli found but B. Morgan No. 1 present in stool	59.
" " " B. "C.L.A." No. 1 (v. table 3) " " "	6.
" " " B. "C.L.A." No. 2 " " "	26.
" " " B. faecalis alkaligenes " "	77.
" " " B. paracolon types " "	80.

In a few cases (in the absence of other findings) the only unusual organism present was B. proteus, and in some cases also plate cultures yielded an abundant growth of enterococci. In a considerable number of cases B. Morgan No. 1, B. "C.L.A." 1 and 2, B. faecalis alkaligenes and B. paracolon types were present along with B. dysenteriae, and in many cases from which B. dysenteriae was absent mixed infections with two or even three of these organisms were noted. In the table these cases

TABLE 1 (Continued).

are recorded under the organism found to be most numerous in the particular case. (In 168 cases also flagellate protozoa, lamblia intestinalis, Tetramitus Mesnili (Wenyon), Trichomonas hominis were noted in the absence of other findings.)

TABLE 2A.

CLASSICAL DYSENTERY BACILLI

Gram-negative, non-sporing, aerobic bacilli;

	Motility.	Glucose.	Lactose.	Dulcite.	Saccharose.	Mannite.	Maltose.	Indol.	Gelatin.
B. dysenteriae									
Shiga	—	⊥	—	—	—	—	—	—	—
Flexner	—	⊥	—	—	—	⊥	⊥	+	—
Y	—	⊥	—	—	—	⊥	—	+	—

" TYPICAL " DYSENTERY BACILLI.

Shiga	—	⊥	—	—	—	—	—	—	—
	Agglutinated by antiserum to classical strain.								
"Flexner-Y"	—	⊥	—	—	—	⊥	⊥ —	+ —	—
	Agglutinated by antiserum to classical Y strain.								

⊥ = Acid no gas.

— = No acid or gas production.

TABLE 2B.

ATYPICAL DYSENTERY BACILLI.

Gram-negative, non-sporing, aerobic bacilli.

Types	Motility	Glucose	Lactose	Dulcite	Saccharose	Mannite	Maltose	Indol	Gelatin	
1.	—	⊥	—	—	—	—	—	—	—	(Corresponds to B. dys. (Shiga but not agglu- (tinable by an Anti-Shiga (serum.
2.	—	⊥	—	—	—	—	—	+	—	
3.	—	⊥	—	—	—	—	⊥	—	—	
4.	—	⊥	—	—	—	—	⊥	+	—	
5.	—	⊥	—	—	—	⊥	—	—	—	) Correspond to B. dys.
6.	—	⊥	—	—	—	⊥	—	+	—	) Flexner-Y but not
7.	—	⊥	—	—	—	⊥	⊥	—	—	) agglutinated by the
8.	—	⊥	—	—	—	⊥	⊥	+	—	) anti-Y serum.
12.	—	⊥	—	—	⊥	—	⊥	+	—	
16.	—	⊥	—	—	⊥	⊥	⊥	+	—	
17.	—	⊥	—	⊥	—	—	—	—	—	
20.	—	⊥	—	⊥	—	—	⊥	+	—	
22.	—	⊥	—	⊥	—	⊥	—	+	—	
24.	—	⊥	—	⊥	—	⊥	⊥	+	—	
32.	—	⊥	—	⊥	⊥	⊥	⊥	+	—	
40.	—	⊥	⊥	—	—	⊥	⊥	+	—	
48.	—	⊥	⊥	—	⊥	⊥	⊥	+	—	
56.	—	⊥	⊥	⊥	—	⊥	⊥	+	—	
64.	—	⊥	⊥	⊥	⊥	⊥	⊥	+	—	

⊥ = acid no gas.

The numerical classification is based on the various possible combinations of cultural reactions.

TABLE 3.

CONCOMITANTS OF B. DYSENTERIAE.

	Motility	Glucose	Lactose	Dulcite	Saccharose	Mannite	Maltose	Indol	Gelatin
1. B. Morgan No. 1	+	⊥ +	−	−	−	−	−	+	−
2. B. "C.L.A." 1	+	⊥ +	−	−	−	−	−	−	−
" 2	−	+	−	−	−	−	+	+	−
" 3	−	+	−	−	−	−	−	+	−
" 4	+	+	−	−	−	−	+	−	−
" 5	−	+	−	−	−	−	+	−	−
" 6	+	+	−	−	−	−	+	+	−
" 7	−	+	−	−	−	−	−	−	−
3. B. faecalis	+	−	−	−	−	−	−	−	−
alkaligenes types	−								
4. B. paracolon types	+ −	+	−	+ −	−	+	+	+ −	−
5. B. proteus types	+	⊥ +	−	+ −	+ −	−	+ −	+ −	+

All gram-negative, aerobic, non-sporing bacilli.

6. B. pyocyaneus.

7. A gram-negative, non-carbohydrate fermenting, non-motile, cocco-bacillus.

8. Enterococci.

Table 1 indicates generally the frequency of these organisms in dysentery cases; those referred to above but not represented in table 1 were only noted in the subsequent investigations and were of rare occurrence.

The percentage of acute cases of non-amoebic dysentery, therefore, from which classical dysentery bacilli could be isolated was relatively low and as many atypical organisms were noted as typical Flexner-Y types; in a large number of acute cases also only organisms such as B. Morgan No. 1 and allied types, B. faecalis alkaligenes, B. paracolon types were present. In short, the more extensive investigation of dysentery cases confirmed the original observations.

Continued investigations with particular reference to the types of dysentery bacilli that could be isolated from acute cases yielded the following total results: in 451 cases from which these organisms were isolated

99 were B. dysenteriae Shiga infections

179 " " Flexner-Y "

and in 173 atypical varieties were found.

In the majority of cases with obvious dysentery or diarrhoea from which these organisms or entamoebae were absent the following types were present in the stools, and often in large numbers:

B. Morgan No. 1)
B. " C.L.A. " types)
B. paracolon types)
B. faecalis alkaligenes)- see table 3.
B. proteus)
B. pyocyaneus)
Enterococci)
A gram-negative non-motile non-carbohydrate-fermenting cocco-bacillus.

Often two or three of these organisms were present together in the stools. The most prevalent types have already been

indicated (table 1) — B. Morgan No. 1, B. faecalis alkaligenes, B. paracolon and B " C.L.A." No. 2.

B. DYSENTERIAE SHIGA STRAINS.

The Shiga strains isolated invariably corresponded in their cultural and biochemical reactions to the classical type and showed a specific agglutination reaction (i.e. approximately to end-titre) with a homologous agglutinating serum. Only a few strains which corresponded in cultural reactions to the Shiga type and did not agglutinate in primary culture, became agglutinable after repeated subculture. No non-mannite-fermenting strains which resembled the Shiga type in most of their cultural reactions but differed as regards the fermentation of dulcite, saccharose, maltose, or the production of indol ever agglutinated even in low titres with a Shiga serum. Thus the only organisms of the dysentery group which reacted to the Shiga serum were those which corresponded exactly with the classical type in cultural reactions.

B. DYSENTERIAE, FLEXNER-Y STRAINS.

Originally the Flexner and Y (Hiss and Russell) types were described as separate varieties but it proved difficult to identify by serological reactions new strains with these classical types. In routine diagnostic work such differentiation appeared unnecessary and the system adopted was to classify all mannite fermenting types which reacted with the

Y serum as Flexner-Y organisms in virtue of the fact that this serum also agglutinated in high titres all organisms which reacted to the anti-Flexner serum. The Y serum used was that of the Lister Institute and at that time was prepared by immunizing with the classical Y strain of Hiss and Russell (v.p. 17). The strains of this type invariably corresponded in their cultural reactions to the classical types, varying only in fermentation of maltose and as regards the formation of indol. All the strains irrespective of these variations agglutinated approximately to end-titre with the Y serum. No strains of the dysentery group which differed in biochemical characters from the typical varieties reacted with the Y serum. Saccharose-fermenting strains which reacted with a Y serum have been described by other observers (Martin and Williams, Glynn and others) but in these investigations in Egypt it was invariably found that the Flexner-Y strains failed to ferment saccharose or lactose and that mannite-fermenting strains which after some days incubation fermented saccharose or lactose were not agglutinated by the Y serum and were therefore classified as atypical organisms.

An anti-Flexner serum (Royal Army Medical College) was also used in parallel series in the agglutination tests of a considerable number of mannite-fermenters and only a small proportion of these reacted to it even in low titres. A

strain which was agglutinated by the Flexner serum was invariably agglutinated by the Y serum.

A high-titre agglutinating serum was obtained to a strain which reacted with the Y serum but not with the Flexner serum; it was found that this serum only agglutinated a small number of strains which reacted up to end-titre with the Y serum and none of these reacted with the Flexner serum.

Thus <u>at least</u> three serological types could be recognised in the group:

	Anti-Y serum (Lister Institute)	Anti-Flexner serum (R.A.M. College)	Serum to strain agglutinated by Y serum but not by Flexner serum
1. (Majority of strains of Flexner-Y group).	+	—	—
2.	+	+	—
3.	+	—	+

+ indicates agglutination approximately up to end-titre of serum.

It was apparent therefore that the Flexner-Y group was not serologically homogeneous and that it included different serological types all capable of reacting to the particular Y serum; thus the classical strain with which the agglutinating serum was prepared appeared to represent antigenic properties common to the whole group. The serological problem in this

group seemed therefore a complex one, but at that time further study of the subject was interrupted by the routine work of the laboratory.

[Subsequently the serological characters of the group were studied in detail by Murray, and by Andrewes and Inman. Murray found that four antisera prepared from selected members of the Flexner-Y group were required to " bring down the whole of the series of Flexner-Y's studied," but noted that there was a considerable amount of " overlapping " in the agglutination results between the different serological types. Andrewes and Inman indicated from their observations the existence of " at least four distinct antigenic components in the group," designated V, W, X and Z and that " any one of these four may so predominate in different strains as to impart a distinct serological facies." They also showed as regards the Y type of Hiss and Russell that there was not sufficient evidence of its representing a fifth antigenic component since the agglutinin could be absorbed by a combination of V, W, X and Z. They indicated also that the V type corresponded with the original Flexner strain.

They recommended for the identification of the Flexner-Y group a polyvalent agglutinating serum prepared by pooling monovalent sera to these different types.]

It was noted that certain Shiga strains were agglutinated approximately to full titre by the Y serum; these strains

corresponded in cultural reactions with the classical Shiga type and reacted with an anti-Shiga serum. Chick has drawn attention to the fact that the Y serum exerts a well marked " group " agglutination towards B. dysenteriae Shiga, but with an end-titre lower than that for the homologous organism.

On the other hand no Flexner-Y strains reacted to any degree with the Shiga serum.

Only a small number of strains found to be inagglutinable in primary culture became agglutinable after repeated subculture.

THE " ATYPICAL DYSENTERY BACILLI."

The " atypical " varieties owing to their close biological relationship to the classical organisms appeared of considerable significance from the aetiological standpoint especially when they occurred in large numbers in the excreta during the earlier phase of an acute case in which no amoebae or typical dysentery bacilli could be detected.

In these investigations organisms of this type were so frequently met with in acute dysentery that their occurrence could not be ignored. They had apparently no place among the normal intestinal flora as judged by a considerable personal experience of intestinal bacteria in Great Britain and in Egypt. Moreover in a series of 438 specimens of faeces from enterica cases cultured by direct plating on the same medium as that used for the isolation of dysentery bacilli, organisms of this type were never met with though all types of pale colonies on these plates were fully investigated.

In only two cases of amoebic dysentery were these organisms noted; of course classical dysentery bacilli have also been found in amoebic cases.

These facts together with their close similarity to the typical varieties suggested the likelihood of their being dysentery producers.

Bahr has suggested that the atypical organisms might be concomitants of the typical varieties and not primary causal agents themselves; from my own experience I am unable to agree with this view; it was quite evident, however, that certain other organisms which appeared in the stools of dysentery cases (B. Morgan No. 1, etc. - v. supra) could be regarded as concomitants (v. infra).

The group of atypical dysentery bacilli may be defined as: gram-negative, non-motile, non-sporing, aerobic bacilli, not

liquefying gelatin, always fermenting glucose (without gas production), different strains varying as regards the fermentation of lactose, dulcite, saccharose, mannite, maltose, (in all cases without gas production), and the formation of indol from peptone, and not agglutinated by a Y, Flexner or Shiga serum, even after repeated subculture. The reactions of the types met with are shown in table 2B.

Some of them corresponded in all their cultural reactions to the Shiga or Flexner-Y types but failed to react with the respective agglutinating sera; these were at first described as " inagglutinable B. dysenteriae Shiga or Flexner-Y." Martin and Williams also described organisms of this type and regarded them as true dysentery bacilli. Only a few strains which at first appeared to belong to this category became agglutinable after repeated subculture on artificial media, and were of course classified as typical varieties; certain strains which in primary culture might have been classified as inagglutinable B. dysenteriae rapidly underwent spontaneous mutation and displayed fermentative characters which clearly differentiated them from the typical organisms. Thus, strains which at first appeared to correspond to the Flexner-Y type in biochemical characters, developed in lactose, saccharose and less frequently in dulcite variants which rapidly fermented these sugars just as in the case of the mutating coliform

bacilli (v. Part 1, p. 55). These all proved inagglutinable by the Y serum. Other strains retained their original characters and remained stable. Certain also, when first tested, exhibited fermentative reactions after 24 hours which differentiated them from the classical types and all of these proved inagglutinable by the Shiga, Flexner and Y sera.

Some doubt has been cast on the fermentative characters of organisms recorded by workers in the East during the war owing to the doubtful purity of " sugars " supplied to the Military Laboratories from Army Stores. Specimens of impure sugars were occasionally supplied, but only in the hands of inexperienced workers could such impurities escape notice: it therefore became a routine method at the Base Laboratory in Alexandria to test specimens of lactose, dulcite, saccharose, mannite and maltose with known strains of B. typhosus, B. paratyphosus A, B. dysenteriae Shiga, B. dysenteriae Flexner and B. dysenteriae Y. If any aberrations were noted, the specimen in question was discarded. In 1916 also practically all the sugars used by me were pre-war preparations of Merck or Kalbaum.

A number of representative strains of atypical organisms were sent to me in South Africa from Egypt in 1918 and on retesting with pre-war Merck preparations gave the same reactions as those recorded in Egypt.

ANIMAL EXPERIMENTS.

Representative strains * of these various types were proved to be markedly virulent by intravenous and intraperitoneal injection of rabbits, producing a characteristic haemorrhagic enteritis. The organisms were tested shortly after isolation. The effects corresponded to those produced by typical Shiga and Flexner-Y strains.

At first emulsions of living organisms were injected intraperitoneally but it was observed that intravenous injection produced the same effect and demonstrated a highly selective action of the organism or its toxin on the intestinal mucosa.

The dose injected was usually $\frac{1}{8}$th - $\frac{1}{4}$th of a 24 hours agar slope culture emulsified in saline and the animals usually died within 24 to 48 hours. Some strains of atypical varieties, however, exhibited a much higher degree of virulence and the intravenous injection of $\frac{1}{40}$ th of a 24 hours agar slope culture produced the characteristic effect.

Comparative tests with a recently isolated Shiga, and a type No. 2 strain showed the latter was distinctly more virulent i.e. smaller doses were lethal than in the case of the particular Shiga strain.

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*In the case of the commoner types at least 3 strains were tested; only one strain of certain types was met with (3, 12, 17, 20) and this of course was the only strain tested.

On autopsy the mucosa of the small intestine was found to be intensely inflamed and usually showed massive haemorrhages in the tissues; the lumen of the small intestine was distended with blood stained muco-purulent material containing degenerate polymorph leucocytes, macrophage cells and masses of exfoliated epithelium. The haemorrhagic lesions different from the punctiform haemorrhages sometimes seen in the intestinal wall in certain experimental septicaemias. The large intestine was generally less affected and in some cases the changes were confined to the small intestine. In certain of the experiments, however, the stomach, the small and large intestine were uniformly affected, and exhibited the characteristic haemorrhagic inflammation.

The organism, injected intravenously, could also be demonstrated, often in almost pure culture, in the contents of the intestine. This has also been shown by Besredka.

Of course the degree of effect produced by different strains varied considerably but not according to their cultural type. The same variation was noted in the case of the typical organisms tested and as might have been expected depended on the virulence of the individual strains. In comparing the effects of animal inoculation by typical and atypical strains no essential differences could be established, and as already shown a particular atypical strain might be even more virulent than a typical organism (v. supra).

None of the various types recorded were non-virulent on testing shortly after isolation, but it was noted that certain strains which had been cultivated artificially for 2 to 3 months were non-virulent even in large doses.

The animal experiments demonstrated a highly selective effect on the intestinal mucosa and the capacity for producing haemorrhagic enteritis may be regarded as an attribute characteristic of the dysentery group. A number of other types of intestinal bacteria found in dysentery cases - coliform bacilli, B. Morgan No. 1 and similar types, B. faecalis alkaligenes. B. paracolon types, etc, were tested (v. p. 39) but failed to produce similar effects. The V. cholerae and the paracholera vilrios also exhibit a selective toxic action on the intestinal mucosa, but in this case haemorrhage is absent or only punctiform haemorrhages are noted (v. Part 3 p. 13).

From their occurrence, often in large numbers, in early cases of acute dysentery (in the absence of classical types) and their characteristic effects in animal experiment (which were similar to those produced by typical Shiga and Flexner-Y strains) together with their close biological similarity to organisms of the group of typical dysentery bacilli, it was concluded that these atypical organisms were to be regarded as true dysentery bacilli.

Additional Note: It was thought that further confirmation of the pathogenic role of these organisms might be obtained if the sera of patients were found to exert a specific agglutination reaction to the infecting organism. At my request Capt. Storer, R.A.M.C., undertook this enquiry: in only a small proportion of cases of proved Shiga and Y infections could a definitely specific reaction be obtained; in some cases the reaction occurred in such relatively low titres as to introduce the fallacy of a normal serum effect, (as shown originally by Lentz). A few cases of atypical B. dysenteriae infections were examined but in only 2 was there any evidence of a specific reaction to the associated organism. As a considerable number of cases due to Shiga and Flexner-Y types failed to show a definitely specific agglutination reaction, the negative result in cases with which atypical organisms were associated could not be regarded as of any significance and as this line of investigation did not promise any further elucidation of the aetiological problem, it was not pursued further.

In 1918 Andrewes studied a number of strains of " atypical dysentery bacilli " submitted to him from other laboratories and classified them into 3 main types designated " B. ambiguus " (corresponding to type 2), " B. alkalescens " (corresponding in cultural reactions to type 24), " B. dispar " (corresponding to lactose fermenting types shown in table 2B). He concluded from his observations that the first two varieties had " no connection with dysentery " but committed himself to no definite conclusion with regard to the " B. dispar " type. He found that the " B. ambiguus " and " alkalescens " types were non-virulent to rabbits but the " B. dispar " was markedly pathogenic. Such conclusions arrived at from the mere laboratory study of a number of strains, without reference to other data in favour of their aetiological relationship to dysentery seem hardly justifiable. Andrewes reported that the " B. ambiguus " type was practically non-virulent; yet this type in my own experiments proved highly virulent on intravenous injection of the rabbit; of course my own strains were tested as regards their virulence immediately after isolation. Conclusions drawn from negative animal tests with strains that have been growing on culture medium for some time can hardly be regarded as reliable.

An organism corresponding to type 2 and Andrewes' B. ambiguus was described by Schmitz in a localized epidemic among Rumanian prisoners of war. Schmitz found this organism virulent to rabbits.

Two outbreaks of mild dysentery with which this type of organism was associated have also been recorded by Broughton-Alcock.

The War Office Committee on Dysentery reported that this organism could not be excluded as a cause of the disease though they concluded it was of little epidemiological importance in comparison with bacilli of the Shiga and Flexner type.

As regards the lactose-fermenting types the Committee was not prepared to dismiss these organisms as aetiological factors but regarded them also as of less epidemiological importance than the typical dysentery bacilli.

Atypical varieties corresponding to certain of those classified in the table were also isolated from acute cases of non-amoebic dysentery in Egypt by J. G. Thomson and Hirst. They found that of 100 strains of the dysentery group, 29 were typical Shiga organisms, 32 were Flexner-Y types and 39 atypical varieties.

In my own investigations atypical organisms were isolated from stools within a few minutes from the time they were passed and were the predominant organisms present. Thus, there was no support for the suggestion that they were " products of decomposition of faeces kept for some time " (Bahr). Moreover specimens of faeces containing typical B. dysenteriae were kept at room temperature (Egypt in winter)

for 24 hours and it was found that the dysentery bacilli generally died out after 6 to 8 hours, but no organisms capable of growing on MacConkey's medium appeared after 24 hours which were not originally present in the specimen; B. coli and the various " concomitant " organisms if present persisted after the dysentery bacilli had disappeared.

In only 2 of the cases investigated were mixed infections with typical and atypical organisms noted; these were too relatively uncommon to indicate that the atypical bacilli were only concomitants. A case of mixed infection with typical Shiga and Flexner-Y types was noted; this has also been recorded by Bahr.

CLINICAL AND EPIDEMIOLOGICAL SIGNIFICANCE OF ATYPICAL DYSENTERY BACILLI.

Dysentery has usually been defined as a condition of diarrhoea characterized by the presence of blood and mucus in the stools. Leaving out of consideration amoebic dysentery, the accepted typical dysentery bacilli were not infrequently met with in cases which were regarded clinically as " simple " diarrhoea without obvious blood or mucus in the discharges; these cases were aetiologically of the same nature as the more typical cases and had to be regarded as examples of " bacillary dysentery "; the stools usually showed the same type of cellular exudate as in the more acute cases.

Thus, " bacillary dysentery " comprises not only the typical acute cases but also the less severe conditions - clinically diarrhoea without the usual dysenteric signs. This was recognised by the War Office Committee on dysentery. Ryle has also drawn attention to these cases of mild bacillary dysentery. Thus, in assessing the part played by an organism in the causation of dysentery it seemed necessary to consider the possible clinical conditions with which such organisms might be associated.

As regards the type of infection due to atypical varieties, the greater proportion of the cases considered collectively were of the milder type (diarrhoea with or without mucus in the stool but little or no blood), and the proportion of cases with the typical acute signs i.e. passing the characteristic blood and mucus stools was lower. Nevertheless severe cases of dysentery were not infrequently met with apparently due to these varieties and fatal cases were noted in which atypical dysentery bacilli were isolated from the dysenteric lesions post mortem.

The Shiga infections were mostly of the severe type and the atypical B. dysenteriae infections of the milder type, while the Flexner-Y infections occupied an intermediate position in this respect. These facts were determined by a careful review of the records of a large series of unselected cases from which these organisms had been isolated.

FREQUENCY OF DYSENTERY BACILLI IN THE FAECES AT DIFFERENT STAGES OF THE DISEASE.

During the first few days of the illness, the dysentery bacilli were usually present in considerable numbers and often in almost pure culture; after this they tended to disappear from the discharges and to be replaced by " concomitant " organisms (table 3.). Thus at a later stage of a dysenteric illness plate cultures often showed large numbers of colonies of these concomitants and the dysentery bacilli were absent. This explained to some extent how in a large proportion of cases only such organisms as B. Morgan No. 1, B. faecalis alkaligenes, B. paracolon, etc. were found, in the absence of organisms of the dysentery group.

The following record of a case examined daily exemplifies the change in the flora during the dysenteric illness:

Days from commencement of illness.	Organisms present in cultures from stools.
2.	Almost pure growth of B. dys. Shiga; and a few colonies of B. coli.
3.	Do.
4.	B. dys. colonies not so numerous; a few colonies of B. Morgan No. 1 and B. coli.
5.	No B. dys.; large numbers of colonies of B. Morgan No. 1; a few B. coli colonies.
6.	Do.
7.	Do.
8. 9. 10. 11.	No B. dys.; B. Morgan No. 1 colonies less numerous: B. coli colonies more numerous.
12.	B. coli colonies only.

Case not treated by antidysentery serum.

Thus, as the case tended to recovery these concomitant organisms also diminished in numbers and ultimately gave place to the normal organisms of the bowel. It may be said that in general if cases are examined within the first two or three days, the infecting organism can be isolated without difficulty, but at a later stage only the concomitant may be found. In a number of autopsies also in which cultures were made directly from the intestinal lesions no dysentery bacilli were isolated but cultures of these concomitants were obtained.

The organisms which may be grouped together as " concomitants " of B. dysenteriae are indicated in table 3, and their cultural reactions are shown.

It was not infrequently noted in protracted cases that large numbers of colonies of enterococci developed on the plates; a case has however been recorded in which enterococci were present in large numbers at an earlier stage of the illness. (v. p. 8).

It was of course difficult to determine the actual part played by these organisms, but it might appear as if the dysentery bacilli proper only initiated the lesions and that other organisms such as these referred to acted by/aggravating or maintaining the pathological condition.

It is to be noted that many of these types also appeared in enterica cases (table 4.).

TABLE 4.

Unusual aerobic organisms present in faeces of enterica cases from which enterica organisms absent.

Series of 438 cases of enterica.

(Enterica organisms isolated from - - - -	52.)
- B. typhosus, B. paratyphosus A and B.	
B. Morgan No. 1 present - - - - - -	5
B. "C.L.A." 1 - - - - - - - -	7
B. "C.L.A." 2 - - - - - - - -	2
B. faecalis alkaligenes - - - - - -	10
B. paracolon types - - - - - - -	10
Non-motile gram-negative cocco-bacillus at first not fermenting any sugars but later producing acid (no gas) from glucose - - - - - - - -	1
B. pyocyaneus - - - - - - - -	5
Motile gram-negative non-liquefying bacillus fermenting glucose and saccharose without gas, not fermenting lactose, dulcite, mannite, not producing indol - - -	1
Enterococci (present in large numbers) - - -	26

In many of these cases the organisms referred to were present in large numbers.

Virulence tests were carried out with representative strains of these organisms; strains of B. Morgan No. 1 and B.C.L.A. 1 and 2 were found to be virulent to rabbits on intraperitoneal injection, but produced no specific effects; intravenous injection apart from a transitory toxaemia was without effect.

These results yielded a significant contrast with those following intravenous or intraperitoneal injection of the typical and atypical dysentery bacilli.

Several strains of B. faecalis alkaligenes were similarly ~~treated~~ tested but proved non-pathogenic both by intraperitoneal and intravenous injection; the paracolon strains were also non-virulent.

Thus it was impossible by animal inoculation tests to demonstrate any specific pathogenic effects on the part of these concomitant organisms.

It seems a likely possibility that these bacteria are inhabitants of the intestinal tract which are present in minimal numbers under normal conditions, and therefore are not usually detectable in ordinary cultures from faeces; under certain conditions (e.g. in enterica and particularly in bacillary dysentery) they may be able to multiply in such large numbers as to represent the predominant faecal organisms. Moreover, if these organisms are potentially pathogenic, when

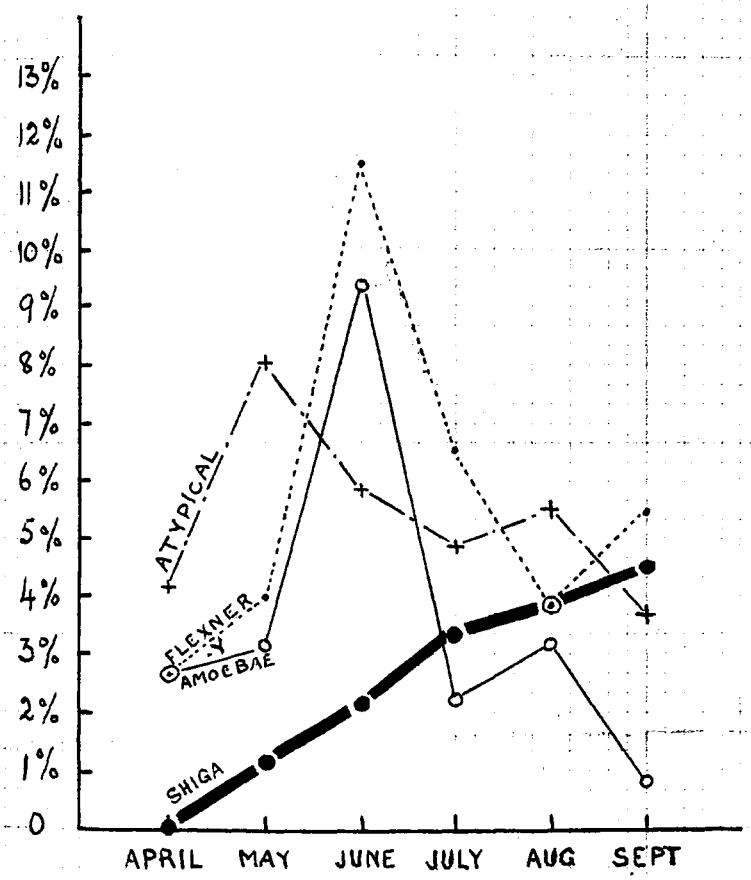
they are present in large numbers they may play an adjuvant part in the pathogenesis of dysentery.

RELATIVE PREVALENCE OF THE DIFFERENT INFECTIONS AT DIFFERENT PERIODS.

The chart shows the curves of relative prevalence of the different bacillary infections, B. dysenteriae Shiga, Flexner-Y, atypical bacilli and also those due to Entamoeba histolytica during the six months from April to September. (inclusive) of 1916. The data from which the curves were plotted out were the number of cases in which the respective organism was found stated as a percentage of the total number of cases examined during the particular month. This allowed for variation due to differences in the total number of cases examined; thus the number examined and therefore the number of cases in which positive findings were recorded varied each month.

No relationship was noted between the prevalence of the different bacillary infections. In April no infections with the Shiga type were noted and more atypical strains were isolated than those of the Flexner-Y type. In June on the other hand, the proportion of Flexner-Y strains isolated was greater; in September the infections with the Shiga type exceeded those with atypical bacilli.

Chart to show the relative prevalence of the different infections at different periods



On the other hand it was found that in certain cases a few colonies of dysentery bacilli could be detected in plate cultures made from the excreta at a later stage of the disease. Thus, in 15 out of 28 cases in which the stools were examined repeatedly dysentery bacilli were noted in the 2nd week, and in 3 cases in the 3rd week. In one case, the organisms were still detectable until the 18th day, and subsequently reappeared on the 25th day.

Thus the excretion of these organisms may be intermittent; in one case dysentery bacilli were present on the 4th and 5th days, absent on the 6th and present again on the 8th and 9th days; in another case dysentery bacilli were present on the 6th and 7th days, absent on the 8th and present again on the 9th day.

The Occurrence of Healthy Carriers.

With a view to determining the existence of healthy carriers both among troops and natives in Alexandria, the faeces of a number of healthy individuals were examined; the results of this investigation are shown:

	Number examined.	Carriers.	
Dysentery convalescents in Convalescent Hospital	100	8 i.e. 8%	Flexner-Y - 4 Shiga - 3 Atypical - 1
Orderlies in a General Hospital	60	4 i.e. 6.6%	Flexner-Y - 2 Atypical - 2
Personnel of Army Service Corps Motor Transport Unit	53	2 i.e. 3.7%	both Flexner-Y organisms
Native prisoners in Egyptian Prison, Alexandria	100	1 i.e. 1%	a Flexner-Y type.

The convalescent cases were recently recovered patients but at the time of examination were in good health. In 8% dysentery bacilli were detected. The organisms were not present in large numbers in the excreta. It is possible that this proportion of carriers among recent convalescents was a low estimate as only one examination was made; if the

excretion of these organisms is intermittent as seems likely, the proportion of convalescents that remain carriers for some time after recovery may be much higher.

In a series of 60 orderlies at the 21st General Hospital, Alexandria, whose faeces were examined, 4 proved to be carriers (6.6%) and 2 carriers were also detected among 53 officers and men of an Army Service Corps unit encamped in Alexandria.

Among 100 Egyptian natives at the Egyptian Government Prison, Alexandria, 1 carrier was detected.

It was found that dysentery bacilli might be present in the excreta of men who presented no history of dysentery or diarrhoea. For example, a man in the Steward's Store of the 21st General Hospital, who apparently had never suffered from diarrhoea or dysentery while in the Mediterranean Force, was found to be a carrier, and the dysentery bacillus (Flexner-Y type) was isolated on two separate occasions at an interval of 21 days.

Most of the carriers however, had suffered at a previous date from either dysentery or diarrhoea. In one case the dysenteric attack had occurred 18 months previously and there had been complete freedom from intestinal disturbance for 9 months before the date of examination.

It was therefore apparent that dysentery bacilli may be present not only in the excreta of persons suffering from

dysentery and diarrhoea but also in the stools of those who have suffered from these conditions at some previous date and also those who have not had any intestinal illness.

In a country like Egypt where bacillary dysentery is endemic and especially in the absence of adequate sanitation, a certain proportion of the population must represent foci from which this infection is spread.

Length of time during which excreta are infective.

This probably varies under different conditions and while as a general rule the dysentery bacilli tend to disappear rapidly (in 6 to 8 hours) as judged by cultures made at intervals from stools kept at room temperature, in certain cases both the Shiga and Flexner-Y types have been found to survive as long as 24 hours. Thus the stool is infective for a period sufficiently long to allow of the dissemination of the organisms by some vehicle of infection.

The possibility of infection by water, sand, food and flies.

The water supply of Alexandria was obtained from the Mahmoudieh Canal (which was known to be heavily polluted). The water was filtered through sand by a " rapid filtration " process before distribution to the city, but even the filtered water contained " typical " B. coli often in as small quantities as 0.1 c.c. and invariably in quantities of 5 c.c.

No dysentery bacilli were ever found in the filtered water and there was no epidemiological evidence of dysentery being a " water-borne " disease. On the other hand, it might be said that the City supply was potentially infective in view of the fact that the intake water was heavily polluted and the filtration was imperfect as determined by the frequent high B. coli content. Certain experiments were carried out to ascertain whether B. dysenteriae survived for any length of time in a polluted water to which they had been added; it was found that they did not persist for more than 48 hours in the unfiltered Mahmoudieh Canal water kept in flasks in the laboratory and exposed to light during the day. The canal water contained large numbers of coliform bacilli.

Around the native dwellings of Alexandria (and also in other Egyptian towns and villages) the ground is liable to frequent faecal contamination (D. Thomson and Mackie) from human sources; during dust storms, dust and sand readily gains access to food, milk, water, etc., (unless carefully protected) and the question was raised as to whether in Egypt dysenteric infection might be conveyed in this way. 34 specimens of sand from various sources were examined including specimens from polluted ground in certain native quarters, but no dysentery bacilli were ever detected.

Specimens of dried sterilized sand placed in Petri dishes were artificially contaminated with B. dysenteriae

Shiga and Flexner-Y types, and then placed where they were directly exposed to the sun. B. dysenteriae Shiga died out after six hours and the Flexner-Y organism survived for 24 hours but was not recoverable after 48 hours.

Milk was the only specimen of food which was systematically examined; large numbers of specimens of milk supplied to the Military Hospitals were examined bacteriologically in the course of the laboratory routine, but though these specimens almost invariably were heavily contaminated with coliform bacilli, no dysentery bacilli were ever detected.

It seemed likely that the house-fly might carry dysentery bacilli from excreta to food (where insanitary conditions prevailed) and act as a vehicle of infection.

Flies were experimentally allowed to contaminate themselves from dysenteric stools and were then transferred to Petri dishes containing MacConkey's agar in which they were kept for 1 to 2 hours. In this way the medium was inoculated by the fly in the same way as it would infect food material. This method had been utilized in a previous investigation on the duration of infectivity of flies after feeding on sugar containing typhoid and paratyphoid bacilli; the organisms deposited by the fly could be determined by the examination of colonies developing after incubation of

the plate. It was found in this way that flies infected with enterica organisms remained infective for 4 to 7 days. Flies infected in the same way with dysentery bacilli remained infective for not more than 24 hours. A small proportion of the flies fed on dysenteric stools were found to be infective and dysentery bacilli were isolated from the medium with which they had been subsequently in contact.

56 " wild " flies caught in the Native Quarters were examined in the same way but no dysentery bacilli were isolated from them. These flies usually produced abundant contamination of the plates with which they had been in contact; the organisms grown were generally coliform bacilli but not infrequently colonies of B. Morgan No. 1 and allied types, B. faecalis alkaligenes and B. proteus were noted i.e. organisms found associated with dysentery and diarrhoea (v. supra).

While it is possible that flies convey dysentery bacilli, no direct evidence was obtained to show that this was a frequent mode of transmission and infection.

These investigations also show that dysentery bacilli do not persist for long periods outside the body in excreta, polluted water, and sand, and it seemed likely that under active service conditions direct or indirect personal contact was the most important factor in the spread of bacillary

dysentery in view of the frequency of healthy carriers, the fact that many cases of simple diarrhoea without characteristic dysenteric symptoms were due to dysentery bacilli and the probability that these cases often escaped notice, the infected person continuing on duty or recovering without being admitted to hospital.

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PART 3

THE

V. PARACHOLERAE GROUP.

INTRODUCTION.

At the Base Laboratory of the Egyptian Expeditionary Force (1916-1918), I had the opportunity of investigating 57 strains of " non-cholera " vibrios isolated from typical choleraic cases, from cases of acute diarrhoea (not choleraic in type) and also from healthy carriers. These organisms in their general characters all closely resembled one another and evidently belonged to a well defined biological group; in many respects they corresponded closely to the classical V. cholerae but were differentiated by serum reactions.

It has been recognised by other workers in the East that apart from the typical epidemic cholera of Asia due to a vibrio with the biological characters of the classical vibrio cholerae of Koch, choleraic conditions may be produced by different vibrio types (Castellani, Chalmers and Waterfield). As suggested by Castellani, a choleraic illness due to a vibrio which cannot be identified with the V. cholerae might be designated " paracholera "

In the identification of vibrio strains isolated from choleraic cases, the possible occurrence of variants of the V. cholerae must be considered. Thus the El Tor vibrio of Gotschlich differed from the classical type in producing an active haemolysin but was agglutinated by a specific anticholera serum, and, in view of its serological characters,

has been generally regarded as a variety of the typical V. cholerae. It has also been claimed that strains of V. cholerae may under certain conditions lose their specific serological characters (e.g. agglutinability by a homologous antiserum) and thus simulate non-cholera types. It is, however, generally agreed that the failure of a vibrio strain (especially after repeated subculture) to react with an anticholera agglutinating serum is evidence that it belongs to a different species. Moreover, the serological differentiation is confirmed if an agglutinating antiserum to this strain fails to react with a known V. cholerae.

THE OCCURRENCE OF PARACHOLERA VIBRIOS IN A LOCALIZED CHOLERAIC OUTBREAK.

My attention was first drawn to organisms of this type by their occurrence in certain choleraic cases forming a small localized outbreak in one of the Convalescent Hospitals in Egypt.

The following clinical summaries will serve to indicate the conditions with which these organisms were associated:

On October 26, Pte. G., a patient convalescing from " pleurodynia ", developed a sudden acute illness which presented the characteristic clinical signs and symptoms of

Asiatic cholera. The condition started with severe colic, followed by intense diarrhoea and vomiting, and severe cramping pains in the thighs and legs. The evacuations were typically " rice-water " in character. The acute stage was followed by a state of extreme collapse with subnormal temperature, low blood pressure, sunken eyes, cold livid skin, and a weak husky voice. He was treated in the collapsed state by intravenous injection of hypertonic saline solution and subsequently made an uninterrupted recovery. It is of interest to note that this patient had been suffering from digestive disorder for six days previous to the onset of the choleraic illness.

The same day, October 26, another patient Pte. B., in the same hospital suddenly developed acute diarrhoea with abdominal pain and cramps in the legs, but the illness was not so severe as in the first case, and was not associated with any marked degree of collapse, though the blood pressure was relatively low. This man had been suffering for some months past from recurrent mild diarrhoeal attacks.

On the following day, October 27, three other patients in the hospital, Pte. C., Trpr. W., and Pte. P., developed acute choleraic diarrhoea. In the case of Pte. C., the condition was associated with abdominal pain and vomiting but without marked collapse. The intestinal discharges were

of the " rice-water " type. Trpr. W's. illness was simply an acute but transient diarrhoea with little general disturbance. This patient had an " enteric " illness in August. The day before the acute attack he had slight diarrhoea. Pte. P. suffered from a more acute diarrhoea, with vomiting, cramps in the legs and some degree of collapse.

On October 28, at midnight, an R.A.M.C. orderly, Pte. L., who had been in attendance on Pte. B. the same day, suddenly developed an acute choleraic condition which was more or less similar to that of Pte. G., and was associated with marked collapse. His evacuations during the acute stage were typically " rice-water " in character. He, however, made a good recovery after two days' illness.

From all these cases vibrios were isolated.* In the cases, G., L., and P. large numbers of vibrio-like organisms were quite evident on microscopic examination of the discharges.

On November 1, another patient in the hospital developed an acute cholera-like illness. The diarrhoeal stage, which lasted about eleven hours, was followed by a collapsed state with complete cessation of the diarrhoea. The first stool passed after this, about 24 hours after the onset of the illness, was examined but no vibrios were detected. Specimens of the earlier evacuations were not obtained for bacteriological examination.

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* In the cases Pte. C. and Trpr. W. vibrios were first detected in primary culture by Captain E. J. Storer of the Military Bacteriological Laboratory, Alexandria. The subsequent isolation and investigation of these strains were carried out by myself.

In the case of G., vibrios were detected on the first and second days but were found to be absent on the third day from the onset of the illness. In the other cases also vibrios disappeared rapidly from the faeces.

Appropriate preventive measures were at once taken by the D.A.D.M.S. (Sanitation) when vibrios were found in the first case and after November 1 no further choleraic cases were reported.

The presence of vibrios in large numbers during the acute phase and their rapid disappearance after recovery were regarded as suggestive of their aetiological relationship to the disease.

Clinically, certain of the cases, especially that of Pte. G., were indistinguishable from Asiatic cholera. Others were milder in type.

Isolation of the Vibrios.

Peptone-water tubes were inoculated from the intestinal discharges and incubated at 37° C. for 6-8 hours. On examination of the cultures large numbers of actively motile vibrios were found, considerably in excess of the other organisms present. A loopful of the thin pellicle-growth on the surface of the medium was introduced into fresh peptone-water tubes which were incubated for a period of about 12

hours. A practically pure vibrio culture was obtained in this way by " enrichment " in peptone-water and the strains were isolated from colonies on plates of Dieudonné's medium, inoculated from the first or second peptone tubes.*

Characters of the Vibrio Strains Isolated.

The six strains corresponded closely in their morphological and cultural characters.

Morphological Characters: Actively motile " comma "-shaped organism, 1·3 to 2 microns in length and 0·4 micron in breadth; there was, however, considerable variation in size, and "S" forms were noted in young cultures. Films from older cultures showed the characteristic involution forms of the V. cholerae. In preparations stained to show flagella by the method of Nicolle and Morax, a single terminal flagellum about three times the length of the vibrio was demonstrated.

Staining reactions: the vibrio was gram-negative. In young cultures it stained well with the ordinary stains but in older cultures the staining reaction was faint.

Cultural characters: all the strains were readily cultivated on ordinary media under aerobic conditions, both at 37° C. and 22° C.

Stroke cultures on sloped agar, after 24 hours at 37° C. consisted of a moist whitish growth slightly more opaque and

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*Further details of the technique employed are given below (p. 28).

more abundant than that of a V. cholerae. After 2-3 days the growth became slightly brownish-yellow in colour (like V. cholerae).

Colonies on agar, after 24 hours at 37° C., were moist, white, somewhat raised, with a completely circular well-defined margin, and slightly more opaque than those of V. cholerae. The average diameter of 24 hours colonies was 2-3 m.m. After 48 hours they began to assume a brownish-yellow colour.

In gelatin stab cultures (15% gelatin) at 16° - 18° C., a white line of growth was apparent in 24 hours. After 36-48 hours, liquefaction began at the upper end of the stab, and by the 3rd or 4th day a " cup " formed with a funnel of liquefaction extending down along the line of inoculation. Liquefaction occurred more rapidly than in the case of a V. cholerae strain but otherwise the type of growth was the same.

In peptone-water, the organism grew abundantly producing a general turbidity of the medium and a characteristic " pellicle " on the surface. There was no apparent pigment-production.

On MacConkey's and Dieudonné's media, the vibrio grew well, producing colonies which were somewhat larger than those of V. cholerae. The colonies on MacConkey's agar, after 48

hours showed a characteristic yellowish-brown colour and were less raised and opaque than the colonies on ordinary agar.

On alkaline potato medium, an abundant moist white growth developed at 37° C. after 24 hours. In 4 or 5 days it became brownish-pink in colour.

On solidified blood serum at 37° C. abundant growth and rapid liquefaction of the medium occurred, so that in 36 hours about three-quarters of the sloped medium was liquefied. A V. cholerae strain in the same time liquefied about one-quarter of the same amount of serum medium.

Biochemical reactions: the results of the fermentative tests were as follows:-

Glucose	acid;	no	gas		
Galactose	"	"			
Laevulose	"	"			
Maltose	"	"			
Saccharose	"	"			
Mannite	"	"			
Dextrine	"	"			
Glycerin	"	"			
Dulcite	no	acid	production	after	10 days
Adonite	"	"	"	"	"
Rhamnose	"	"	"	"	"
Raffinose	"	"	"	"	"
Inulin	"	"	"	"	"
Salicin	"	"	"	"	"

Sorbite	- - -	slightly acid 1st day; later alkaline.
Milk	- - -	slightly acid; no clot.
Lactose	- - -	at first no fermentation; after 7-10 days distinctly acid except in the case of strain W.

Cholera-red reaction (depending on the formation of nitrites and indol) was markedly positive after 18 hours growth. The test consisted in adding about 2 c.c. of 50% sulphuric acid to a peptone-water culture. Haemolysis, tested by cultivation on blood agar, was evident after 36 hours' growth. Phosphorescence was absent.

Serological Reactions

V. Cholerae Antiserum.

Agglutination. There was no agglutination of any of the strains by a 1:50 dilution of an anti-cholera serum (Lister Institute) whose end-titre was 1:2000. The tests were repeated after the strains had been subcultured several times, with negative result. Similarly, the strains after recovery from experimental animals (v. infra) still failed to react with the V. cholerae antiserum.

Complement Deviation. No complement deviation reaction was obtained in the case of any of the strains along with the anticholera serum, though a marked reaction was elicited in the case of a known V. cholerae strain.

Patients' Sera.

Agglutination. The sera of the six cases were tested 7 days after the onset of the illness, both with the particular strain isolated and also with a V. cholerae. Pte. G's. serum agglutinated the respective strain and also V. cholerae in dilutions up to 1:200 after 4 hours.

None of the other sera reacted to the strain isolated or to V. cholerae. G. had been inoculated twice with cholera vaccine 9 months previously. The serum of a healthy person inoculated three months previously with cholera vaccine did not agglutinate the same V. cholerae strain in dilutions higher than 1:20 and did not act on strain G. even in a 1:20 dilution.

The occurrence of specific agglutinins in the serum of infected persons is strongly indicative of the causal relationship of the vibrio to the illness with which it was associated. Only one of the six cases (Pte. G.) reacted in this way; the negative reaction in the other cases could not, however, be considered of any significance (see also Part 2 p.31A). Thus, even in true cholera the agglutinin reaction is variable and though well marked in certain cases is frequently quite absent (as shown by the protocols of Greig's investigations).

As regards the " concomitant " reaction of G's. serum to V. cholerae, it might be supposed that an increase of the post-inoculation agglutinins had occurred in an analogous fashion to that noted in the case of paratyphoid patients previously inoculated with B. typhosus vaccine (v. Part 5 p. 3).

G's. serum was retested a month after the onset of the illness. It was noted that the end-titre of the agglutinins for strain G. and V. cholerae was now 1:100.

Antiserum to Strains G. and L.

Rabbits were immunized by intravenous injection of increasing doses of agar slope cultures emulsified in normal saline and sterilized at 65° C. An agglutinating antiserum was first obtained for strain G. and tested with the other strains and also a typical V. cholerae. The results are shown in the following table:-

Agglutinating Serum V. Strain G.

	Serum Dilution.						
Strain	1:100	1:200	1:400	1:800	1:1600	1:3200	No serum
G.	++++	++++	++++	+++	+	—	—
W.	++++	++++	++++	++++	+++	—	—
P.	++++	++++	++++	++++	++	—	—
C.	++++	++++	++++	++++	++	—	—
L.	—	—	—	—	—	—	—
B.	—	—	—	—	—	—	—
V.cholerae	—	—	—	—	—	—	—

Readings made after 4 hours.

(++++ = complete agglutination and the various degrees of partial agglutination are represented by +++, ++, +.)

When it was found that strains L. and B. did not react with the antiserum to G. even in low dilutions, an immune serum to strain L. was obtained and tested with the other strains and V. cholerae. The results are shown in the following table:-

Agglutinating Serum V. Strain L.

Strain	Serum Dilution. 1:100	1:200	1:400	1:800	1:1600	1:3200	No serum
G.	—	—	—	—	—	—	—
W.	—	—	—	—	—	—	—
P.	—	—	—	—	—	—	—
C.	—	—	—	—	—	—	—
L.	++++	++++	++++	++++	++	+	—
B.	++++	++++	++++	++++	+++	++	—
V.cholerae	—	—	—	—	—	—	—

Readings made after 4 hours.

These results clearly showed that the six strains represented two different serological types. The antiserum to G. agglutinated G., W., C., and P., but had no effect on L. and B., while the immune serum to L. agglutinated L. and B., but did not react with the strains of the other species (G., W., C., P.). It was also apparent that both these types were serologically different from V. cholerae. It is of interest to note here that Pte. L. was probably infected from Pte. B. (vide clinical summaries).

Pathogenic Effects on Animals.

Guinea pigs: all the strains were tested by intraperitoneal injection of one-half of a 24 hours' agar slope culture emulsified in saline. The animals showed signs of collapse after 2-3 hours and died in 4-6 hours. At autopsy the abdomen was markedly distended; the peritoneal cavity contained considerable excess of fluid which was turbid and in some cases blood-stained. Small intestine - the mucosa and whole intestinal wall was inflamed; there was no faecal material in the bowel but the lumen was distended with an opaque yellow mucous fluid slightly blood-stained; microscopic examination revealed large numbers of vibrios and an abundant cellular exudate. Vibrios were recovered by culture from the peritoneal exudate, the intestinal contents and also the heart blood. These effects were similar to those produced by two V. cholerae strains which were similarly tested for purposes of comparison.

Pigeons: two of the strains were tested by injection of $\frac{1}{40}$th of a 24 hours' agar slope culture into the pectoral muscle. No pathogenic effects were noted.

Rabbits: striking results were obtained in these experiments and it was found possible to reproduce a typical cholera picture, post-mortem by intravenous injection of even $\frac{1}{80}$th of an agar slope culture. The six strains all exhibited

the same effects. In general, after intravenous injection of $\frac{1}{20}$th of a culture, the animal died in about 24 hours or less. In one case there was a marked diarrhoeal condition before death. Post-mortem - the small intestine contained no faecal material; the mucosa was intensely inflamed and the lumen of the bowel was distended with a white milky mucous fluid (in some cases with blood-stained muco-purulent material). The intestinal fluid contained an abundant cellular exudate consisting of degenerate leucocytes and masses of exfoliated epithelium. The pathological condition was most marked in the lower part of the small intestine. Cultures of the intestinal contents on MacConkey's agar in some cases represented a pure growth of vibrios.

These animal experiments showed a highly selective toxic action on the mucosa of the small intestine. The results also corresponded to the pathogenic effects similarly produced by a known V. cholerae.

The Identity of the Strains Isolated.

From the bacteriological findings it was apparent that these vibrio strains closely resembled the V. cholerae in many of their characters; they differed, however, in their serological reactions. As regards their general cultural characters, no essential difference could be established; the

liquefaction of gelatin and solidified serum was, however, more marked. The biochemical reactions were similar to those of V. cholerae. The haemolytic test differentiated these strains from the classical cholera vibrio (which is non-haemolytic) but as is well known, varieties of the V. cholerae (identified by certain specific serological reactions) have been shown to be haemolytic, e.g. Ruffer's Groups II and III. [Group II was agglutinated by an antiserum to the typical cholera vibrio and also reacted positively in the Pfeiffer test but the complement-fixation reaction was absent. Group III failed to react in the Pfeiffer test but was agglutinated by the antiserum and also gave the complement-fixation reaction.]

Strains were also met with in Egypt (1916-1918) from choleraic cases which, though markedly haemolytic, still reacted up to end-titre with a V. cholerae serum,* and these were classified as " V. cholerae."

It has been suggested that agglutinability of a cholera vibrio by an anti-cholera serum may not be a constant character. Thus, it was shown by Crendiropoulo how agglutinable vibrios in the faeces might be succeeded after a time by inagglutinable organisms in the bile. Greig has also demonstrated how a non-agglutinable vibrio isolated from water, after inoculation into an experimental animal and

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* These strains were submitted to me for investigation from the Field Laboratories.

subsequent isolation from the bile tended to alter its morphological and serological characters so that it resembled more closely the standard V. cholerae.

While strains of V. cholerae may be met with which in primary culture fail to agglutinate with a specific antiserum - as in the case of B. typhosus, B. dysenteriae, etc., - there can be little doubt that the agglutination test with a V. cholerae serum is generally a suitable means of differentiating the cholera group from other vibrios (v. also Chalmers and Waterfield). Moreover, the fact that antisera to strains G. and L., while strongly agglutinating the homologous strains, had no effect on a typical V. cholerae (which reacted to an anticholera serum) was conclusive proof that these vibrio strains were specifically different from the V. cholerae.

As regards the general classification of vibrios, Chalmers and Waterfield have recognised the following groups:-
(1) Albensis (2) Cholera (3) Metschnikovi (4) Gindha
(5) Finkler and Prior (6) Drennani (7) Terrigenus.

The " Gindha " group was defined as follows: " vibrios, motile, aerobic, non-phosphorescent, growing in and liquefying gelatin, not producing pigment in peptone, not agglutinated by true cholera immune serum in 1:200 or greater dilutions, with growths in gelatin stabs resembling those of V. cholerae, and

not causing death in 24 hours in pigeons when injected into the pectoral muscles in small quantities."

Chalmers and Waterfield described a vibrio strain of this type causally associated with a case of paracholera at Port Sudan in December 1915, and identified it with the V. Gindha (Pfeiffer) which was originally found in the water of a well at Gindha (in Erythrea) after an epidemic of " cholera." Chalmers and Waterfield also classified the various vibrio species which they recognised as belonging to the "V. Gindha group." These included among other species the V. Gindha and the V. Kegallensis. The latter was described by Castellani as a paracholera vibrio and was found by him to be associated with a cholera-like disease in Ceylon. Non-cholera vibrios have also been described from time to time in choleraic and diarrhoeal cases by different workers; Greig observed non-cholera vibrios in cases of " cholera " but as he failed to obtain any agglutination reaction by the patients' serum to these strains and as they were also noted as concomitants of the V. cholerae, he apparently disregarded them as choleragenic organisms.

It is significant that a human experiment with the original V. Gindha resulted in a severe cholera-like illness (v. Macé).

The V. Gindha was differentiated from the other members of the group according to Chalmers by (1) the power of reducing nitrates and forming indol - on which the cholera-red reaction depends; (2) the rapid liquefaction of solidified blood serum. In the case of the V. Kegallensis the cholera-red reaction was absent. According to these criteria the " paracholera " strains described above would correspond to the V. Gindha; on alkaline potato medium, however, they produced a characteristic pinkish growth while the classical V. Gindha as described develops a " maize-yellow " growth. It is to be noted, however, that the type of growth on potato is somewhat variable; it is uncertain therefore if this can be regarded as a differential criterion. Further reference is made later to the serological identity of Chalmers and Waterfield's strain. The cholera red reaction differentiated them from the V. Kegallensis.

It appears doubtful if this system of classification is sufficiently complete for the identification of the paracholera vibrios. Thus of the six strains isolated from these choleraic cases referred to above, it has been shown that four were specifically different from the other two, though all presented similar cultural characters. This group therefore comprises different serological types (as will be shown more fully later) and any system of classification must be based both on cultural characters and also specific serum reactions.

The evidence was strongly in favour of these strains being causally related to the choleraic disease with which they were associated, and they have therefore been classified as paracholera vibrios. For further reference, the two types represented respectively by strains G. and L. have been designated V. paracholerae A. and B.

FURTHER INVESTIGATIONS.

A few days prior to the occurrence of these choleraic cases a number of patients in the convalescent hospital had suffered from an acute diarrhoeal attack but as the condition proved transitory no bacteriological investigation had been requested.

At that time also a considerable proportion of the patients were convalescents from the Mesopotamia Expeditionary Force including men who had suffered from a choleraic illness.

It was therefore decided to carry out a systematic examination of all the patients in the hospital (about 900) with a view to ascertaining if any were vibrio carriers.

The faeces of each patient was examined on two occasions by the technique described later (p.28) and the investigation led to the demonstration of vibrio carriers among (1) cholera convalescents; (2) men who had suffered a few days previously from acute diarrhoea contracted in the hospital (already

referred to); (3) cases with a history of " diarrhoeal " or " dysenteric " attacks in Mesopotamia or Egypt (Sinai Peninsula); (4) men who presented no history of previous intestinal illness (including both convalescents from the Mesopotamia and Egyptian Forces). There had been of course free " contact " among the patients in the hospital.

The systematic examination* revealed the presence of vibrios in the faeces of 34 cases.

None of these strains corresponded serologically with the V. cholerae and in their general cultural characters and biochemical reactions were identical with the strains described in the paracholera cases. The only variation noted was in the case of lactose fermentation; certain strains developed acid from lactose after 7-10 days; most of the strains, however, failed to ferment lactose.

Their pathogenic effects on laboratory animals were also identical with those of the original strains described above.

Their serological characters were studied and it was found that only a small proportion of the strains corresponded serologically with the A and B types found in the paracholera cases and among the remainder it was possible to differentiate 8 additional serological types

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* In the preliminary work of detecting the vibrios in primary culture, I was assisted by Captains Storer and Hirst; the isolation and detailed study of these organisms was carried out by myself.

This differentiation was well marked, i.e. the antiserum to one type agglutinated organisms of the same type in high titres (the end-titre corresponding to that for the strain used for immunization) and had no effect on strains of other types even in low titres; the serological distinction was thus similar to that described between the A and B types.

Among the cases from which these vibrio strains were isolated, 10 were found to have suffered from acute diarrhoea a few days before the paracholera outbreak.

The strains from these 10 cases represented types serologically different from A and B; 4 corresponded to one serological type and 6 to another, classified respectively as C and D.

Among the remaining 24 strains, types A, B, C and D were all represented and also six other serological types designated E, F, G, H, I and J. 4 strains respectively of types A, E, G and H were found in " cholera " convalescents. The cases from which the other strains were isolated gave no history of a choleraic illness but included men who had suffered from " diarrhoeal " or " dysenteric " attacks in Mesopotamia and also cases from the Egyptian Expeditionary Force presenting a similar history. In others again no history of intestinal illness was obtainable.

Of course, any of these cases might have been infected by contact in the hospital and it is well known how, during an actual cholera epidemic, contacts may be infected and become carriers without suffering from the disease.

Thus the findings may be summarized as follows*:-

Paracholera cases from which vibrios were isolated	- 6 -	(4 of type A (2 " B.
Cases convalescent from acute diarrhoea (contracted in hospital) in which vibrios were found	- 10 -	(4 of type C ((6 " D.
Cases free from diarrhoea or other intestinal illness (while in hospital) in which vibrios were found i.e. healthy carriers	- 24 -	(the 24 strains (represented 10 (different sero-(logical types (A, B, C, D, E, (F, G, H, I, J.

The question also arose at that time as to the occurrence of vibrios of the same type in the native Egyptian; specimens of faeces from 100 healthy natives in the State prison at Alexandria were examined with negative result in all cases; the native staff at the convalescent hospital were also examined on two occasions but no carriers were detected. It was known of course that non-cholera vibrios had been frequently met with in pilgrims from Mecca at the quarantine stations of the International Quarantine Board.

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* It is impossible to give fuller details as the laboratory records were lost at sea due to the sinking by submarine action of the transport on which I was travelling from Egypt in 1918.

Choleraic disease had apparently been prevalent in Mesopotamia and as certain carriers of the different types presented a history of " cholera " or " diarrhoea " in Mesopotamia during the previous three months, it was presumed that these organisms might be of Mesopotamian origin.

Some of the carriers were investigated at intervals; in certain cases, vibrios were found in the faeces over a period of one month; in other cases they apparently disappeared rapidly from the stool.

Among the carriers were men who had suffered from " diarrhoeal " or " dysenteric " illnesses in Egypt; a few months previously a limited cholera epidemic had occurred in the Egyptian Expeditionary Force but it was known that the causative organism in this case corresponded to the classical V. cholerae * and none of the patients in the hospital had been in the particular area in which cholera occurred or had been in contact with men of the particular military units affected. At that time no "non-cholera vibrios" had been isolated from diarrhoeal cases in any of the military laboratories of the Force but until two months prior to this there were no facilities for laboratory examination in the field. It was only at the Base that detailed examinations could be carried out.

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* I had the opportunity of studying certain of the strains.

It was assumed from the evidence available, that vibrios of the type described were responsible for an intestinal condition, varying from a simple diarrhoea to an acute illness clinically indistinguishable from Asiatic cholera; that these organisms represented a group not serologically homogeneous but including different serological types; that they were, like V. cholerae, apparently of Asiatic origin.

During 1917, I had a further opportunity of investigating another series of 17 vibrio strains all presenting the same general characters as those originally investigated; with one exception, these were isolated in the field laboratories from sporadic cases of acute choleraic diarrhoea, some of which simulated clinically true cholera. At that time the Force was operating in Palestine. The cultures were submitted to me for investigation by officers in charge of Field Laboratories.

Two of these strains corresponded to the A type and 3 to B.

Two strains were found to belong to another serological type different from the series A to J and therefore designated K. A culture of the V. Gindha type isolated by Chalmers and Waterfield was obtained from Dr. Chalmers at Khartum and it was found to belong to this serological type (K).

Two strains submitted for investigation from one of the field laboratories were also found to represent a further serological type, designated L.

A fatal case of choleraic type similar clinically to that of Pte. G. (v. supra) occurred in an officer a few days after his arrival in Alexandria* from England; vibrios were present in large numbers in the stools and on isolation of the strain, it was found to be similar in cultural characters to the other vibrios but proved on serological investigation to belong to a different type (designated M.)

7 other strains were investigated; none of them agglutinated even in low titres with antisera to types A - M, and antisera prepared by immunizing rabbits with these strains agglutinated only the corresponding strain, i.e. the particular strain used for immunization.

The question arose as to whether this group of vibrios was analogous in serological reactions to the B. coli group (v. part 1) in which agglutinating antisera are specific for the individual strain. Strains of types A and B were isolated, however, in different places and at intervals of 6 months. Lt. Colonel Ledingham, Consulting Bacteriologist of the Mesopotamia Expeditionary Force also informed me that he had isolated a strain in Mesopotamia (1917) corresponding to the B type (identified by an agglutinating antiserum supplied to him by me). Moreover, the " V. Gindha " of Chalmers and Waterfield isolated at Port Sudan was found to be serologically identical with 2 strains isolated in 1917 in Palestine (v. supra).

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* At a Base Depot Camp.

Though certain of the serological types, in the series of strains investigated were represented by only one strain, other serological subgroups included a number of strains, e.g. A, B, etc.

Among the 57 strains studied, 20 different serological types were recognised.

CONCLUSIONS.

Besides the typical epidemic Asiatic cholera due to the V. cholerae, choleraic and diarrhoeal conditions may be due to a group of vibrios of the type described, conveniently classified as V. paracholerae.

These organisms closely resemble V. cholerae in morphological, cultural, and biochemical characters and as regards the effects of experimental inoculation in animals but are easily distinguished by serological tests.

The paracholera group is not serologically homogeneous but represents a considerable number of serological types precisely differentiated by agglutination reactions.

In general paracholera cases are of less severity than typical cholera but on the other hand in certain instances may closely simulate true cholera.

Fatal cases are uncommon; of those known to me only 2 proved fatal (due to types K and M).

The diarrhoeal and choleraic cases with which these organisms are associated are either sporadic or occur in the form of small localized outbreaks.

These organisms are like V. cholerae transmitted by carriers and are probably of Asiatic origin.

Individuals may be infected without contracting any intestinal illness and become carriers.

A NOTE ON THE METHODS ADOPTED FOR THE ISOLATION AND SEROLOGICAL IDENTIFICATION OF VIBRIOS.

In studying the technique of isolation of V. cholerae and the paracholera vibrios from faeces, it was found that successful results depended on the careful observance of certain details. The method adopted consisted first in " enriching " the vibrio in alkaline peptone water - a medium known to be specially favourable to the growth of these organisms - and then sub-inoculating on a selective alkaline solid medium (Dieudonné's).

1. A tube of 10 c.c. peptone water was inoculated with a flake of mucus or in the case of a fluid faecal stool with a large loopful of the specimen. In examining possible carriers the stool if solid or semisolid was thoroughly emulsified in sterile salt solution and a large loopful added to the peptone water. The peptone water used was 1% peptone with 0.5% sodium chloride standardized so as to be neutral to phenol-phthalein, i.e. distinctly alkaline to litmus.

The tube was incubated at 37° C. for 6 to 8 hours.

2. A plate of Dieudonné's medium was also inoculated directly from the stool and incubated for 12-18 hours.

Dieudonné's Medium: defibrinated ox's or sheep's blood was mixed with an equal volume of normal sodium hydroxide solution and steamed in the Koch's sterilizer for 1 hour on

3 successive days. This blood-alkali mixture was then added to neutral peptone agar (3% agar) in the proportion of 3 parts of the former to 7 parts of the latter. The medium was again steamed for 1 hour. It proved essential that the medium should be tested before use by inoculating it with a strain of V. cholerae as when freshly prepared it was often extremely inhibitory, due to the presence of a volatile ammoniacal substance in the blood-alkali mixture which could be removed by repeated heating. If found inhibitory the medium was re-steamed and the plates were exposed to air for a short time covered by a sheet of sterile paper.

Dieudonné's medium proved highly selective for vibrios and almost completely inhibited the growth of the usual coliform bacilli of the faeces. The only other intestinal organisms found to be capable of growing well on this medium were:

Certain B. faecalis alkaligenes types;
some enterococcus types;
certain unusual types of coliform bacilli of rare occurrence.

Thus a practically pure culture of a cholera or para-cholera vibrio could be obtained on this medium from a mixture containing other intestinal organisms.

3. The peptone-water culture was examined after 6-8 hours by means of stained films made from a drop of the surface layer of the culture: a large loopful was placed on a slide and slowly dried at room temperature; the film was then fixed by heat and washed in a stream of water to remove the dried peptone particles which stain deeply and obscure the film; the preparation was then stained with dilute carbol-fuchsin for 1 minute and examined microscopically. At the same time a hanging drop preparation was examined. At the edge of the drop vibrios were easily detected by their characteristic morphology and " scintillating " or darting motility.

In general, however, the fuchsin-stained film could be relied on alone for the detection of vibrios.

4. If vibrios were present a subinoculation was made on a Dieudonné's plate. If no vibrios were detected a subinoculation was made into a second peptone water tube; this tube was incubated for 6 to 8 hours and a film from it was then examined as in the case of the primary culture; if vibrios were present, a Dieudonné plate was inoculated from the peptone culture. If no vibrios were detectable the result was regarded as negative.

If vibrios were present in large numbers in the specimen of faeces, an abundant growth could be obtained on the Dieudonné plate inoculated directly from the specimen. As

this growth was practically pure, an emulsion could be prepared from it for agglutination tests with an anticholera serum so that within 18 to 24 hours it was possible to determine whether the causative vibrio was V. cholerae or a " non-cholera " organism. In this way the bacteriological diagnosis could be expedited.

Where vibrios were relatively less numerous and were not cultured directly on a Dieudonné plate, the organism could be isolated after enrichment in either one or two peptone-water cultures.

In two instances it was noted that though negative results followed the peptone-water enriching process, a growth was obtained on a Dieudonné plate directly inoculated from the faeces. It seemed therefore advisable to carry out both procedures to ensure the maximum success in the isolation of these vibrios.

A film made from the growth on Dieudonné plates (inoculated directly or from peptone-water cultures) usually revealed a pure growth of vibrios.

Pure cultures on agar slopes were obtained from isolated colonies; the morphological, cultural and biochemical characters of the strains were then studied and animal inoculation and serological tests were carried out as indicated above.

The technique of agglutination, agglutinin absorption tests and complement deviation tests was that described in Part 1, pp. 27, 30, 36.

Pfeiffer's reaction: the technique adopted was as follows:

4 large loopfuls of a 24 hours' agar slope culture were emulsified in 4 c.c. of normal saline; to 2 c.c. of this emulsion 0·002 c.c. of the anticholera serum was added (A); the remaining 2 c.c. were retained for a control test (B); 4 loopfuls of a 24 hours' agar slope culture of a known V. cholerae strain were emulsified in 4 c.c. normal saline and to 2 c.c. 0·002 c.c. of the same serum was added (C), the remaining 2 c.c. being retained (D).

A, B, C and D were injected into the peritoneal cavities of small guinea-pigs of approximately equal weights; the peritoneal fluid of each animal was examined after ½ hour and also after 2 hours. Specimens were most easily obtained by introducing the needle of a 2 c.c. syringe into the peritoneum and drawing up a small quantity of fluid with the syringe. A drop was then ejected on to a coverglass and examined as a hanging drop preparation.

If the unknown vibrio was V. cholerae - Animal injected with A: the vibrios became immobile and globular and finally became broken up and disappeared from the fluid. The animal was also protected from the lethal effect of the organism;

animal injected with B: the vibrios remained actively motile, showed no bacteriolysis and the animal died usually within 24 hours;

animal injected with C: the result was the same as in the case of A;

animal D: the result was the same as in the case of B.

Experiments C and D were included as controls.

In the case of the paracholera vibrios - in experiment A: the organisms remained actively motile after 2 hours and the animal died within 24 hours; while in experiment C bacteriolysis and protective action occurred.

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PART 4

THE ISOLATION OF PARATYPHOID BACILLI FROM FAECES BY ENRICHMENT WITH BRILLIANT-GREEN IN FLUID MEDIUM; WITH ADDITIONAL NOTES ON THE ISOLATION OF PARATYPHOID BACILLI FROM URINE AND THE RECOGNITION OF B. PARATYPHOSUS B COLONIES IN PLATE CULTURES.

In 1913 Browning, Gilmour and Mackie introduced a method for the isolation of B. typhosus from faeces by enrichment of this organism with brilliant-green in fluid medium. It was shown that those types of B. coli which are commonly found in faeces, sub-group A (Mackie) - vide Part I - are more susceptible to the inhibitory effect of brilliant-green than B. typhosus and the paratyphoid bacilli; thus in fluid medium containing brilliant-green, inoculated from faeces, B. typhosus if present is capable of outgrowing the coliform bacilli. It was recognised, however, that some coliform types, less frequently met with in faeces were even more resistant than the typhoid bacillus, e.g. certain sub-group B types and the inosite-fermenters (sub-group C) - v. Part I p. 46 .

Browning, Gilmour and Mackie claimed for the method that (1) it rendered the isolation of B. typhosus and B. paratyphosus possible when these organisms could not be detected on plates inoculated directly from faeces, and (2) that frequently, when only scanty colonies of B. typhosus were present in direct cultures, the brilliant-green fluid medium yielded an almost pure growth of this organism, thus facilitating its detection and isolation.

The following are the details of the method originally advocated: a peptone-water medium was prepared in the usual way, containing 2% peptone (Witte's) and 0·5% sodium chloride;

5 c.c. were distributed in 6 inches by $\frac{5}{8}$ inch test tubes plugged with cotton-wool, and sterilized at 120° C. for 15 minutes in the autoclave. From a stock 1% solution of brilliant-green in distilled water, a 1:10,000 dilution was made up by adding 0·1 c.c. to 9·9 c.c. of distilled water. Of this dilution the following amounts were added to tubes of the peptone-water;

1	2	3	4	5	6
0·04	0·08	0·12	0·16	0·22	0·3 c.c.

A large loopful of the faeces (which was emulsified if necessary in sterile salt solution) was added to each tube and thoroughly mixed with the medium. After 20 to 24 hours' incubation at 37° C. subinoculations were made from each tube on plates of MacConkey's medium. These plates were incubated for about 24 hours and the resulting growths were examined in the usual way.

The brilliant-green enrichment method has been accepted by various workers as superior to the ordinary methods of direct plating on differential solid media and has been most extensively employed with generally favourable results (v. Browning, Applied Bacteriology).

In the original papers by Browning, Gilmour and Mackie, the use of varying concentrations of brilliant-green was described as an essential feature of the method owing to the

variation in the optimum amount for obtaining the maximum growth of typhoid bacilli from different specimens of faeces. The procedure has been abbreviated, however, by certain workers (v. Browning) owing to the additional time required for carrying out the extended method; this modification has generally consisted of limiting the inoculation to one tube containing a 1:200,000 or 1:250,000 concentration of brilliant-green (in 10 c.c. of peptone water).

While this procedure has yielded better results than those obtained by direct plating, it is open to fallacy and if the maximum success is to be obtained, it is essential to use a series of amounts of brilliant-green as in the original method. Moreover, where it is important that the most thorough technique should be employed in the isolation of typhoid and paratyphoid bacilli, no reasonable amplification of an isolation method can be neglected, provided that the extra time devoted to it is likely to repay the worker by his obtaining more successful results. Thus, a further modification of the original method intended to obviate the possibility of overgrowth of B. typhosus by certain types of B. coli (e.g. sub-group C) which are more resistant to brilliant-green than the typical coliform bacilli, i.e. the use of tellurate of soda (or telluric acid) in conjunction with brilliant-green, was demonstrated by Browning, Mackie and

Smith to be a further advance in the selective enrichment of B. typhosus in fluid medium. The importance of this modification was further emphasized by Browning and Thornton.

When brilliant-green-resistant coliform organisms are present in faeces containing B. typhosus or paratyphoid bacilli, they are of course likely to overgrow the latter organisms in certain concentrations of brilliant-green, but on the other hand if a series of different concentrations of the dye are inoculated, it may be found that there is an optimum concentration in which the typhoid-paratyphoid organism grows abundantly and the enrichment of the more resistant coliform type occurs in higher concentrations. This was noted by Browning, Mackie and Smith and is also well exemplified in table 6 of this paper (v. infra). Thus, if varying concentrations of brilliant-green are employed, relative enrichment of B. typhosus or B. paratyphosus may occur in a particular concentration even when resistant B. coli strains are present in the faeces.

Browning, Mackie and Smith (1914) indicated that the use of tellurate was applicable only as regards the isolation of B. typhosus and B. paratyphosus A, and it was shown that B. paratyphosus B. and B. Gaertner were less resistant than B. typhosus to tellurate by itself. *

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*It was found later by Smith that B. paratyphosus B was more resistant to the mixture of brilliant-green and telluric acid than either B. typhosus or B. paratyphosus A. (v. Browning, Applied Bacteriology, p. 112).

In selecting a routine method, therefore, for the bacteriological investigation of faeces carried out at the Central Bacteriological Laboratory, Alexandria, during the enteric prevalence in the earlier stages of the Mediterranean Campaign when the great majority of enteric infections were due to B. paratyphosus A and B, it was decided to employ the original brilliant-green method.

In all cases varying concentrations of brilliant-green in peptone water were inoculated. As regards the details of the technique: a dense and uniform emulsion of faeces was made in sterile 0·85% salt solution and from this tubes of peptone-water (2% peptone, 0·5% sodium chloride, neutral to litmus) were inoculated. In general a large loopful (0·4 cms. diameter) of the emulsion was added so that a heavy inoculation resulted. In the case of diarrhoeal stools the amount added to the tubes depended on the density of the specimen; if watery in consistence, three or four loopfuls were used for the inoculation of each tube; if the specimen was of greater density, one loopful was sufficient. The tubes were then incubated at 37° C. Subcultures were made never later than 12 hours (and sometimes as early as 7 or 8 hours) on MacConkey's agar. Clarke and Stokes who made extensive use of the brilliant green enrichment method inoculated the tubes heavily and subcultured not later than

9 hours after. Torrey used a fixed amount of brilliant-green but varied the quantity of faeces added to the medium. Though here allowance is made for variation in the optimum adjustment of the amount of faeces in relation to the amount of brilliant green, it is doubtful if this procedure is likely to elicit the best results if the enteric organisms are scanty. It seemed more rational to use varying amounts of brilliant-green and inoculate each tube heavily. Where the number of typhoid or paratyphoid organisms in the specimen is likely to be relatively small, it is undoubtedly essential to inoculate with the maximum amount of faeces; if the typhoid bacilli are present in the amount of faeces used for a " light inoculation " they could probably be isolated by the direct methods, so that in such cases the only advantage of employing an enrichment process would be increased facility of " picking off " suspicious colonies on the subinoculated plates owing to their relatively greater number or their presence in practically pure culture. It is also more essential if heavy inoculation is resorted to that varying amounts of brilliant-green should be used; the organic matter added may be sufficient to interfere with the inhibitory properties of the dye so that in the lower concentrations the typhoid-paratyphoid bacilli are overgrown by coliform bacilli especially if the bacterial content is great; as the influence

of this factor must vary with different specimens of faeces, it is obvious that the necessary allowance for it can only be made by varying the concentration of brilliant-green.

The amounts of brilliant-green generally employed were:

1	2	3
0·25 c.c.	0·4 c.c.	0·6 - 0·7 c.c.

of a 1:10,000 solution in distilled water freshly made from the stock 1% solution.

The following examples from my own experience of the bacteriological results obtained in cases where B. paratyphosus A and B were isolated by the brilliant-green method serve to illustrate the importance of the actual method of application of the enrichment process. In the cases quoted, the brilliant-green method was also controlled by direct plating on MacConkey's medium and the results show the striking superiority of the enrichment method.

Table I.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 8 hours(when plated on MacConkey's medium).
1.	0·25 c.c.	Practically pure growth of B. paratyphosus A.
2.	0·4 c.c.	Scanty growth of atypical B. coli.
3.	0·6 c.c.	No growth.

On direct plates no B. paratyphosus A colonies detectable.

Here a " one-tube " method in which a 1:250,000 concentration of brilliant-green (i.e. 0·4 c.c. 1:10,000 in 10 c.c. peptone-water) was inoculated would have completely failed to yield any result, whereas a practically pure culture of B. paratyphosus A was obtained in the tube containing 0·25 c.c. of the dye. On the plate inoculated directly from the faeces no paratyphoid bacilli were detectable showing that this organism was present in relatively small numbers. It is to be noted also that an atypical B. coli was grown in the 2nd tube, i.e. it was apparently capable of growing in stronger concentrations and was more resistant than the paratyphoid bacillus; in a lower concentration of the dye, however, the paratyphoid bacillus was not overgrown by it (see also table 6).

Table 2.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 12 hours (when plated on MacConkey's medium).
1.	0·25 c.c.	Typical B. coli.
2.	0·4 c.c.	Mixed growth of B. coli and B. paratyphosus A.
3.	0·7 c.c.	Practically pure growth of B. paratyphosus A.

On direct plate no B. paratyphosus A colonies detectable.

In this case 0·4 c.c. of the brilliant-green solution was sufficient to enrich the paratyphoid bacillus to a certain extent, but a larger amount (0·7 c.c.) elicited an almost

pure culture of this organism. This result was also obtained from a specimen of faeces which showed no suspicious colonies on direct plating.

Table 3.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 8 hours (when plated on MacConkey's medium).
1.	0·25 c.c.	B. coli - atypical
2.	0·4 c.c.	B. coli - atypical
3.	0·65 c.c.	Atypical B. coli and B. paratyphosus B in mixed growth.

On direct plate no B. paratyphosus B colonies detectable.

This result is interesting in that no typical B. coli grew in the brilliant-green medium, but in all the tubes there was an abundant growth of an atypical B. coli which was relatively resistant to the dye. The highest concentration of brilliant-green, however, seemed to be sufficiently inhibitory to allow the paratyphoid bacilli to multiply though not sufficient to completely stop the growth of the coliform organisms. No paratyphoid bacilli were detectable on the direct plate.

Table 4.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 10 hours (when plated on MacConkey's medium).
1.	0·25 c.c.	B. coli - atypical.
2.	0·4 c.c.	B. coli - atypical.
3.	0·6 c.c.	Atypical B. coli and B. paratyphosus A mixed.

2 B. paratyphosus A colonies detectable on direct plate.

This shows a similar result in the case of B. paratyphosus A. Here it was possible to isolate the paratyphoid bacillus from the direct plate but in the highest concentration of brilliant-green, marked enrichment of this organism occurred in spite of the presence of a resistant coliform bacillus and on the plates subinoculated from the brilliant-green culture, large numbers of colonies developed.

Table 5.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 8 hours (when plated on MacConkey's medium).
1.	0·25 c.c.	Practically pure growth of B. paratyphosus B.
2.	0·4 c.c.	Do.
3.	0·6 c.c.	Do.

On direct plate a few colonies of B. paratyphosus B.

In this case though the paratyphoid colonies were present on direct plate, all the concentrations of brilliant-green produced an almost pure growth of this organism, thus rendering the detection of the organism much easier than by the ordinary method.

Table 6.

Tube.	Brilliant-green 1:10,000 in 10 c.c. peptone water.	Resulting growth after 8 hours (when plated on MacConkey's medium).
1.	0·25 c.c.	B. coli (typical)
2.	0·4 c.c.	Mixed B. coli (typical) and B. paratyphosus A.
3.	0·6 c.c.	Growth of atypical B. coli (inosite-fermenting type - sub-group C, Mackie).

No B. paratyphosus A colonies detectable on direct plate.

In this case the optimum amount of brilliant-green for the enrichment of the paratyphoid bacillus was 0·4 c.c.; a higher amount 0·6 c.c. produced complete inhibition of the paratyphoid bacillus and favoured the growth of the more resistant coliform bacillus present in the faeces. This is a striking instance of the careful adjustment necessary for a satisfactory result. It is remarkable how slight variations in the amount of brilliant-green affect the character of the resulting growth, certain concentrations favouring the growth

of one type of organism at the expense of another. In the case quoted in table 6, though a highly resistant coliform bacillus was present in the faeces, it did not interfere with the growth of the paratyphoid bacillus in a particular concentration of brilliant-green (tube 2) which represented the optimum for the enrichment of the latter organism; the optimum concentration for the enrichment of the coliform type was higher (tube 3). Thus, when varying concentrations of brilliant-green are used, even if highly resistant coliform bacilli are present in the faeces it may still be possible to obtain in one concentration relative enrichment of the typhoid-paratyphoid bacillus. This has already been alluded to. Table 1 exemplifies an analogous result.

As will be seen from these examples the optimum amount of brilliant-green necessary to elicit the maximum growth of the paratyphoid bacilli varies with each specimen of faeces, though lying within a certain range, i.e., from 0·25 c.c. to 0·7 c.c. of a 1:10,000 solution in 10 c.c. medium, and the resulting growth may be completely altered by even slight variations in the amount of the dye used. On what this variability depends it is difficult to say - probably qualitative and quantitative variations in the bacterial content; difference in the resistance to brilliant-green of various strains of B. typhosus and the paratyphoid bacilli.

and possibly differences in the chemical content of the faeces especially where heavy inoculations are made. Differences in the reaction of the medium and the character of the peptone may also affect the results (Browning); in the cases quoted, however, a pre-war Witte's peptone was used and the reaction of the peptone water was always rendered approximately neutral to litmus.

These results in which the brilliant-green method was controlled by simultaneous direct plating represented a further demonstration of the superiority of this process, provided a complete method is used.

In cases 2 and 6 the abbreviated procedure would have been equally successful with the original method, but in cases 1, 3 and 4 the " one-tube " modification would have failed to produce a positive result. Moreover, in case 4 a positive result would have been obtained by direct plating, while the " one-tube " method would have yielded a negative result.

These observations clearly demonstrated the necessity of adhering to the original method if uniformly satisfactory results are to be obtained and the fallacy of using an abbreviated or one-tube method.

Of course in a great many instances the typhoid and paratyphoid bacilli can be isolated by direct plating, but in

all such cases the preliminary enrichment in brilliant-green peptone water is likely to render the detection of suspicious colonies much easier and so ensures greater certainty in the isolation of the enteric organisms. This feature alone, e.g. as shown in table 5, has proved of the greatest importance with the brilliant-green process and represents, apart from other considerations, a striking advantage of the method.

THE ISOLATION OF PARATYPHOID BACILLI FROM URINE.

The attempt to isolate the specific organism is undoubtedly the most important diagnostic method that can be applied in a suspected enterica case. In the early stages of the illness this may be achieved in a large proportion of cases, as is well known, by the method of blood-culture; when cases come under observation only at a later stage of the disease, for the isolation of typhoid or paratyphoid bacilli it is necessary to resort to cultivation from the faeces and urine.

The essential difficulty in isolating the enterica organism from faeces is due to their being relatively scanty in the majority of cases and therefore considerably outnumbered by coliform bacilli. Hence special methods, such as the brilliant-green enrichment process, have been devised to overcome this difficulty.

In the case of cultures from urine the isolation of a typhoid-paratyphoid bacillus, if present, is comparatively easy, since, unless the urine is contaminated, these organisms occur in practically pure culture. On the other hand a single examination of urine is often of little value. Repeated examinations, however, may yield successful results. During an enterica illness transient bacilluric periods occur, and in a large proportion of cases if daily examinations are made over a period of 7 to 10 days, on a particular day large numbers of organisms are present in the urine and can be easily isolated. As the bacilli are present in considerable numbers it is not even necessary to centrifugalize the specimen; an abundant growth can be obtained by simply plating out two or three loopfuls of the urine on a MacConkey plate.

The possibility of recovering enterica organisms in this may when other methods had failed was noted in the course of routine observations and to test the efficiency of the method, a series of 12 cases in the 2nd and 3rd weeks were examined; these cases all presented clinical evidence of enterica and their sera exhibited specific agglutination reactions with B. paratyphosus A or B. No growths were obtained by blood culture and the faeces had been examined with negative results. Daily examinations of the urine were made until organisms were detected and in each case the specific organism (B. paratyphosus A or B) was isolated in this way.

Though this method may necessitate a considerable number of examinations, the whole procedure is comparatively uncomplicated and quickly carried out; moreover the results are likely to repay the worker for the additional labour and time involved. It is unnecessary to centrifugalize the urine and provided fresh uncontaminated specimens are submitted a negative result is at once indicated by the absence of growth on the plate.

In connection with this diagnostic method, it has been noted that bacteriuria in enterica may occasionally be due to organisms other than the enterica group, e.g. B. paracolon types, " B.C.L.A. types " (v. part 2, table 3), a non-motile cocco-bacillus similar to that described in table 3 part 2, and enterococci. These organisms when they occurred were present in large numbers in freshly passed specimens. Patrick in 1914 (v. part 1, p. 21) working in Great Britain also noted in cases of typhoid fever the occurrence of bacilluria due to certain B. paracolon types.

Thus in carrying out bacteriological examination of specimens of urine in enterica the possible occurrence of organisms other than the enterica group must be considered.

THE RECOGNITION OF B. PARATYPHOSUS B COLONIES IN PLATE CULTURES.

The colonies of the typhoid-paratyphoid bacilli in plate cultures after 24 hours incubation at 37° C. show no morphological characters which differentiate them from a number of other non-lactose fermenting intestinal bacilli. Therefore, in picking-off suspicious colonies on MacConkey's medium, it is necessary to subculture several of the pale colonies present for investigation, as other non-lactose-fermenters may grow whose colonies cannot be distinguished by naked-eye appearances from those of the enterica group.

In the case of B. paratyphosus B it was noted that a striking feature of the colonies could be elicited which in several cases facilitated their recognition. After incubation of the plates for 18-24 hours at 37° C., if the culture is allowed to remain at room temperature for a further 24 hours, a raised opaque border develops round the colony. This character, though not absolutely specific for B. paratyphosus B., proved so distinctive as to enable scanty colonies of this organism to be easily picked out in plate cultures containing other non-lactose-fermenters.

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PART 5

A STUDY OF THE

AGGLUTINATION REACTIONS OF THE

SERUM IN PARATYPHOID INFECTIONS

OCCURRING AMONG TYPHOID INOCULATED TROOPS.

The following observations represent the results of a systematic study of the agglutination reactions of a series of paratyphoid cases occurring among troops of the Mediterranean Expeditionary Force (1915) who had been previously inoculated with the original typhoid vaccine in general use in the British Army during 1914-15. This study * was carried out by me as part of an extensive investigation of cases of " enterica " at that time so prevalent in the Force, and the practical importance of the work lay in the diagnostic interpretation of results obtained by the usual agglutination tests in typhoid inoculated persons subsequently contracting an " enterica " illness. The serological findings were of course controlled by isolation of the causal organism in as many as possible of the cases examined and in this way the significance of the different reactions was confirmed.

Apart from the practical value of these observations at a time when paratyphoid fever was prevalent among troops inoculated with only the typhoid vaccine and when it was essential to ascertain the statistical prevalence of paratyphoid as compared with typhoid infections,** this study elicited an interesting and important immunological phenomenon.

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*100 cases with positive agglutination results were studied.

**The determination of the prevalence of paratyphoid infections led to the application of the " triple vaccine " in the M.E.F.

Certain of these observations were also confirmed later by Captain H. G. Wiltshire in another series of cases and the whole subject of the diagnostic interpretation of agglutination tests in typhoid and paratyphoid fever among typhoid vaccinated troops was dealt with by Mackie and Wiltshire in a paper published in the Journal of the Royal Army Medical Corps (1917) - Vol. 29, No. 3, p. 276.

The methods employed were similar to those already described in Part 1; emulsions of living organisms were preferred and the " macroscopic " technique was used. The bacillary emulsions were freshly prepared in 0·85% saline solution from 18-24 hours' agar-slope cultures; these emulsions were standardized so that their opacity was approximately equal to that of a 0·1% suspension of very finely powdered pure chalk in distilled water. The strains of B. typhosus, B. paratyphosus A and B were those specially selected in the Central Laboratory, Cairo (State Institute of Hygiene) for use in all the laboratories of the Mediterranean Expeditionary Force.

The diagnostic system adopted was to test varying dilutions of the patient's serum with each of the three enterica organisms in parallel series. The dilutions tested were as follows:

1	2	3	4	5	6
1:50,	1:100,	1:200,	1:500,	1:1000,	1:2000

and in the case of certain powerfully agglutinating sera, 1:4000 and 1:8000.

These dilutions were obtained by placing in a series of test-tubes a fixed quantity (0·4 c.c.) of

the serum diluted as follows:

1	2	3	4	5	6
1:25,	1:50,	1:100,	1:250,	1:500,	1:1000,

and then adding an equal volume of bacillary emulsion.

The tubes were incubated for 2 hours at 37° C. They were then removed from the incubator and allowed to stand at room temperature till next day. Readings were made after 2 hours and finally after 20-24 hours.

General results obtained in paratyphoid cases among men previously inoculated with B. typhosus vaccine.

In the great majority of these cases a specific agglutination reaction occurred with one of the paratyphoid bacilli, and in practically every case where the paratyphoid bacillus was agglutinated by the serum there was also a definite reaction with B. typhosus; in most instances, the end-titre of the reaction with B. typhosus was lower than that with the paratyphoid organism but it was frequently equal to, and in a few cases actually higher than the end-point of the reaction with the paratyphoid bacillus.

Tables 1, 2, 3 and 4 show characteristic examples of these results; in table 1 the highest titre of the paratyphoid A agglutinins is 1:2000, that of the concomitant typhoid agglutinins, 1:1000; after 2 hours there was no reaction with B. paratyphosus B in even a 1:50 dilution of the serum.

Table 2 shows an analogous effect in a case of B. paratyphosus B infection. Table 3 exemplifies a case where a more marked reaction occurred with B. typhosus than with the infecting organism. In table 4 are shown reactions equal in degree with both organisms.

Table 1.

	Serum dilutions.						Control; No Serum.
	1:50	1:100	1:200	1:500	1:1000	1:2000	
B. typhosus	++++	++++	++++	+++	+	—	—
B. paratyphosus A	++++	++++	++++	++++	+++	+	—
B. paratyphosus B	—	—	—	—	—	—	—

Readings after 2 hours at 37° C. B. paratyphosus A isolated. Inoculated with B. typhosus vaccine (2 doses) 8 months previously. Agglutination test on 7th day of illness.

(In this and in subsequent tables the degree of reaction is signified by: ++++, +++, ++ and +; — signifies absence of agglutination.)

Table 2.

	Serum dilutions.							Control; No Serum.
	1:50	1:100	1:200	1:500	1:1000	1:2000	1:4000	
B. typhosus	++++	++++	+++	++	+	—	—	—
B. paratyphosus A	—	—	—	—	—	—	—	—
B. paratyphosus B	++++	++++	++++	++++	++++	++	++	—

Readings after 2 hours at 37° C. Inoculated with B. typhosus vaccine (2 doses) 15 months previously. Agglutination test on 14th day of illness.

Table 3.

	Serum dilutions.						Control; No Serum.
	1:50	1:100	1:200	1:500	1:1000	1:2000	
B. typhosus	++++	++++	++++	+++	++	—	—
B. paratyphosus A	++++	++++	+++	—	—	—	—
B. paratyphosus B	+	—	—	—	—	—	—

Readings after 2 hours at 37° C. B. paratyphosus A isolated. Inoculated with B. typhosus vaccine 10 months previously.

Agglutination test on 21st day of illness.

Table 4.

	Serum dilutions.				Control; No Serum.
	1:50	1:100	1:200	1:500	
B. typhosus	++++	++++	+++	—	—
B. paratyphosus A	++++	++++	+++	—	—
B. paratyphosus B	—	—	—	—	—

Readings after 2 hours at 37° C.

B. paratyphosus A isolated.

Inoculated with B. typhosus vaccine 1 year previously.

Agglutination test in 3rd week of illness.

Thus, at the very outset, the constant occurrence of these " double " reactions introduced a serious difficulty in the diagnosis of the infection, where the specific organism could not be isolated from the body. It was well known, of course, that inoculation with B. typhosus vaccine leads to the appearance of a specific agglutinin in the blood serum which for a short time after the injection may act in relatively high titres. In the majority of the cases dealt with in this investigation, a period of at least 9 months had elapsed since the typhoid inoculation; a series of 20 healthy individuals inoculated 9 to 12 months previously were examined as regards the reaction of their sera to B. typhosus (using the same technique as that employed in the diagnostic tests) but the highest end-titre noted was 1:200 after 2

hours at 37° C. Yet in the cases quoted in tables 2 and 3 the typhoid agglutinin acted in titres up to 1:1000 and these cases had been inoculated respectively 15 and 10 months previously. This degree of reaction with B. typhosus could not therefore be regarded as due entirely to the mere persistence of the post-inoculation agglutinin.

It is noteworthy that the reaction with the particular paratyphoid bacillus was highly specific; thus in the results shown in tables 1 to 4, while the reaction to the infecting organism occurred in titres as high as 1:2000 and 1:4000, even 1:50 dilutions of the serum failed to produce any degree of effect with the other paratyphoid bacillus.

Absorption Tests.

The question arose as to whether the concomitant reaction with B. typhosus was due to coagglutinins. Absorption tests were therefore carried out in a number of cases; these proved that the serum contained independent agglutinins to the respective organisms. Table 5 shows the results of one of these experiments.

Technique of absorption tests: 4 c.c. of a 1:25 dilution of the serum was made up and divided into two equal parts. Two 24 hours' agar slope cultures of B. typhosus were emulsified in one part, and two similar agar cultures of the particular paratyphoid bacillus in the other. The emulsions

were incubated for 3-4 hours; the treated serum was then separated by centrifugalization and pipetted off from the bacillary sediment.

The usual series of dilutions were then made from each portion of the serum and both were tested with B. typhosus and the paratyphoid bacillus. Table 5 shows the results of an absorption test with a serum which agglutinated both B. typhosus and B. paratyphosus B.

Table 5.

Primary agglutination test

	Dilutions of Serum.				
	1:50	1:100	1:200	1:500	1:1000
B. typhosus	++++	++++	++++	+++	—
B. paratyphosus A	—	—	—	—	—
B. paratyphosus B.	++++	++++	++++	++++	++
Serum absorbed with B. typhosus					
B. typhosus	—	—	—	—	—
B. paratyphosus A	—	—	—	—	—
B. paratyphosus B	++++	++++	++++	+++	+
Serum absorbed with B. paratyphosus B.					
B. typhosus	++++	++++	+++	++	—
B. paratyphosus A	—	—	—	—	—
B. paratyphosus B	—	—	—	—	—

Readings after 2 hours at 37° C.

Having excluded coagglutination effects two interpretations of these double reactions seemed possible: (1) the cases were mixed infections with B. typhosus and B. paratyphosus; (2) the B. typhosus agglutinin still present in the serum as a result of vaccination had been increased in amount during the subsequent paratyphoid infection. In the great majority of cases in which well developed agglutinins to the paratyphoid bacillus were detected, a corresponding effect with B. typhosus was also observed; in fact the frequency with which a double agglutination result of this kind was noted, indicated the improbability of the first interpretation; there was no reason to believe from the data obtained by isolation of enterica organisms that double infections were so relatively frequent. The second interpretation seemed a more likely explanation of the concomitant reaction.

Absence of the Concomitant Reaction with B. typhosus.

In occasional cases the agglutination test showed the presence of agglutinins to the paratyphoid bacillus only, without any concomitant reaction to B. typhosus. Tables 6 and 7 exemplify such results. In these cases the reaction with the paratyphoid bacillus did not occur in titres higher than 1:500; distinct concomitant reactions were noted, however, along with even less marked agglutination of B. paratyphosus (v. tables 3 and 4). On the other hand, as indicated later, the concomitant reaction only occurred in paratyphoid cases in which there was a definite reaction to the infecting organism. Both these cases had been inoculated with B. typhosus vaccine but the

intervals between the inoculation and the paratyphoid infection were relatively long - 13 and 14 months respectively; table 2 however shows a well marked concomitant reaction in a case inoculated 15 months before the enteric illness. It cannot therefore be concluded that the concomitant agglutination of B. typhosus is dependent on the interval between inoculation and the occurrence of the paratyphoid infection.

Table 6.

	Dilutions of Serum.				
	1:50	1:100	1:200	1:500	1:1000
B. typhosus	—	—	—	—	—
B. paratyphosus A	++++	++++	++++	++	—
B. paratyphosus B	—	—	—	—	—

Readings after 2 hours at 37° C. Inoculated with B. typhosus vaccine (2 doses) 13 months previously.

Agglutination test on 10th day of illness.

Table 7.

	Dilutions of Serum.				
	1:50	1:100	1:200	1:500	1:1000
B. typhosus	—	—	—	—	—
B. paratyphosus A	—	—	—	—	—
B. paratyphosus B	++++	+++	+++	+++	—

Readings after 2 hours at 37° C. Inoculated with B. typhosus vaccine (2 doses) 14 months previously.

Agglutination test on 10th day of illness.

Reactions with both B. paratyphosus A and B.

In two cases moderately high titre agglutinins to both B. paratyphosus A and B were observed. Absorption tests were carried out, the serum being saturated with B. typhosus, B. paratyphosus A and B, and the " absorbed " sera were tested each with these three organisms. The results (as exemplified in table 8) showed the presence of independent agglutinins to all three enterica organisms.

Table 8.

	Primary agglutination test			
	Dilutions of Serum.			
	1:50	1:100	1:200	1:500
B. typhosus	++++	++++	+	—
B. paratyphosus A	++++	++++	+++	++
B. paratyphosus B	++++	+++	++	+
	Serum after absorption with B. typhosus			
B. typhosus	—	—	—	—
B. paratyphosus A	++++	++++	+++	+
B. paratyphosus B	++++	+++	+	—
	Serum after absorption with B. paratyphosus A			
B. typhosus	++++	++++	—	—
B. paratyphosus A	—	—	—	—
B. paratyphosus B	++++	+++	+	—
	Serum after absorption with B. paratyphosus B.			
B. typhosus	++++	+++	—	—
B. paratyphosus A	++++	++++	+++	+
B. paratyphosus B	—	—	—	—

Readings after 2 hours at 37° C.

Inoculated with B. typhosus vaccine (2 doses) 10 months previously.

Agglutination test about 14th day of illness.

It was concluded from the results of this experiment that the case represented a mixed infection with B. paratyphosus A and B; no specific organisms were however isolated.

Degree of Agglutination Reaction -

Absence of Specific Reaction.

In a considerable number of the cases studied, the end-titre of the reaction with the specific organism was not lower than 1:1000. The highest agglutination titre observed in B. paratyphosus A infections was 1:4000, in B. paratyphosus B infections 1:8000. The reactions with B. paratyphosus B were generally more marked than in the case of B. paratyphosus A.

It had been suggested by some bacteriologists in the Mediterranean Expeditionary Force that the agglutination reaction was developed at too late a stage of the disease to be of diagnostic value. It is to be noted, however, that high-titre agglutinins to B. paratyphosus A and B. paratyphosus B have been observed as early as the 7th day (e.g., v. table 1.)

It has been frequently noted that especially in paratyphoid A infections the agglutinin development is slight (v. table 9) even in the later stages of the illness and not infrequently in proved B. paratyphosus A cases, specific agglutinins cannot be demonstrated in the serum. In many instances among this series of cases the B. paratyphosus A agglutinins were poorly developed (with end-titres of 1:50

or 1:100) and in certain cases from which this organism was isolated specific agglutinins could not be demonstrated even in convalescence. In such cases the only result of the agglutination test with the three enterica organisms was a reaction with B. typhosus in relatively low titres, e.g. 1:100 or 1:200 (v. table 10). This was interpreted as due to the persistence of the post-inoculation agglutinin and designated the " vaccine-effect." Table 10 shows the " vaccine-effect " and absence of B. paratyphosus A agglutinins in a case from which this organism was isolated.

Table 9.

	Dilution of Serum.			
	1:50	1:100	1:200	1:500
B. typhosus	++	—	—	—
B. paratyphosus A	+++	++	+	—
B. paratyphosus B	—	—	—	—

Readings after 2 hours at 37° C.

B. paratyphosus A isolated.

Agglutination test on 14th day of illness.

Date of typhoid vaccination unknown.

Table 10.

	Dilution of Serum.			
	1:50	1:100	1:200	1:500
B. typhosus	+++	+++	++	—
B. paratyphosus A	—	—	—	—
B. paratyphosus B	—	—	—	—

Readings after 2 hours at 37° C.

B. paratyphosus A isolated.

Agglutination test 4 weeks after onset of illness.

Inoculated with B. typhosus vaccine (2 doses) 9 months previously.

In many cases diagnosed clinically as " enterica " agglutination tests yielded results similar to that shown in table 10, even when repeated at later stages of the disease. Where no specific organism was isolated, such cases presented a difficult problem in laboratory diagnosis, and at that time, they were accepted by some bacteriologists and clinicians as B. typhosus infections. Agglutination of B. typhosus in titres up to 1:200 within 12 months after inoculation was however compatible with a vaccination effect (v. supra) and in vaccinated cases under these conditions could be of no diagnostic significance. In view of the prevalence of B. paratyphosus A infections and the observation of similar agglutination results in cases from which this organism was

isolated (e.g. case shown in table 10) it seemed probable that many cases of this type were paratyphoid A infections in which agglutinins were not developed. Of course some of these cases may have been B. typhosus or B. paratyphosus B infections in which there was a similar non-development of agglutinins. The possible absence of a specific agglutination reaction during the whole course of an enteric illness is certainly a factor which limits the diagnostic value of the serological test but, provided this possibility is fully appreciated, there is not likely to be any misinterpretation of results and " negative " effects may simply be disregarded as being of no diagnostic significance.

The question also arose as to whether any " exaltation " of the B. typhosus post-inoculation agglutinin was likely to be produced in paratyphoid cases in which the homologous agglutinin was absent.

In the case shown in table 10, the agglutination reaction with B. typhosus was not greater than that which might have occurred in a healthy individual as a vaccination effect. In the case shown in table 1 where there was a marked reaction to B. paratyphosus A on the 7th day, the B. typhosus agglutinin reacted in titres up to 1:1000; in the case quoted in table 3, the concomitant reaction with B. typhosus also occurred in high titres. Apparently this exaltation of the post-inoculation B. typhosus agglutinin only resulted in paratyphoid

cases in which there was a definite reaction to the infecting organism. Occasional cases were observed in which a high-titre (1:1000, 1:2000) reaction was obtained with B. typhosus without any reaction to a paratyphoid bacillus; the possibility of this result being due to an increase in the amount of the post-inoculation agglutinin in a paratyphoid case without a specific reaction, was considered. In the case shown in table 11, B. typhosus was isolated and such results were generally interpreted as signifying a B. typhosus infection.

Table 11.

	Dilution of Serum.					
	1:50	1:100	1:200	1:500	1:1000	1:2000
B. typhosus	++++	++++	++++	+++	++	+
B. paratyphosus A	—	—	—	—	—	—
B. paratyphosus B	—	—	—	—	—	—

Readings after 2 hours at 37° C.

B. typhosus isolated.

Agglutination test 21 days from onset of illness.

Inoculated with B. typhosus vaccine 6 months previously.

In general, the lowest serum dilution tested was 1:50; in paratyphoid A infections, agglutination in still lower dilutions has been accepted by some observers as of diagnostic

significance. Of course a particular standard is applicable only to the method employed and the effect of a certain concentration of serum tested by one method may vary considerably from that elicited by a different technique. According to the method employed in these investigations there was apparently no advantage to be derived by testing dilutions lower than 1:50 for B. paratyphosus A agglutinins. Thus in the cases from which B. paratyphosus A was isolated and in which no agglutination of this organism occurred in a 1:50 concentration of the serum, 1:20 dilutions were also tested with negative result.

The Importance of Second Readings after 24 Hours.

While it was usually possible to elicit decisive results after 2 hours at 37° C., in all cases readings were also made after 20-24 hours (v. p. 3), and in certain instances more definite results were obtained in this way. (v. tables 12 and 13).

Table 12.

		Dilution of Serum.				
		1:50	1:100	1:200	1:500	1:1000
After 2 hours at 37° C.	B. typhosus	++	—	—	—	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++	—	—	—	—
After 24 hours.	B. typhosus	++++	++	—	—	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++++	+++	++	—	—

Inoculated with B. typhosus vaccine (2 doses) 5 months previously.

Agglutination test on 16th day of illness.

Table 13.

		Serum Dilution.				
		1:50	1:100	1:200	1:500	1:1000
After 2 hours at 37° C.	B. typhosus	+	—	—	—	—
	B. paratyphosus A	+++	+	—	—	—
	B. paratyphosus B	—	—	—	—	—
After 24 hours.	B. typhosus	+++	++	—	—	—
	B. paratyphosus A	+++	+++	+++	++	—
	B. paratyphosus B	++	—	—	—	—

Inoculated with B. typhosus vaccine (2 doses) 11 months previously.

Agglutination test on 7th day of illness.

B. paratyphosus A isolated.

The 24 hours' readings were generally higher than those made after 2 hours; coagglutination effects were often noted after 24 hours but only in low titres (1:50 or 1:100) even when a marked reaction occurred with the infecting organism (v. table 14 - B. paratyphosus A infection showing weak coagglutination of B. paratyphosus B after 24 hours).

Table 14.

		Dilution of Serum.					
		1:50	1:100	1:200	1:500	1:1000	1:2000
After 2 hours.	B. typhosus	++++	+++	+	—	—	—
	B. paratyphosus A	++++	++++	++++	+++	++	—
	B. paratyphosus B	—	—	—	—	—	—
After 24 hours.	B. typhosus	++++	++++	+++	+	—	—
	B. paratyphosus A	++++	++++	++++	++++	+++	—
	B. paratyphosus B	+++	+	—	—	—	—

Inoculated with B. typhosus vaccine (2 doses) 7 weeks previously.

Agglutination test on 12th day of illness.

B. paratyphosus A isolated.

The progress of the reaction over a period from 2 to 24 hours is shown in table 15; the effect apparently reached its maximum in 9 hours, but it was found more convenient to make the second reading on the following day after the tubes had been allowed to stand overnight at room temperature.

Table 15.

		Dilution of Serum.				
		1:1000	1:2000	1:4000	1:8000	1:16000
After 2 hours at 37° C.	B. typhosus	++++	++	+	—	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++++	++	+	—	—
After 6 hours.	B. typhosus	++++	+++	+	—	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++++	++++	++	—	—
After 9 hours.	B. typhosus	++++	+++	+++	+	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++++	++++	+++	++	—
After 24 hours.	B. typhosus	++++	+++	+++	+	—
	B. paratyphosus A	—	—	—	—	—
	B. paratyphosus B	++++	++++	++++	++	—

Inoculated with B. typhosus vaccine 12 months previously.

Agglutination test in 3rd week of illness.

Repetition of the Test during the Course of the Illness.

When an indefinite or negative reaction was elicited on first testing, the examination was if possible repeated later - often with striking results. Table 16 illustrates how a repeated test in a case of B. paratyphosus A infection with at first poorly developed agglutinins, conclusively proved the interpretation of the first test.

Table 16.

		Dilution of Serum.					
		1:50	1:100	1:200	1:500	1:1000	1:2000
About 5th day.	B. typhosus	+	—	—	—	—	—
	B. paratyphosus A	+++	+	—	—	—	—
	B. paratyphosus B	—	—	—	—	—	—
About 13th day.	B. typhosus	+++	++	+	—	—	—
	B. paratyphosus A	++++	++++	++++	++++	+++	++
	B. paratyphosus B	++	—	—	—	—	—

Inoculated with B. typhosus vaccine 11 months previously.

Anomalous Reactions.

Anomalous reactions were occasionally reported by different bacteriologists in the Mediterranean Force; one of these apparently contradictory results was also noted in the course of this investigation - B. paratyphosus A was isolated, yet on carrying out the serological test, high-titre agglutination of B. paratyphosus B was observed though no reaction occurred with B. paratyphosus A (table 17). The agglutination test was repeated with similar result; the identity of the strain isolated was carefully determined by the usual biochemical and serological tests; the identity of the paratyphoid strains used in the agglutination test was also confirmed.

The possibility of this result being due to an unusual paragglutination phenomenon (v. part 1 p.29-30) could not altogether be excluded but it was assumed that the reaction with B. paratyphosus B was the result of infection with this organism. B. paratyphosus A was isolated from the case but even after 3 weeks from the onset of the illness no homologous agglutinins appeared in the serum. As already shown this is a not uncommon feature of paratyphoid A cases. Presumptive evidence of the occurrence of mixed paratyphoid infections has been noted (v. p. 11 table 8) and it seemed a possible explanation of this anomalous result that the case represented a double infection with B. paratyphosus A and B in which only one of the causal organisms was detected.

Table 17.

	Dilution of Serum.						
	1:50	1:100	1:200	1:500	1:1000	1:2000	1:4000
B. typhosus	++++	++++	++++	+++	+++	+	—
B. paratyphosus A	—	—	—	—	—	—	—
B. paratyphosus B	++++	++++	++++	+++	+++	+++	++

B. paratyphosus A isolated from urine (present in large numbers).

Inoculated with B. typhosus vaccine (2 doses) 5 months previously.

Agglutination test on 18th day of illness.

Correlation of Bacteriological and Serological Results.

It was of course impossible to confirm all the serological results by isolation of the infecting organism; in certain cases, however, the inferences made from various types of reaction were confirmed in this way (v. tables 1, 3, 4, 9, 10, 11, 13, 14).

In the total series of cases studied by Mackie and Wiltshire, of 32 proved B. paratyphosus A infections, 23 showed a specific agglutination reaction with the infecting organism, 8 showed only a " vaccine effect " or a negative result (as shown in tables 10 and 11) and 1 case yielded the anomalous result referred to above; of 8 cases from which B. paratyphosus B was isolated all showed well marked homologous agglutinins in the serum.

The duration of the interval between the typhoid inoculation and the illness had apparently no influence on the results. The longest interval among the cases investigated was 14 months; in some instances only about 3 months had elapsed since inoculation and in one case the interval was only 7 weeks. The concomitant reaction with B. typhosus was not however more marked in the recently inoculated cases (v. tables 14 and 2) although the cases in which no concomitant reaction was observed had been inoculated over a year previously.

Diagnostic Standards.

From these observations it was concluded that in the paratyphoid infections marked agglutination (+++), elicited by the technique described, in a 1:50 dilution of the serum after 2 hours at 37° C. is of diagnostic significance. In the majority of cases, however, more conclusive evidence was elicited by titrating to the end-point of the reaction.

The concomitant reaction with B. typhosus in typhoid vaccinated cases apparently represented a re-development of the post-inoculation agglutinin and was therefore of no diagnostic importance. The phenomenon, however, is of the greatest immunological interest in showing the tendency on the part of the tissues which have once produced specific antibodies to again develop similar antibodies in response to a suitable stimulus not necessarily of the same specific nature as that which called forth the original immunity reaction. Thus even after immune substances have almost disappeared from the blood of a previously immune individual, there may still be a persistence of immunity in the sense that the body cells which have previously responded may more readily react subsequently to an immunizing influence.