ON SLOW LACTOSE FERMENTING *B. COLI* IN URINARY AND INTESTINAL INFECTIONS.

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CONTENTS.

Introduction.

In a paper published in this *Journal* in 1924, Dudgeon described a haemolytic slow lactose fermenting bacillus which had been isolated from the urine of patients suffering from acute infections of the urinary tract. Attention was drawn to the fact that these bacilli produced the most severe attacks of urinary fever met with, and that the urinary manifestations might be masked at the outset owing to the severe general reaction which occurred, so that a diagnosis of "enterica" had been made on clinical evidence. Further experience has confirmed these views and in several instances failure to realise them has led to errors in the diagnosis of these cases. The pyrexial period is longer and more severe, as a rule, than in true *coli* fever. In women recently confined, bacilli with the cultural and serological characters about to be referred to have been isolated from the urine and from the lochia. The absence of urinary symptoms has led to the clinical diagnosis of puerperal septicaemia. In spite of the severity of the symptoms at the outset, the

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tendency is for complete recovery to ensue and for the urine to be declared free from bacilli. This we know from experience is unusual in cases of true *coli* fever. Fifty-one cases were recorded, in the paper previously referred to, due to bacilli which were actively haemolytic, serologically similar, formed blue colonies on the surface of litmus-lactose-agar plates, fermented lactose broth slowly, and gave an alkaline reaction in saccharose. In spite of the severity of the acute febrile illness, the bacillus was not isolated from the blood stream or faeces.

In an interval of two years since this paper was published, slow lactose fermenters have been isolated from 300 cases of acute and chronic infections of the urinary and intestinal tracts, and the investigation of these bacilli forms the subject of this communication.

1. Clinical Records.

(*a*) *Urinary infections.* We again lay emphasis on the fact that at the onset of the acute illness produced by these bacilli the general symptoms may be so severe that the urinary condition is entirely overlooked and consequently the clinical diagnosis of enterica has been made on several occasions. The presence of a bacilluria due to slow lactose fermenting bacilli in the urine of a patient with high temperature and severe general reaction, even in the absence of urinary symptoms, may be the only indication of the nature of the illness at the outset. It may be two or three days, or even longer, before the true urinary symptoms appear, and pus is found in the urine.

In patients recently confined the clinical diagnosis of puerperal septicaemia has been made, because of a sudden rise of temperature and severe general symptoms, without urinary manifestations. This mistake can be avoided by a bacteriological examination of the urine. In the original paper on this subject by Dudgeon it was pointed out that the urinary infection gradually disappeared with the establishment of convalescence, and greater experience has confirmed this view. We now know that complete recovery usually occurs, but a few instances of persistence of the urinary infection have been observed. When contrasted, however, with true *B. coli* infections the results are infinitely more satisfactory. In the previous paper one fatal case was referred to. Since then two other cases have occurred. One of these patients was an infant who had multiple septic foci in the cortex of both kidneys due to one of these strains of bacilli, while the twin sister of the child who died contracted, a few days after the death of her sister, a very severe illness with acute urinary manifestations due to the same bacillus, but she eventually recovered. In the early stages of the illness in both these children severe screaming attacks occurred which led to the diagnosis of acute meningitis. Lumbar puncture was performed but the cerebro-spinal fluid was found to be normal. Subsequently other cases have occurred with profound cerebral symptoms.

Pyrexia at the onset of the illness and for the first two or three days

ranges from 101–104° F., but may reach 105 or 106° F., and may be associated with one or more rigors which may be severe.

Certain complications have occurred similar to those met with in other urinary infections. One patient developed acute epididymitis seven days from the onset of the acute illness, and another an abscess of the bladder wall due to the same bacillus as produced the acute cystitis.

(*b*) *Intestinal infections.* Five cases are included here among patients and nurses at an obstetric hospital who were suffering from acute diarrhoea with mucus present in the faeces. One patient had a temperature of about 102° F. for 48 hours. All these cases occurred during a period of three weeks and all made a complete recovery.

From two patients in St Thomas's Hospital slow lactose fermenting bacilli were isolated; in one case from the interior of the appendix in association with thread worms, and from the faeces of the other patient who was suffering from ulcerative colitis. From two cases at the obstetric hospital the bacillus isolated belonged to Group *A* −, from two other cases the bacillus belonged to Group *C* +, and from the fifth case to Group *D*. In four of the cases the bacillus was obtained in pure culture from the faeces, but in the fifth case only a few colonies were present.

If these organisms were the cause of the diarrhoea, which is possible, as the bacilli were present in pure culture in the faeces in four out of the five cases, then the source of the infection must have been multiple as different strains were isolated. On the other hand, the diarrhoea may have been due to an unrecognised cause or causes which allowed a very active multiplication of the slow lactose fermenters in the intestinal canal. None of these patients developed a urinary infection, but the bacilli isolated from the faeces in these seven cases belonged to the same groups as the slow lactose fermenting bacilli obtained from the urinary tract.

2. BACTERIOLOGY.

(*a*) *Abstract of original investigation.* The cultural reactions of the bacilli isolated from the urine in the first group of 51 cases are given in the paper by Dudgeon (1924). The colonies on litmus-lactose-agar were *large* and blue, and acquired a greenish-blue colour after a few days' incubation at 37° C. Marked haemolysis occurred in 0·9 and 0·5 per cent. sodium chloride in 5 c.c. of peptone water with 2·0 per cent. of human red cells added. The bacilli were gram negative, motile, and fermented mannitol, dextose, and maltose. Lactose broth was acidified without gas formation in the first 24 hours by a few of the strains, but in the majority of instances acidification with gas formation occurred during the first five days and subsequently alkalinity may occur. A very small percentage of strains did not ferment lactose. Saccharose was rendered alkaline in 48 hours by every strain and the alkalinity gradually increased during a period of ten days. Milk was acidified and clotted by most strains in 72 hours, while a solid clot was subsequently formed, but with

three strains after the preliminary acidification the milk became alkaline and no clotting occurred. Gelatine was not liquefied.

Three anti-sera were prepared in the original investigation, but every strain was agglutinated by these anti-sera and each anti-serum was partially or completely desaturated by any of the 51 cultures. The cultural, haemolytic, and serological reactions obtained led to the view that these bacilli belonged to one group of organisms.

(*b*) *Present investigation.* During the last two years every strain isolated from the urinary tract, and a few strains from the intestinal tract, have been subjected to a complete bacteriological investigation, with the result that seven groups of slow lactose fermenting bacilli will be described, based on cultural, haemolytic, and serological tests.

Before detailing the exact method of classification, it may be as well to define the methods of grouping which have been adopted, and are recorded in Table I.

Table I. *The grouping of the slow lactose fermenting bacilli.*

Groups	Percentage total of each group %	Haemolysis	Carbohydrate reactions			
			Lactose			
			Agar	Broth	Saccharose	Dulcitol
A. + (original)	82	+	Blue	Variable either Alk., or delayed A., or delayed A. and G.	Alk.	A. and G.
−	4		,,	,,	,,	,,
B. +	2	+	,,	,,	A. and G.	Alk.
−	1	−	,,	,,	,,	,,
C. +	4	+	,,	,,	A. and G.	A. and G.
−	4	−	,,	,,	,,	,,
D. −	3	−	,,	,,	Alk.	Alk.

A. = Acidity. A. and G. = Acid and Gas formation. Alk. = Alkalinity.

Dudgeon, Wordley and Bawtree (1921) stated that the cultural reactions of *B. coli* strains isolated from the urinary tract did not assist in the grouping of *B. coli* when employed in conjunction with the haemolytic tests, and that the great majority of these strains of *B. coli* did not ferment cane sugar. In their second communication (1922), they arrived at the following conclusion: "As a result of a very large number of observations, we have been unable to correlate the cultural reactions and the serological findings." Their chief classification of colon bacilli was into haemolytic and non-haemolytic strains.

Certain carbohydrate media, lactose, saccharose, and dulcitol, have been employed for the differentiation of the slow lactose fermenting bacilli, and by this means a subdivision into four main groups, *A*, *B*, *C* and *D*, has been adopted. Three groups *A*, *B* and *C* have been further subdivided into haemolytic and non-haemolytic, while group *D* includes only non-haemolytic strains.

The original group described in this *Journal* in 1924 which is now referred to as *A* + comprises the vast majority of these bacilli, 82 per cent., the six

remaining groups only supplying 18 per cent. The severe acute infections, as in the case of *B. coli*, mostly belong to the haemolytic groups. The percentage total of haemolytic bacilli included in our seven groups is 88 per cent. which shows that the great majority of these slow lactose fermenting bacilli, isolated more especially from the urinary tract, are haemolytic.

In November 1920, Dr Castellani sent one of us (L. S. D.) a culture of a bacillus described by him in 1905 as *B. columbensis* which he had isolated from cases of enteric-like fever in Ceylon. In his text-book on Tropical Medicine it is stated that this bacillus had been the cause of certain urinary complications of the febrile illness. Spaar reported a case in 1915 of fever due to *B. columbensis* in a woman from whom this organism was isolated from the faeces. The T.A.B. agglutination reaction with the patient's blood was negative although a reaction of 1 in 80 was obtained with *B. columbensis*.

Lurie, in 1915, recorded a case of febris columbensis in the Balkans from whom four colonies of this bacillus were obtained from the faeces. There was no bacteriological evidence of typhoid or paratyphoid fever. *B. columbensis* Castellani has been fully investigated by us. It corresponds in its cultural reactions to our group *A*, and is strongly haemolytic to group *A* +. The cultural and other reactions of *B. columbensis* Castellani, as studied by us are as follows: it forms large permanent blue colonies on litmus-lactose-agar. The colonies are larger than those of *B. typhosus* and have an unpleasant odour. Lactose and saccharose both are rendered permanently alkaline and the alkaline reaction is very marked, while mannitol, dextrose, maltose and dulcitol are fermented. Well-marked indol formation occurs. Milk is temporarily acidified and then becomes strongly alkaline without clotting. Jelly is not liquefied. Complete haemolysis occurs in 24 hours at 37° C. in both tubes of salted peptone water according to the technique described by us. This bacillus is not agglutinated by para-β or *C* anti-sera and the agglutination titre of these sera is not reduced when saturated with *B. columbensis*. This organism is agglutinated $\frac{200}{8000}$ by anti-serum *B. 5659* of group *A* + to which it belongs on cultural and haemolytic investigation, but it is agglutinated by anti-serum Dow which was prepared from a true haemolytic colon bacillus to full titre $\frac{20{,}000}{20{,}000}$ and it partially desaturates this anti-serum.

An anti-serum prepared from *B. columbensis* agglutinates this organism and *B. dow* equally well and *B. 5659* very slightly, while both *B. dow* and *B. columbensis* desaturate the *columbensis* anti-serum.

B. columbensis therefore belongs on cultural evidence to Group *A* + and serologically to the haemolytic true colon bacillus. This close serological relationship is shown by many other strains of these organisms.

There are only seven intestinal cases included in this series, and of these in three cases the bacilli were haemolytic. Our investigations are chiefly

based on the slow lactose fermenting bacilli isolated from acute urinary infections, and to a much less degree from chronic infections. In our opinion the relative infrequency of chronic cases supports the view to which attention has already been drawn that the acute cases usually make a complete recovery. Dudgeon (1924) states that the bacillus (group *A* +) had not been isolated from the blood, but subsequently Hope Simpson, while working in his laboratories, isolated such a bacillus from the blood of a patient suffering from an acute attack of urinary fever and the same bacillus from the urine and faeces. This is the only instance so far of this organism having been isolated from the blood stream.

In Table II is recorded a list of the seven intestinal cases from which these bacilli have been recovered from the faeces and their chief points of importance.

Table II. *Intestinal cases from which a bacillus belonging to one of the seven groups has been recovered.*

Case	Source	Symptoms	Urine	Day of exam. of faeces	Bacillus isolated
1	Faeces	Acute diarrhoea mucus +temp. 102° F.	Sterile	5th	Pure growth of bacillus Group *A* – (N.H.)
2	,,	Acute diarrhoea apyrexial	,,	1st	Pure growth of bacillus Group *C* + (H.)
3	,,	,,	,,	1st	Pure growth of bacillus Group *A* – (N.H.)
4	,,	,,	,,	1st	Few colonies of bacillus Group *D* – (N.H.)
5	,,	,,	,,	1st	Pure growth of bacillus Group *C* + (H.)
6	Interior of appendix	Acute appendicitis	,,	?	Abundant growth of bacillus Group *C* + (H.)
7	Faeces and wall of colon	Ulcerative colitis	,,	?	Pure growth of bacillus Group *B* – (N.H.)

Blood reactions. Large numbers of tests have been made with the blood of patients suffering from acute or chronic infections due to these bacilli. It is very uncommon for a reaction of over 1 in 500 ever to occur, although a reaction of 1 in 2000 has been met with, while it can be stated definitely that a reaction under 1 in 150 is of *no* diagnostic value.

(*c*) *The cultural reactions.* Although the paratyphoid group can be readily distinguished from any of the seven strains of these slow lactose fermenting bacilli, yet mistakes have occurred. The bacilli which are included in Group *A* – ferment dulcitol, but do not acidify saccharose, and are non-haemolytic. Lactose may not be acidified for two or three days, or even longer. The colonies are larger than those of the paratyphoid group as a whole and are blue to blue-green in colour on litmus-lactose-agar. They have an unpleasant odour. These colonies form indol within 24 hours, or the reaction may be delayed for two to three days. It is this reaction which serves to distinguish these bacilli from the paratyphoid group apart from serological tests. It is very probable that many strains isolated from the faeces of soldiers during

the Great War and regarded as atypical strains of *B. paratyphosus* β were really cultures of these bacilli, as such strains formed indol and were inagglutinable with para β antiserum. The importance of this question, however, is well illustrated in the case of a patient suffering from acute cystitis whose urine contained thick pus and bacilli. A pure culture of a bacillus was obtained from the urine which gave blue colonies on litmus-lactose-agar, did not ferment lactose or saccharose within two days, but fermented dulcitol, and was non-haemolytic. This organism was not agglutinated by any of the anti-sera prepared from the bacilli grouped by us, but reacted up to end point with *B. gärtner* anti-serum. The possibility of such a mistake is of considerable practical importance.

The patient whose case has been briefly referred to was suffering from acute cystitis, with high temperature, and had been abroad. Our investigations with these slow lactose fermenting bacilli have led us to regard the three carbohydrate media lactose, saccharose, and dulcitol employed in conjunction with the haemolytic tests as essential in the primary grouping of these bacilli, and our serological investigations have confirmed this view. The carbohydrate reactions, however, must be taken in conjunction with the haemolytic tests. It is a matter of great interest that 82 per cent. of the slow lactose fermenting bacilli are haemolytic and react in the same manner in saccharose and dulcitol, although the lactose reactions are variable. Cross serological reactions occur between this group and the commonest haemolytic *B. coli* strains which ferment lactose and dulcitol, but not saccharose, and as Dudgeon, Wordley and Bawtree have shown, it is the haemolytic *B. coli* with these cultural reactions that occur most frequently in acute urinary infections.

Litmus-lactose-agar. Every strain has given blue colonies on litmus-lactose-agar which are large and opaque, and a greenish coloration after several days at 37° C. which may extend into the surrounding medium. They were red on phenol red lactose-agar. Bacilli which form blue colonies on litmus-lactose-agar and turn red 24 hours later are not discussed in the present communication.

Preparation of liquid carbohydrate media. Our liquid carbohydrate media contain 1 per cent. of lemco in distilled water, 1 per cent. of May and Baker's peptone, 0·25 per cent. pure sodium chloride, 1 per cent. of carbohydrate, and 1 per cent. of a 0·06 per cent. solution of phenol red in distilled water. The final reaction of the medium is 7·5 by the Universal Indicator.

Lactose lemco broth. In the majority of instances this medium is unaffected, or rendered slightly alkaline, during the first 24 hours at 37° C. Subsequently, it is acidified and gradually gas formation occurs, although some strains may take several days to produce true fermentation. At the end of five to ten days at 37° C. the medium may be rendered distinctly alkaline. Four strains which belonged to groups $A+$, $C+$ and $D-$ did not ferment lactose in ten days, although one of these strains acidified the medium. Some strains produced a very definite alkalinity during the first few days incubation, although

subsequently the medium was fermented. In spite of the fact that 96 per cent. of the strains ultimately fermented lactose with gas production, every strain formed blue surface colonies on litmus-lactose-agar which remained constant for ten days, and such strains as have been tested have given red colonies on phenol red lactose-agar.

The action of young agar cultures on lactose lemco broth. It was thought that although this lactose medium was not fermented when incubated from broth or agar cultures which had been growing for 24 hours or longer at 37° C., yet fermentation of the lactose medium might occur if it was inoculated from young agar cultures. For these experiments, four haemolytic strains from group $A+$ were employed, and each experiment was repeated. Agar cultures were inoculated with strains known as *5659*, *6489*, Williams and Freeman, and tubes of lactose broth were inoculated from their agar cultures which had been incubated for 3, 5, 7, 9 and 24 hours at 37° C. The inoculated media were immediately incubated at 37° C. and the results were read each day for four days. The results of these observations will be readily understood from reference to Table III.

Table III. *To show the action of young agar cultures of the slow lactose fermenting bacilli on lactose broth.*

Age of agar cultures in hours	Three				Five				Seven				Nine				Twenty-four			
No. or name of organism from Group $A+$	6489	5659	Freeman	Williams	6489	5659	Freeman	Williams	6489	5659	Freeman	Williams	6489	5659	Freeman	Williams	6489	5659	Freeman	Williams
(1) Records taken 10.30 a.m. following day	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(2) 10.30 next day	—	—	Alk.	$+_1$	—	—	Alk.	$+_1$	—	—	Alk.	$+_1$	—	—	Alk.	$+_1$	Alk.	Alk.	Alk.	+
(3) ,, ,,	$+_1$	$+_1$	Alk.	$+_2$	$+_1$	$+_1$	Alk.	$+_2$	$+_1$	$+_1$	Alk.	$+_2$	$+_1$	$+_1$	Alk.	$+_2$	Alk.	N	Alk.	$+_3$
(4) ,, ,,	$+_2$	$+_2$	Alk.	$+_2$	$+_2$	$+_2$	Alk.	$+_2$	$+_2$	$+_2$	Alk.	$+_2$	$+_2$	$+_2$	Alk.	$+_2$	$+_2$	$+_2$	Alk.	$+_3$

+ = acidity and trace of gas formation. $+_1$, $+_2$, $+_3$ = acidity and degrees of gas formation.

It would thus appear that there is no apparent difference between the results obtained with lactose broth inoculated from agar cultures of 3, 5, 7, 9 and 24 hours' duration, even after making full allowance for the age of the growth in the lactose medium.

When bacilli from group $A+$ were subcultured twice daily in lactose for ten days the fermentation of the medium was not accelerated.

Saccharose lemco broth. Eleven per cent. of the strains which are included in groups $B+$ and $B-$, $C+$ and $C-$, fermented saccharose with gas formation, while 89 per cent. produced no acidity.

Dulcitol lemco broth. Ninety-four per cent. of the strains which are included in groups $A+$ and $A-$, $C+$ and $C-$ fermented dulcitol with gas formation. This medium in some cases was rendered alkaline at the outset, in others alkaline after eight to ten days at 37° C.

The other "sugars" which included mannitol, dextrose and maltose, were fermented with gas production in every instance.

Indol was produced by every strain within three days, usually within 24 hours.

Gelatine (15 *per cent.*) was not liquefied. Gelatine cultures of the various strains were also incubated at 37° C. for seven to ten days, but the gelatine was no less viscid.

Milk was acidified in every case, although in a few instances the acidification was very transitory and was rapidly changed to alkalinity. In such instances clotting did not occur. In 94 per cent. of the strains milk was acidified and clotted.

(*d*) *Regular and irregular colonies.* In 1905, Dudgeon and Sargent described and figured two types of colonies on the surface of gelatine plates grown direct from the peritoneal fluid in peritonitis. One had an irregular edge and surface, the other was perfectly regular. These colonies correspond in appearance to the rough and smooth colonies to which so much attention has been given since Arkwright's work was published in 1921. Savage, in 1904, described and figured 28 surface colonies of *B. coli* on gelatine showing widely different appearances. The regular and irregular colonies also occur with the slow lactose fermenting *B. coli* groups if the inflammatory exudate is plated direct on agar, litmus-lactose-agar, or gelatine. Three haemolytic strains of the slow lactose fermenting bacilli from groups $B+$ and $C+$ were grown for one week at 37° C. in beef broth. From the $B+$ broth culture regular and irregular colonies were present in about equal numbers. Two regular and two irregular colonies were planted in veal broth from the $B+$ and $C+$ agar plates grown at 37° C., and examined after 24 hours. It was then found that the regular colonies gave a uniform growth, and the irregular showed large clumps in the medium. Both types of colonies were found to be equally haemolytic and when the irregular colonies were filtered through linen the clear emulsion which was left gave good agglutination with the anti-sera.

(*e*) *Haemolysis.* Evidence of haemolysis was obtained from surface colonies on blood agar plates, and by growing the organism in two tubes of 5 c.c. of peptone water one of which contained 0·5 per cent. pure sodium chloride, and the other 0·85 per cent. To each of these tubes solid human red cells were added to the extent of 2·0 per cent. The liquid media were inoculated from a 24 hours' growth on agar at 37° C., which were then incubated at 37° C. for 24 hours, when the readings were taken. Owing to the rapidity with which the red cells fall in the peptone salt medium, 0·1 per cent. agar was added for all the more recent experiments. This allows the red cells to remain suspended in the fluid and thus the degree of haemolysis is more readily appreciated. The slow lactose fermenting bacilli and the true *B. coli* give better evidence of haemolysis in liquid than on solid media. Every strain was tested by the above methods as soon as isolated in pure culture, and subsequently at various intervals, and they have all been found to retain the haemolytic properties after long periods of isolation from the body.

(*f*) *Haemolysis and the liquefaction of gelatine.* As none of these slow

lactose fermenting bacilli liquefied gelatine, a simple test was undertaken to see the effect on a gelatine medium containing red cells. This medium contained 15 per cent. of gelatine, and 0·5 per cent. pure sodium chloride to which was added human red cells to give a 2 per cent. suspension in the medium. Various haemolytic strains of these organisms were used for the inoculation of the red cell gelatine medium, also *S. aureus* and haemolytic strains of *B. coli* and *B. proteus*. All the tubes were grown at 22° C., and duplicates at 37° C., for seven days. Haemolysis occurred with the slow lactose fermenters and with *B. coli*, but no liquefaction of gelatine; the *B. proteus* cultures produced haemolysis and liquefaction of gelatine, and the *S. aureus* liquefaction of gelatine without haemolysis.

(*g*) *Filtered toxin and haemolysis.* Bacilli from groups *A* +, *B* + and *C* + which are the haemolytic strains of the slow lactose fermenting bacilli, were grown for 24 hours and for one week at 37° C. in a liquid medium prepared from ox heart muscle together with casein digest. Haemolytic strains of *B. coli*, *S. aureus*, streptococcus, and *B. welchii* isolated from the human intestinal tract, were grown in this medium for the control experiments. Each culture was filtered through a German Berkefeld W. candle. The filtrates were then tested for haemolysis with the following results as shown in Table IV.

Table IV. *Filtrates and haemolysis.*

Filtrate						
1. B. welchii	C.	C.	C.	I.C.	M.	Tr.
2. B. coli (Dow)	–	–	–	–	–	–
3. B. 5659 Group *A* +	–	–	–	–	–	–
4. B. 6489 Group *A* +	–	–	–	–	–	–
5. B. gale Group *C* +	–	–	–	–	–	–
6. B. ewen Group *B* +	–	–	–	–	–	–
7. S. aureus	–	–	–	–	–	–
8. Streptococcus (H.)	–	–	–	–	–	–
No. of tubes	1	2	3	4	5	6
Amount of filtrate	1 c.c.	0·9	0·75	0·5	0·24	0·1
,, saline	—	0·1	0·25	0·5	0·75	0·9
,, red cells		1 per cent. in each tube				

Abbreviations used: C. = Complete haemolysis.
I.C. = Incomplete. Some red cells left.
M. = Marked. Many red cells left.
Tr. = Trace. Only few red cells destroyed giving only tingeing.
– = No haemolysis.

There was no evidence of a haemolysin in the filtrates prepared in the manner indicated, except in the *B. welchii* filtrates. Haemolytic strains were grown for one month and for one week at 37° C. in beef broth, but a haemolysin was not demonstrated in the filtrate.

(*h*) *Pathogenicity.* In spite of the fact that these bacilli produce such a severe general and local reaction when growing in the urinary tract of man, yet, large doses can be injected intravenously into rabbits without ill effect, although immune substances are rapidly formed. If, however, the bacilli are held up in the kidney owing to blocking of a ureter, then septic nephritis may be produced; or if the bacilli are injected into the pleural cavity, suppuration

in the chest may occur. A preliminary intravenous injection of 250 millions of live bacilli from a 24 hours' growth on agar of any of these organisms may produce little or no effect, while an intravenous injection of 100 million live bacilli has been employed for the immunisation of a large number of normal rabbits without producing a suppurative process. When, however, we consider these results, they are not so widely different from what occurs in man. The acute symptoms in cases of pyelitis are due to inflammation of the pelvic epithelium of the kidney and subepithelial tissues and are of greatest intensity if the outflow of urine is more or less completely obstructed. A similar although less common process may develop from cystitis. If, however, the bacilli are living and multiplying in the urinary tract of man without any hindrance to the outflow of urine, there may be little or no discomfort. It is our belief that the acute febrile illness produced in man by these bacilli, and also by true colon bacilli, occurs when the outflow of urine is temporarily obstructed.

3. SEROLOGICAL REACTIONS.

(*a*) *Precipitin reactions.* Type cultures of the haemolytic groups *A*, *B* and *C*, were grown for one month aerobically at 37° C. in beef broth containing 1 per cent. of May and Baker's peptone, and with a reaction of 7·2. Antigens prepared by growing the bacilli for 24 hours in beef broth were useless for these experiments, and seven and fourteen day antigens were not as satisfactory as the one month. The flasks of broth were well shaken on several occasions during the month's incubation and the very abundant growth which was obtained was filtered through German Berkefeld candles, type "W." The candles were sterilised and then washed through with 200 c.c. of sterile normal saline by means of a vacuum pump connected with the main water supply. To the clear filtrate obtained from filtering the broth flasks 0·1 per cent. formalin was added. The filtrates were stored in sterile glass bottles, closed with waxed corks, in the dark at room temperature. Anti-sera were obtained by immunising rabbits with vaccines, or with live

Table V. *To show the quantities of anti-serum and antigen employed in each tube for the precipitin reactions.*

No. of tube	1	2	3	4	5	6	7	8
Anti-serum:								
Quantity	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	—
Dilution	1 in 5	10	20	40	80	160	5	Saline only 1 c.c.
Antigen:							Saline	
Quantity	0·5 c.c.	0·5 c.c.	0·5 c.c.	0·5 c.c.	0·5 c.c.	0·5 c.c.	0·5 c.c.	0·5 c.c.

Total amount in each tube is 1·5 c.c.

The results of the reactions are recorded as follows:

$+_3$ = Thick deposit covering convex end of tube.
$+_2$ = Less abundant precipitate formed.
\+ = Slight deposit.
Trace (Tr.) = Granularity in suspension.
Faint trace (F.Tr.) = Faint granularity in suspension.
\− = No reaction. 0 = Reaction not done.

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cultures so that strong anti-sera were obtained. The reagents consisting of an anti-serum and antigen were incubated together in varying dilutions in small glass tubes in a water bath at 52° C. for 24 hours. The results were then read by the aid of a hand lens in a suitable light against a black background. The amount of antigen in each tube was a constant factor (0·5 c.c.), while the anti-serum was added in varying quantities diluted with normal saline. In each experiment six tubes of diluted anti-serum were used, and two control tubes of anti-serum with saline, and antigen with saline. The dilutions of anti-serum with saline are shown in Table V.

A certain number of the experiments will be recorded in detail, and subsequently the value of this reaction will be discussed.

Exp. 1. A rabbit was immunised with a haemolytic bacillus No. 5659 group *A* +. The anti-serum when prepared agglutinated its own bacillus to an end point of 1 in 40,000, and gave strong specific precipitin reactions as recorded in the following table:

			Dilutions of anti-serum					
Anti-serum	Antigen	Group	1 in 5	10	20	40	80	160
5659 (H.) (Group *A* +) (Sample A.)	B. 5659 (H.)	*A* +	$+_3$	$+_3$	$+_2$	$+_2$	+	Tr.
		−	−	−	−	−	−	−
	B. 6489 (H.)	*A* +	$+_3$	$+_3$	$+_2$	+	+	Tr.
	B. ewen (H.)	*B* +	−	−	−	−	−	−
	B. paratyphosus *β* (N.H.)	−	−	−	−	−	−	−

Exp. 2. Another supply of anti-serum 5659 prepared from a rabbit was tested with various antigens to show the precipitin reactions which are recorded below:

			Dilutions of anti-serum					
Anti-serum	Antigen	Group	1 in 5	10	20	40	80	160
5659 (H.) (Group *A* +) (Sample B.)	B. 5659 (H.)	*A* +	$+_3$	$+_3$	$+_2$	+	+	+
	B. williams (H.)	*A* +	$+_3$	$+_3$	$+_2$	$+_2$	+	+
	B. 6489 (H.)	*A* +	$+_3$	$+_3$	$+_2$	+	+	+
	B. ewen (H.)	*B* +	−	−	−	−	0	0
	B. gale (H.)	*C* +	Tr.	−	−	−	0	0
	B. fincham (H.)	*A* +	$+_2$	$+_2$	$+_2$	+	+	+

The effect of storage on an anti-serum. Anti-serum *5659* as employed in Exp. 2 was *stored* undiluted in the ice safe without the addition of an antiseptic for ten days, and also when diluted 1 in 5 with formol saline (0·1 per cent.) at room temperature. The *storage* of anti-serum *5659* in the manner indicated did not reduce its efficiency on the above-mentioned antigens at the end of ten days.

Exp. 3. A rabbit was immunised with a haemolytic bacillus from group *C* + (gale). This anti-serum, when finally prepared, agglutinated *B. gale* up to 1 in 10,000.

Precipitin reactions with this anti-serum gave the following results:

			Dilution of anti-serum					
Anti-serum	Antigen	Group	1 in 5	10	20	40	80	160
Gale (H.) (Group *C* +)	B. ewen	*B* +	−	−	−	−	0	0
	B. williams	*A* +	+	−	−	−	−	
	B. 5659	*A* +	+	F.Tr.	−	−	−	−
	B. gale	*C* +	$+_2$	$+_2$	+	+	Tr.	Tr.

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Exp. 4. This experiment was carried out on similar lines to the previous experiment with an anti-serum prepared from *B. ewen* of group *B* +. The agglutination end-point of the ewen anti-serum on *B. ewen* antigen was 1 in 20,000.

			Dilution of anti-serum					
Anti-serum	Antigen	Group	1 in 5	10	20	40	80	160
Ewen (H.) (Group *B* +)	B. ewen (H.)	*B* +	$+_3$	$+_3$	$+_2$	$+_2$	+	Tr.
	B. gale (H.)	*C* +	+	+	Tr.	–	–	–
	B. 5659 (H.)	*A* +	–	–	–	–	–	0
	B. fincham (H.)	*A* +	Tr.	–	–	–	–	0
	B. wilson (N.H.) (appendix)	*B* –	+	Tr.	–	–	–	–

Inoculation of filtered antigens into rabbits. The intravenous injection of the filtered beef broth extracts into rabbits was found to produce agglutinins and precipitins, but no ill effect occurred with the dosage employed.

Summary. The technical preparation of the antigens, and the methods adopted for carrying out the precipitin reactions, correspond in detail with the methods employed by Dudgeon and Bamforth (1925) in their work on staphylococcal precipitins.

The results of our precipitin experiments with the slow lactose fermenting bacilli shows that strong reactions are obtained which are clearly what may be described as "type specific." In the experiments numbered 1, 2, 3 and 4, the anti-sera were prepared from haemolytic strains, and the majority of the antigens were also prepared from these cultures. In Exps. 1 and 2 the anti-serum No. *5659* was prepared from *B. 5659* which belongs to group *A* +. This anti-serum gives strong precipitin reactions with all antigens prepared from bacilli of group *A* +, but does not react to any appreciable degree with other antigens in the *B* and *C* groups, or with an antigen of *B. paratyphosus β*. In Exp. 3, the anti-serum prepared from *B. gale* (group *C* +) reacted well with its own antigen, and gave either no reaction, or only a slight reaction with antigens prepared in a similar manner from bacilli of other groups. A somewhat similar result was obtained with the anti-serum *B. ewen* (group *B* +) as recorded in Exp. 4. This anti-serum gave a strong reaction with its own antigen, and a relatively slight reaction with antigens prepared from other groups. The precipitin results recorded in Exps. 1, 2, 3 and 4 and numerous other observations not recorded here, would appear to indicate that these reactions carried out by the methods described, are so type specific that they are of greater value for the study of these bacilli than the agglutination reactions. It was, however, entirely with haemolytic strains that satisfactory results were obtained.

(*b*) *Agglutination reactions.* We have found that the slow lactose fermenting bacilli included in groups *A*, *B*, *C* and *D* do not agglutinate with typhoid, para-*β* or Aertrycke (Newport) anti-sera at the end of two hours at 52° C., but may give a slight reaction not exceeding a dilution of 1 in 50 after five hours at 52° C.

The storing of anti-sera. The anti-sera were usually stored at room temperature diluted 1 in 5, or 1 in 10 with saline containing 0·1 per cent. formalin.

By this means they were stored for prolonged periods without deterioration, more especially when the anti-sera were not diluted beyond 1 in 5. If 5 per cent. formalin was added the sera rapidly deteriorated.

Method. The agglutination reactions were carried out in the usual small glass tubes in a water bath at 52° C. for five hours; at the end of this time the tubes were left at room temperature for about 15 minutes, and then the reactions were read and recorded as follows:

C.	Complete agglutination. Bacteria not held in suspension.
I.C.	Incomplete agglutination. Some bacteria in suspension.
M.	Marked agglutination. Less clumping than in I.C.
Trace.	Trace of a reaction. No sedimentation.
Faint trace.	Agglutination just visible with a hand lens.
—.	No agglutination.
0.	Not tested.

Primary testing. When an organism was first isolated from a patient, it was usually tested for coarse agglutination with the various anti-sera which had been prepared. Subsequently subcultures were made in veal broth and on agar twice daily for five days, and the tenth subcultures were used for the agglutination tests. The veal broth antigens were diluted, and the agar cultures emulsified, in normal saline and then tested with the anti-sera.

It was necessary in every case to employ two antigens at the outset of the experiments; (1) live veal broth, and (2) live agar emulsions.

Experience has shown, that while one strain may give a perfect antigen in veal broth, another will not agglutinate in this medium; yet an efficient antigen can be obtained with live or with dead agar emulsions in saline. It is a laborious task, but unless such methods are adopted it is incorrect to describe a bacillus as inagglutinable. Dudgeon, Bawtree and Wordley have already referred to this subject when discussing the agglutination reactions with *B. coli* cultures (1921 and 22).

Saturation of agglutinins. We have adopted various methods for the saturation of the immune sera. Experiments were carried out at 52° C. and at 37° C., but the most satisfactory method is to allow the reaction between serum and bacterial emulsion to take place in the ice safe. When an anti-serum is exposed to a temperature of 52° C. for a long period, there may be considerable reduction in the potency of the serum. The method which we adopted was to inoculate heavily four large agar slopes in 6 × 1 tubes and after 24 hours' incubation at 37° C., 1·5 c.c. of sterile saline was added to one of the tubes. The whole growth was emulsified in the saline which was then pipetted to the second tube when the process was repeated, and then into the third and fourth tube so that finally a very thick emulsion of bacteria was obtained. The water of condensation in each tube was found to balance any loss from the 1·5 c.c. of saline pipetted from tube to tube. The bacterial emulsions which amounted to 1·5 c.c. were mixed with 0·5 c.c. of anti-serum

diluted 1 in 5 with saline, and the mixture was put in the ice safe for a period varying from five to seven days according to circumstance—together with control tubes of serum and normal saline. The tubes which contained the bacterial emulsion and serum were shaken each morning, and at the conclusion of the experiment these emulsions were centrifugalised at high speed until a clear serum was obtained for re-testing. The saturation experiments were of the greatest value, as bacteria which agglutinate feebly may yet desaturate the sera, so that saturation experiments are necessary whenever grouping of bacteria by means of anti-sera is employed.

Andrewes, in a paper on group-agglutination, has elaborated a method by which pure mono-specific sera can be obtained by exhausting ordinary sera of all their group agglutinins. In this paper Andrewes has shown that individual colonies from a plate culture of a bacillus such as *B. paratyphosus C* may react in a widely divergent manner when tested with a specific paratyphoid *C* serum and a group serum. The method of Andrewes which was introduced for the study of the Salmonella group has also the advantage that it dispenses with the adsorption test as previously employed.

Agglutination experiments.

Exp. 1. Anti-serum 5659 (group *A* +) was tested on ten isolated colonies of B. 5659—five regular and five irregular obtained from the surface of agar plates. Each colony gave a perfect emulsion in broth without a trace of auto-agglutination, and also gave almost identical agglutination with the 5659 serum. This anti-serum 5659 was then saturated with two other strains of the same group B. 6489 and *B. williams*, and *B. gale* of group *C* +. After saturation of the serum with B. 6489 and williams, it was found it did not agglutinate any of the regular or irregular colonies of 5659 referred to, but after saturation with *B. gale* which belongs to group *C* +, the serum agglutinated all the 5659 colonies equally well.

Exp. 2. Anti-serum 5659 (group *A* +) was tested on veal broth antigens prepared from 14 strains of bacilli which belong to the same group as B. 5659 with the following results:

	Dilution of serum 5659	
No. of strain	1 in 100	1 in 1000
M.	I.C.	Trace
R.	M.	Trace
W.	I.C.	M.
A.S.	M.	—
N.	I.C.	M.
6752	I.C.	Trace
S.	I.C.	Trace
F.	I.C.	Trace
6614	I.C.	I.C.
7146	I.C.	Trace
Duncan	I.C.	I.C.
27·4935	I.C.	Trace
Salthouse	Trace	Trace
Struckett	I.C.	Trace

The anti-serum 5659 after saturation with two other strains from group *A* + (*B. wilson* and *B. fincham*) was tested on the 14 strains of bacilli in group *A* + previously tested with the unsaturated serum. The results of the experiment showed that complete removal of the agglutinins had been effected in all but three instances, and here the titre of the anti-serum was very considerably reduced as the end-point of the serum had fallen to 100/5000.

Exp. 3. In this experiment, anti-serum 5659 was tested on four separate colonies of *B. duncan* also group *A* + as follows:

	Dilution of anti-serum 5659		
Antigen	1 in 100	400	1000
B. duncan (*A* +)			
Colony 1	C.	C.	C.
,, 2	C.	C.	I.C.
,, 3	C.	C.	C.
,, 4	C.	C.	I.C.

This anti-serum 5659 was then saturated with *B. fincham* and *B. wilson* (group *A* +). The two samples of anti-sera so treated were then re-tested on the same four strains of *B. duncan* (group *A* +) as follows:

	Anti-serum 5659					
	After saturation with *B. fincham*			After saturation with *B. wilson*		
Antigen	1 in 100	200	400	1 in 100	200	400
B. duncan (*A* +)						
Colony 1	M.	Tr.	—	M.	Tr.	—
,, 2	M.	Tr.	—	M.	Tr.	—
,, 3	M.	Tr.	—	M.	Tr.	—
,, 4	M.	Tr.	—	M.	Tr.	—

Exp. 4. Anti-serum *B. ewen* (group *B* +) was tested on the following strains with these results:

			Agglutination reactions	
Organism	Group	Source	Complete or incomplete	End-point
B. ewen	*B* +	Urinary	1000	5000
B. gale	*C* +	,,	50	400
B. wilson	*B* −	Appendix	50	400
B. 5659	*A* +	Urinary	0	0
B. 6489	*A* +	,,	0	0
B. dow	B. coli (H.)	,,	0	0
B. 5651	B. coli (N.H.)	,,	0	0

This anti-serum ewen (group *B* +) was saturated for one week in the ice safe with the following results:

	Agglutination results after saturation of serum Ewen on		
	B. wilson	B. ewen	B. gale
1. Saturated with B. wilson (Appendix) (Group *B* −)	0	0	0
2. ,, B. ewen (Group *B* +)	0	0	0
3. ,, B. gale (Group *C* +)	0	2000	0

Exp. 5. Anti-serum *B. wilson* appendix (group *B* −) was tested on the following bacteria:

			Agglutination reactions	
Organism	Group	Source	Complete or incomplete	End-point
1. B. wilson	*B* −	Appendix	400	2000
2. B. mcfarlane	*B* −	Urinary	—	—
3. B. ewen	*B* +	,,	2000	8000
4. B. 5659	*A* +	,,	—	—
5. B. gale	*C* +	,,	—	50
6. B. wilson	*B* −	Faeces	—	400
7. B. 6489	*A* +	Urinary	—	50

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This anti-serum was then saturated with *B. wilson*, *B. ewen* and *B. gale* for one week in the ice safe with the following results:

	Agglutination results after saturation of the serum on		
	B. wilson	B. ewen	B. gale
1. Saturated with B. wilson (appendix)	—	400	—
2. ,, B. ewen	—	—	—
3. ,, B. gale	2000	6000	—
4. ,, B. mcfarlane	No reduction	No reduction	0
5. ,, B. 5659	,,	,,	0

Exp. 6. Anti-serum gale group $C+$ was tested with six strains of bacilli belonging to the groups indicated, and then re-tested after it had been saturated with four of the strains for seven days in the ice safe.

		Before saturation Anti-serum Gale	
Antigen	Group	Complete or incomplete	End-point
1. B. gale	$C+$	1000	2000
2. B. 7342	$C-$	—	—
3. B. 5659	$A+$	—	50
4. B. cotsford	$C+$	—	400
5. B. ewen	$B+$	—	50
6. B. 6489	$A+$	—	100

	After saturation (antigen)		
Anti-serum Gale saturated with	B. gale	B. 5659	B. cotsford
1. B. gale	—	—	—
2. B. 5659	2000	—	50
3. B. cotsford	400	—	—
4. B. 7342	2000	50	400

Exp. 7. An anti-serum gale (group $C+$) was tested on two other strains belonging to the same group as *B. gale*, with the following results:

		Anti-serum Gale	
Bacillus	Source	Complete or incomplete	End-point
1. B. unit 6	Intestinal	4,000	8,000
2. B. 28/2172	Urinary	4,000	5,000
3. B. gale	,,	5,000	20,000

This anti-serum was then saturated in the ice safe for six days with thick agar emulsions of the bacilli referred to above, with these results:

	Anti-serum Gale after saturation tested on these antigens			
Serum saturated with	B. unit 6	28/2172	"A.W."	gale
1. B. unit 6	—	—	—	—
2. B. 28/2172	—	—	—	—
3. B. gale	—	—	—	—

An anti-serum was made from *B. unit* (3) group $D-$ and tested on a Unit 3 antigen and four other antigens prepared from bacilli which belong to the same group as Unit 3. In spite of the fact that the Unit 3 anti-serum reacted with its own antigen up to 1 in 30,000, yet it failed to agglutinate any of the other strains. A similar result was obtained with anti-serum *6916* prepared from *B. 6916* (group $A-$). This serum would not agglutinate the other strains of group $A-$ and it was not desaturated by any of these strains.

All our experiments are in agreement that serological investigations with all the non-haemolytic strains are too specific to be of practical importance.

The relationship of the slow lactose fermenting bacilli and true B. coli. On cultural evidence these organisms are separated by means of lactose. Serologically they are closely related as shown by the results obtained with immune sera prepared from representative strains. The results of such investigations are recorded in Exp. 8.

Exp. 8. Anti-serum, 5659 (group *A* +):

			Dilution of serum						
Antigen	Group	Haemolysis	100	400	1000	2000	4000	8000	20,000
B. 5659	*A* +	+	C.	C.	C.	I.C.	I.C.	F.Tr.	—
B. dow	True B. coli	+	M.	M.	Tr.	—	—	—	—

Anti-serum Dow (true haemolytic *B. coli*):

			Dilution of serum						
Antigen	Group	Haemolysis	100	400	1000	2000	4000	8000	20,000
B. dow	True B. coli	+	C.	C.	C.	I.C.	I.C.	M.	Tr.
B. 5659	*A* +	+	M.	M.	Tr.	—	—	—	—

Summary.

1. *Clinical.*

(*a*) The slow lactose fermenting bacilli which we have investigated cause an acute febrile illness in man. The general symptoms may be so severe that the diagnosis of typhoid and paratyphoid fever has been made. Such cases are invariably due to an acute infection of the urinary tract, more specially acute pyelitis or pyelo-nephritis, but the general symptoms may be so severe at the onset of the illness that the urinary manifestations are masked.

(*b*) Three fatal cases have occurred.

(*c*) In cases of acute diarrhoea, with or without blood and mucus, these bacilli may be present in pure culture in the faeces.

(*d*) Chronic urinary infections due to these bacilli are uncommon.

(*e*) The bacillus of so-called *columbensis* fever is allied to these bacilli.

2. *Bacteriological.*

(*a*) Bacilli which form blue colonies on litmus-lactose-agar, which remain constant for ten days, and which fail to ferment lactose broth, or in which the fermentation is delayed, have been grouped on their cultural reactions in saccharose and dulcitol, and their power to haemolyse red blood cells suspended in salted peptone water.

Four groups, *A*, *B*, *C* and *D*, are described, while groups *A*, *B* and *C* are subdivided into positive (+) and negative (−) according to whether haemolysis does or does not occur.

The cultural reactions of each group in mannitol, dextrose, maltose and milk, and the formation of indol are the same as with true *B. coli.*

(*b*) Acute urinary infections were due to the haemolytic group (*A* +) in 82 per cent. of the cases.

(*c*) Agglutinins are formed in the blood of patients suffering from these infections, but a reaction below 1 in 150 is of no diagnostic value. The highest reaction met with was 1 in 2000.

(*d*) The delayed reaction in lactose broth is not altered by frequent sub culture of the organism on agar or in lactose broth.

(*e*) Regular and irregular colonies are formed when infected material is plated direct on the surface of agar, blood agar, litmus-lactose-agar and gelatine media. No material difference has been observed when the two types of colonies have been compared culturally and serologically.

(*f*) Every strain was tested for haemolysis in 5 c.c. of peptone water containing 0·5 and 0·85 per cent. of pure sodium chloride, 0·1 per cent. agar, and 2 per cent. of human red cells. The media were inoculated from agar cultures grown for 24 hours at 37° C. Surface colonies on blood agar plates were also examined for evidence of haemolysis, but the results were not as reliable as in the liquid media.

(*g*) A toxin was prepared from haemolytic strains grown in a liquid medium of ox heart muscle and casein digest for 24 hours, and also for one week at 37° C. The growth was filtered through a German Berkefeld W. candle, but the filtrate, and also the filtrate from beef broth cultures, failed to lyse human red cells.

(*h*) The toxin prepared from beef broth cultures of these bacilli cultivated for one month at 37° C. and then filtered through Berkefeld candles can be employed for the immunisation of rabbits.

(*i*) In spite of the acute infective process produced by these bacilli in infections of the urinary tract in man, yet, when rabbits were injected intravenously, no ill effect was produced, but if the inoculation was made into a closed cavity abscess formation occurred. In our opinion, the acute febrile illness in man produced by these bacilli is due to an inflammation of the urinary passages which causes partial or complete obstruction to the outflow of urine.

3. *Serological.*

A. (*a*) Precipitation reactions were carried out at 52° C. for 24 hours with the immune sera of rabbits and the filtered antigens prepared by growing these bacilli in beef broth for one month at 37° C.

(*b*) Strong reactions were obtained which were regarded as type specific and which appeared to indicate that this reaction was of greater value than agglutination experiments for the study of the haemolytic strains of these bacilli. The non-haemolytic strains are much more difficult to investigate serologically as is the case with non-haemolytic *B. coli*.

B. *Agglutination experiments.* (*a*) It was found necessary to ascertain with each strain whether a formalised, or live agar, or veal broth antigen was the most efficient. The antigens were prepared by subculturing the organisms two or three times daily for ten subcultures. Anti-sera were prepared by immunising rabbits with the live bacillus or with vaccines.

The reactions were carried out in a water bath at 52° C. for five hours, and the results were read against a black background.

(*b*) The saturation of anti-sera with very thick emulsions of live bacilli was investigated at 52° C., 37° C., and in the ice safe. The last mentioned method took longer, but was found to be the most efficient.

(*c*) The most satisfactory results were obtained with haemolytic strains, but more especially with group *A* + which comprises 82 per cent. of the total. These results confirm the precipitation reactions.

(*d*) The slow lactose fermenting bacilli are not agglutinated with typhoid, paratyphoid or Aertrycke (Newport) anti-sera.

(*e*) Every anti-serum prepared from the bacilli included in group *A* + was found to agglutinate every member of this group, and with each strain desaturation of the anti-sera could be effected.

(*f*) Bacilli of the same group, haemolytic or non-haemolytic, may be agglutinated by an anti-serum of high titre to a slight degree only, but may desaturate the serum. Saturation experiments were found to be of greater value than ordinary agglutination experiments.

(*g*) Anti-sera prepared from true haemolytic *B. coli* may agglutinate slow lactose fermenting bacilli included in group *A* +, and the converse is equally true.

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