ON *BACILLUS COLI* INFECTIONS OF THE URINARY TRACT, ESPECIALLY IN RELATION TO HAEMOLYTIC ORGANISMS.

By

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INTRODUCTION.

Those who have studied the literature of *Bacillus coli* infections of the urinary tract, over a period of many years, must be fully conscious that the methods employed in such investigations have been on very similar lines. Bacilli isolated from the urine have been classified on the result of various cultural reactions of which the fermentation of cane sugar and dulcitol appears to occupy the most important position. Animals have been inoculated with various strains of urinary coli, while similar inoculation experiments have been made on animals before and after a mechanical injury to one or both kidneys had been produced.

The question whether *B. coli* infections are "ascending" or "haemic" has given rise to considerable discussion of which an undue proportion has been theoretical. For these reasons we decided to investigate this infective condition by entirely different means, with the hope that we might be able to throw light on the various problems which so far had remained unsolved. In our opinion, however valuable experimental observations on animals may have been or may prove to be in the future as regards *B. coli* infections of the urinary tract, yet this condition must be studied fully on the human subject; further, there are few diseases which offer greater opportunities for combined pathological and clinical investigations.

Section I.

The Methods employed in the examination of various strains of *Bacillus coli* for haemolytic properties.

Preparation of media. Tubes containing 5 c.c. of 1 per cent. peptone in 0·5 per cent. and 0·85 per cent. sodium chloride in distilled water were employed throughout.

The human blood was collected by vein puncture in tubes containing 5 per cent. sodium citrate. The blood is well mixed with the citrate, then allowed to stand for about one hour, centrifugalised at high speed, and the supernatant fluid discarded.

Method of testing for haemolysis. Two tubes of each strength sodium chloride in peptone water were inoculated, and 0·1 c.c. of solid red cells were added to one tube of each. The four tubes were then incubated at 37° C. for 24 hours. If haemolysis had occurred, the readings were taken, and 0·1 c.c. of red cells added to the two remaining tubes which were then re-incubated for one hour at 37° C., allowed to stand in the ice-safe over-night, and the degree of haemolysis fully noted. The four tubes were used to obtain a comparison of the degree of haemolysis produced in 0·5 per cent. and 0·85 per cent. sodium chloride, when red cells were added at the time of inoculation and after the bacillus had grown for 24 hours. Control tubes with red cells were employed in each experiment. In reading the degree of haemolysis, the tubes must be slightly shaken when necessary to allow the haemoglobin to diffuse through the medium.

The gradations of haemolysis are recorded as "Trace," "Marked," "Incomplete" and "Complete": a trace is slight tingeing of the media above the red cells, and represents an average of 60 per cent. haemoglobin on the Oliver scale: "Marked" is distinct colouration of the whole media and represents an average of 160 per cent. haemoglobin: "Incomplete" is haemolysis of nearly all the red cells, about 260 per cent. average: "Complete" is haemolysis of all the red cells and represents an average of 300 per cent. haemoglobin.

The strains which possessed active haemolytic properties showed very little difference in the degree of haemolysis effected in 0·5 per cent. and the 0·85 per cent. sodium chloride, or between the tubes to which red cells were added

before inoculation and those to which the cells were added the next day. With the less active strains, haemolysis was generally less marked in the tubes containing 0·5 per cent. sodium chloride.

The routine method for testing the haemolytic properties of the various strains as they were obtained was to take two, three, or more colonies from the MacConkey or blood agar plates. In the urinary cases it was found that if one colony was haemolytic the other colonies from that plate had the same property: similarly if one colony was non-haemolytic, the others were the same: on one occasion only was a haemolytic and a non-haemolytic colony obtained from the same plate: this was a case of an acute coli infection. Blood agar plates were used in the examination of faeces and when haemolysis was present, usually about one half of the colonies from that plate showed evidence of haemolysis.

Persistence of haemolytic properties. Stock cultures of all haemolytic and some non-haemolytic strains were kept and tested from time to time: the haemolytic properties were in every case retained with but slight diminution over periods varying from three months to one year. None of the non-haemolytic strains have shown any trace of haemolytic properties, while two non-haemolytic strains were subcultured ten times on blood agar, but remained non-haemolytic.

Haemolytic properties in the presence of untreated human serum and gum. Several haemolytic and non-haemolytic strains were tested with the addition of untreated human serum, and 6 per cent. gum in physiological saline, to the culture medium.

Experiment 1. In this experiment peptone water containing 0·85 per cent. sodium chloride was used and three tubes for each strain tested:

No. 1 tube contained 5 c.c. peptone water.
No. 2 ,, ,, plus 0·1 c.c. serum.
No. 3 ,, ,, ,, 0·05 c.c. serum.

Each tube was inoculated and incubated at 37° C. for 24 hours: 0·1 c.c. of red cells was then added and the readings taken after one hour at 37° C. Haemolysis was "complete" or "incomplete" in the case of the haemolytic strains in the No. 1 tubes, "none" in the No. 2 tubes, and "none" or "a trace" in the No. 3 tubes.

Experiment 2. To compare the action of untreated human serum and 6 per cent. gum in physiological saline (0·85 per cent.) in peptone water.

Three haemolytic strains of *B. coli* were used in this experiment and the red cells were added before inoculation of the media and the readings taken after 20 hours at 37° C. Each strain gave "complete" haemolysis in the peptone water, "incomplete" or "marked" haemolysis in the tubes to which 0·5 c.c. gum had been added, and "a trace" only in the tubes containing 0·5 c.c. of untreated human serum.

Conclusions. Numerous experiments were made by us on this question, and those quoted above have been selected as typical examples of the limitation of

haemolysis in the presence of normal serum, and little or no effect in the presence of gum: this appears to indicate that the action of the serum is something apart from mere mechanical protection, while in the case of the haemolytic streptococci the addition of blood serum induces an accentuated effect.

B. coli *grown in normal untreated urine.* Experiments were made with several strains of non-haemolytic urinary coli; these were inoculated in normal urine, which had been filtered through a Doulton candle: the urine was very slightly acid or neutral and contained no albumen or sugar: 5 c.c. of urine was used for each tube and these were inoculated with the strains of *B. coli* and grown for 24 hours at 37° C.

Peptone tubes with 0·5 per cent. and 0·85 per cent. sodium chloride were then inoculated from the urine cultures. There was no haemolysis in any tube whether the red cells were added at the same time or after 24 hours' growth in the peptone.

Conclusion. This experiment shows that non-haemolytic strains of *B. coli* when grown in urine do not tend to develop haemolytic properties.

The effect of growing haemolytic strains of B. coli *in peptone water for some days.* This experiment was undertaken to see if the haemolytic properties of *B. coli* were in any way altered by prolonged growth in peptone water without subculture.

Table I.

Shows the diminution of haemolytic properties of *B. coli* when grown in peptone water without subculture.

Organism	Degrees of haemolysis				
4442	I.C.	Trace	Trace	0	I.C.
4879	I.C.	0	Trace	0	I.C.
Dun	I.C.	Trace	Trace	0	I.C.
Dow	I.C.	Trace	Trace	0	I.C.
X 6	I.C.	M	I.C.	Trace	I.C.
Periods of incubation at 37° C. before red cells were added	24 hrs.	3 days	7 days	11 days	24 hours' subculture from the 10 days' old culture. Then red cells added

Conclusions. The above table shows the disappearance or marked diminution of the haemolytic properties of *B. coli* when grown in salted peptone water without subculture, and the reappearance with full activity when subcultured.

Section II.

On *Bacillus coli* infections of the Urinary Tract.

Attention has been drawn to the fact, earlier in this communication, that some strains of *B. coli* are haemolytic while others are non-haemolytic. This difference was observed in the first instance in strains isolated by us from infected urine. Accordingly such infections were investigated more fully with the idea that the incidence of this haemolytic property would help to throw

light on the origin of these infections, about which there has been much controversy.

Method. From women the urine was collected by catheter from the bladder; in the case of men it was passed into a sterile vessel; subsequently it was centrifugalised, examined microscopically and plated direct on MacConkey plates, the amount used for plating varying with the number of bacilli seen under the microscope. Several colonies were taken, and if found to be true colon bacilli, tested for haemolysis as described in Section I. It should be mentioned that only infections caused by *B. coli* are dealt with in these observations, none are included in which "colon-like" bacilli were the infecting organisms. All strains included as colon bacilli by us produced acid and gas in lactose, dextrose, maltose and mannitol, clotted and acidified litmus milk, produced a yellow fluorescence in neutral red broth, formed indol and did not give the carbinol reaction. The fermentation of cane sugar and dulcitol will be discussed later. Inosite was not fermented by any of our strains and such as were tested did not liquefy gelatine.

In all, 69 cases of infections of the urinary tract caused by *B. coli* were examined; of these 27 were men, 42 women.

The number of haemolytic strains isolated from the two sexes is shown in the following table (II).

Table II.

Total number	Sex	Haemolytic	Non-haemolytic	Percentage of haemolytic strains	Percentage of non-haemolytic strains
27	Male	20	7	74	26
42	Female	11	31	26	74

It should be mentioned that, with one exception, all colon bacilli isolated from these infections were of one type as regards their haemolytic properties. That is, all were haemolytic or all non-haemolytic; a mixture of the two varieties was found only in the one instance mentioned above.

There is little mention in the literature of haemolytic strains of *B. coli* or of their significance.

Schottmüller and Much (1908), making a practice of plating faeces both on Conradi and blood agar from cases with any disorder of the digestive tract, found haemolytic coli from time to time. In this paper opsonic indices in various diseases are considered, and a comparison is made between the opsonic index of a patient's serum for the haemolytic and non-haemolytic colon bacillus present in the faeces in a case of gastro enteritis and jaundice.

Schmidt (1909) in a paper devoted to the significance of haemolytic *B. coli* isolated them from both urine and faeces. Most of the urines he examined were from cases of tuberculous infection of the urinary tract. He investigated the faeces also of 73 cases in which there was diarrhoea and 17 normal cases and found 72·6 per cent. haemolytic coli in cases with diarrhoea and 65 per cent. of similar organisms from the 17 normal cases. He did not attach any special

significance to the colon bacillus possessed of haemolytic properties. Haemolysis was judged by changes produced on blood agar plates.

Lyon (1917) describes a case of cystitis which he states was caused by a haemolytic *B. coli*. It was a Gram negative bacillus, but would not, on first isolation, grow on ordinary media, though it would do so later after preliminary cultivation on blood agar.

From a consideration of the previous table it appears that a haemolytic colon bacillus is by far the commonest cause of urinary infection in the case of males, whereas in women exactly the opposite occurs. This at once suggests the possibility of a different mode of infection for the two sexes. A prolonged controversy has arisen on this point in an attempt to explain the admittedly greater liability to coli infections in the female. It has been considered that in females the infection occurs as a result of the direct upward spread of bacteria over the surface of the mucous membrane of the urethra, whilst on the other hand this has been denied, and all infections of this nature have been thought to be due to a blood infection as a result of which the bacteria are excreted by the kidneys, infection extending downwards.

The upholders of the first theory point to the ease of direct contamination by faecal material in the female from anatomical considerations (Box, 1910), while those who uphold the second view consider that as intestinal stasis is more common in women, this favours a blood infection, and Kidd (1920) states that the inability to recognise the primary pyelitis followed by a cystitis later, is due to the fact that all cases have not been examined by means of the cystoscope. A large amount of experimental work has been done on this subject.

Brewer (1906) in attempting to explain acute infections of the kidney without previous cystitis showed that in animals, if the lumbar region is bruised and then living organisms are injected into a vein of the ear, an acute surgical kidney developed on the side previously subjected to trauma.

The same author also showed (1911) that injecting organisms into the circulation without previous trauma did not lead as a rule to any kidney lesion.

Lepper (1921) compressed the ureters for various periods and produced much the same results. Brewer also observed that if virulent organisms were injected into the bladder of an animal and the urethra then tied, a severe cystitis and pyelitis followed, whereas without urethral obstruction no inflammation occurred; in this last case he considered that the pyelitis thus produced was the result of an upward extension of the organism along the walls of the ureter, due to the stagnation of the urine. Thiele and Embleton (1914) showed that if bacteria are painted on the glans penis or anterior part of the urethra, care being taken to cause no abrasion, the infection spread in the following order: in one-and-a-half hours the organisms were chiefly in the iliac and renal glands; three hours later they were in the blood and chyle, while not until 15 hours later were they demonstrated in the urine, so that organisms can pass through uninjured mucous membrane up the lymphatics into the blood

stream and from there are excreted by the kidney into the urine. Further these authors showed that the same sequence of events occurred in intraperitoneal injection of living organisms, and that if the thoracic duct were opened no bacteria appeared in the urine. They could find no evidence of infection ascending along the lumen of the urethra into the bladder.

A great point has been made by those who believe that all urinary infections are primarily *via* the blood stream in that the *B. coli* can be obtained in pure culture from the blood, but the fact that such bacilli may be so recovered does not point to the origin of the infection, since in view of Thiele and Embleton's experiments, even if the colon bacillus did start from the urethral mucous membrane it would still be found in the blood.

A careful examination of the blood in *B. coli* infections of the urinary tract has shown that such bacilli may be isolated very frequently, especially if the blood is taken at the height of the rigor. Cabot and Crabtree (1916) obtained positive blood cultures in 40 per cent. of cases out of 32 examined.

In Section III it is shown that haemolytic coli frequently occur in the faeces and this fact would appear to be of considerable importance in the causation of urinary infections in man, since 76 per cent. of coli infections of the urinary tract in this sex are caused by haemolytic strains. The point therefore arises why the reverse should hold for the female? Only a small proportion of normal individuals have such haemolytic bacilli in their faeces, therefore, it would seem that in the male, and in the female, when this type of infection occurs, this bacillus is especially liable to escape from the gut into the blood stream, whether direct or *via* the lymphatics. In women the greater chance of contamination of the urethra by faecal matter must be admitted, and this being the case the greater frequency of non-haemolytic infections is explained since, as we have shown, only about 11 per cent. of normal individuals have haemolytic strains in their faeces. Whether the infection spreads upwards directly along the urethra or passes directly through the uninjured mucous membrane makes no difference to the fact that from our observations the mode of infection in the two sexes would appear to be different.

In certain instances in the male, local trauma such as a gleet or stricture may play a part in rendering possible an infection from external contamination, and this is suggested from the fact that in four out of seven of our male cases suffering from a non-haemolytic infection, there was a previous history of gonorrhoea or stricture or enlarged prostate, though the number is not great enough from which to draw any certain conclusions. Dudgeon (1908) examined 14 cases with prostatic enlargement and found *B. coli* present in five instances.

In the hope of throwing further light on this point a number of vaginal swabs were examined for the presence of the colon bacillus. They were provided by the kindness of Mr J. M. Wyatt, assistant obstetric physician to St Thomas's Hospital. Precautions were taken to avoid external contamination, the swabs when received were inoculated into a broth tube, incubated overnight, and plated on the following morning. If *B. coli* were isolated they were sub-

sequently tested for evidence of haemolysis. In all, 48 cases were examined in this manner. In 46, from the history and clinical examination, there was no reason to suppose they had a urinary infection, their complaint was, in the majority of cases, of a persistent vaginal discharge. In the remaining two cases there was a known urinary infection which had been shown to be due, in one instance, to a haemolytic *B. coli*, in the other to a non-haemolytic. In these 48 cases the *B. coli* was isolated in 13 instances. In every instance except one, the bacillus was of the non-haemolytic variety. The one exception was the case already mentioned in which the urinary infection was caused by a haemolytic strain of *B. coli*. From the remaining 11, catheter specimens of urine could be secured only in four instances; they were sterile in each case.

In four instances the husbands of women found to be suffering from a haemolytic coli infection were examined to see if they also had a urinary infection of the same type, but in each case the urine was sterile.

In some cases from which *B. coli* had been cultivated from the urine, the ureters were catheterised by Mr Cyril Nitch. *B. coli* was not always obtained from the ureteric urine, but in those instances in which positive results were obtained, the same type of organism was cultivated as in the original examination.

Haemolytic and non-haemolytic strains cultivated from the urine were tested as regards their fermentation of cane sugar and dulcitol for the presence of acid and gas as shown in the following table:

Table III.

	Males haemolytic	Males non-haemolytic	Females haemolytic	Females non-haemolytic
Cane sugar + Dulcitol +	2	1	1	4
Cane sugar + Dulcitol −	4	0	1	5
Cane sugar − Dulcitol −	2	0	4	10
Cane sugar − Dulcitol +	12	4	5	11

The organisms isolated from the vagina gave the following results:

Cane sugar + Dulcitol + 7
Cane sugar + Dulcitol − 0
Cane sugar − Dulcitol − 3
Cane sugar − Dulcitol + 4

It will be seen that cultured characteristics are in no way related to haemolysis or sex, and that the greater majority of strains of *B. coli* isolated from the urine do not ferment cane sugar whether haemolytic or non-haemolytic.

Agglutination reactions. At an early stage in these observations agglutinating sera were prepared from certain urinary and faecal strains and used for testing all colon bacilli isolated from the urine. The strains used for preparing these sera were Dow and Dun, two haemolytic urinary strains, 4869

a non-haemolytic urinary strain, and X 6 and X 9, two haemolytic strains isolated from the faeces.

The results obtained offer additional evidence that infections due to haemolytic and non-haemolytic strains are of different origin. It was found that, with two exceptions, all haemolytic urinary coli were agglutinated by Dow serum. On the other hand, out of 60 non-haemolytic strains, 50 were inagglutinable with any of the anti-sera employed, while ten were agglutinated by the 4869 (non-haemolytic) anti-serum.

None of the urinary strains were agglutinated by X 6 anti-serum, whereas all the haemolytic strains (except the two already mentioned, which were inagglutinable with the Dow anti-serum) were agglutinated by the X 9 anti-serum. This is a very remarkable result and would appear to point to the fact that haemolytic colon bacilli which cause urinary infections belong to a special group of organisms which are met with in the faeces in a small proportion of normal individuals, but more frequently in cases of diarrhoea and colitis, as will be referred to in detail in a later section.

Therefore haemolytic urinary infections would seem to be the result, since they are so much more prevalent in the male where external contamination of the urethra must be uncommon, of a *primary* blood stream infection from the intestine of this special group of colon bacilli, while in the female, whether the infection is a *secondary* blood stream infection from absorption of colon bacilli from the urethral or vaginal mucous membrane, or a direct ascent of colon bacilli along the urethra into the bladder, it is the result of an external urethral contamination, in the majority of instances.

The serum Dow prepared from a rabbit had an end point of $\frac{1}{10{,}000}$ on its homologous antigen, as had also the sera X 6, Dun, and X 9 on their specific antigens.

As has been said all but two haemolytic strains were agglutinated by Dow and X 9 serum, though not to the end point of the serum used, but in many cases to quite a considerable degree. The degree of agglutination, however, was not in any way related to any grouping by virtue of fermentation of cane sugar or dulcitol as is shown in Table IV.

As already mentioned all organisms agglutinated by Dow serum, when tested with X 9 serum were also agglutinated, and it is shown in a later section that conversely all haemolytic faecal strains agglutinated by X 9 serum were also agglutinated by Dow serum. Agglutination of haemolytic urinary strains by X 9 serum was generally in low dilutions, though it always occurred, but one strain showed higher agglutination with X 9 serum than with Dow giving an E.P. of 1 in 3000 with the former anti-serum, but only 1 in 400 by Dow.

It has been mentioned above that two haemolytic strains which were isolated from women were not agglutinated by Dow serum. A potent anti-serum could be prepared from either by animal inoculation, but no cross agglutination between the two strains was found to occur.

Table IV.

Organism	Cultural reaction	Agglutination titre with Dow anti-serum
Parker	Dulcitol + cane sugar −	1/1000
Dun	„ „	1/10,000
Weston	„ „	1/1000
4702	„ „	1/400
4879	Dulcitol + cane sugar +	1/4000
Peters	Dulcitol − cane sugar −	1/1000
4864	„ „	1/400
Haines	„ „	1/1000
4442	Dulcitol − cane sugar +	1/400
5229	„ „	1/1000

Section III.

On the Examination of Faeces for the presence of Haemolytic *Bacillus coli.*

It appears probable that in some cases of bacilluria and pyuria caused by a haemolytic strain of *B. coli*, the infecting organism may gain entrance to the urinary tract *via* the blood stream. If so such organisms may have their normal habitat in the intestinal canal; accordingly the faeces of various classes of patients were examined for the presence of haemolytic colon bacilli.

Method. The faeces were dried on a tile by the method advocated by Dudgeon, and a quantity of the dried powder was evenly spread over a blood agar plate, Wordley (1921), made by adding 1 c.c. of undiluted human oxalated blood to 15 c.c. of melted agar. The plates were incubated over-night and examined next morning for the presence of haemolytic colonies. Haemolytic coli colonies[1] show a narrow, faint, rather irregular zone of haemolysis, quite unlike the well-defined zone round a haemolytic streptococcus colony, and as a rule this can be seen best by looking through the plate. Haemolytic colonies when present were subcultured on to an agar slope and their haemolytic properties were further determined by the methods already described, while the cultural and serological characters were determined also. When haemolytic coli colonies were present on blood agar plates they were found generally in large numbers, at least a half being of this nature. In many instances all coliform

[1] The only evidence of haemolysis may be a greenish discoloration of the colonies.

colonies present showed evidence of haemolysis. While some colonies showing evidence of haemolysis on a blood agar plate did not always do so when inoculated in a peptone water red cell mixture, the reverse of this was not observed. All non-haemolytic colonies on a blood agar plate were found to be of the same nature when tested for haemolysis by other methods.

The cases which furnished material for these investigations are grouped as follows:

I. "*Enterica*" *and Dysentery.* Fifteen patients were included in this group, who, on bacteriological or microscopical evidence, had been infected within three months of the present investigations.

II. Twenty cases were examined. These patients had suffered from diarrhoea and had passed blood or mucus or both, but on repeated examinations of the stools, bacilli of the "enterica" or dysentery group were not isolated, and the entamoeba histolytica was not found.

III. Eleven cases of diarrhoea in infants under two years of age were examined.

IV. Fourteen cases of acute and chronic nephritis.

V. Thirty-three cases are referred to as "unclassified." These include various diseases not especially associated with any obvious intestinal lesion.

VI. Three cases of pernicious anaemia.

VII. Thirty-nine cases labelled as "normal." These patients were admitted to hospital for operation for hernia, deflected nasal septum or for enlarged tonsils.

The final results can best be appreciated if expressed in tabular form.

Table V.

Group	No. of patients examined	Nature of case	Positive results
I	15	"Enterica" and dysentery	5
II	20	Colitis	7
III	11	Diarrhoea in infants	4
IV	14	Nephritis	3
V	33	"Unclassified"	7
VI	3	Pernicious anaemia	1
VII	39	"Normal cases"	5

It will be seen that of these 135 patients, 32 or 24 per cent. had haemolytic strains of *B. coli* in their faeces.

In 39 "normal" cases there were five cases or 13 per cent. from which haemolytic coli were cultivated, while in 31 cases of diarrhoea or colitis, 11 or 35·4 per cent. showed haemolytic coli. The presence of haemolytic coli in the faeces bore no relation to sex.

The cultural characteristics of the haemolytic strains of organisms were fully investigated but no great advantage would be gained by a detailed description. The greater number fermented both dulcitol and cane sugar as can be seen from the following table.

Table VI.

		No.
1	{Cane sugar / Dulcitol} +	16
2	{Cane sugar − / Dulcitol +}	5
3	{Cane sugar + / Dulcitol −}	5
4	{Cane sugar / Dulcitol} −	3

Dr E. W. Todd working in this laboratory permits us to quote from some observations of his, as yet unpublished. Investigating the faeces of infants under one year old not suffering from diarrhoea he found, in 100 cases examined, 13 instances in which haemolytic strains of *B. coli* were present, a proportion identical with that obtained by us in normal adult cases. We examined eight of these 13 strains to determine their serological relationship with the various anti-sera we have prepared. Of these eight, six were agglutinated by X 9 and Dow anti-sera and not by X 6 serum, while two were inagglutinable by any of our anti-sera.

Immune sera for agglutination tests were prepared from rabbits with two of these haemolytic coli (X 6 and X 9) and were employed to test the remainder with the following results. In addition the majority were tested serologically with anti-sera prepared from Dow a haemolytic urinary strain, and "Hyde," a haemolytic culture isolated from the blood.

Cultural characteristics as regards the fermentation of dulcitol and cane sugar bore no relation to agglutination, different types culturally being agglutinated equally well by the same serum.

It was found that eight organisms were agglutinated by X 6 serum and by no other serum. Four strains were agglutinated by X 9 serum and further these four were all agglutinated by Dow serum. None were agglutinated by "Hyde" serum.

Our observations show that haemolytic strains of *B. coli* were most prevalent in cases of diarrhoea or colitis. The initial object of these investigations was to ascertain whether haemolytic coli isolated from the faeces were serologically identical with the Dow urinary strain, since the vast majority of haemolytic urinary strains are agglutinated by an anti-serum prepared from this bacillus. If it was found to be of common occurrence for intestinal coli to be agglutinated by the Dow anti-serum it would help to throw light on the origin of coli urinary infections. Amongst the strains we have examined this phenomenon was not found to be of common occurrence; on the other hand it has been found that a distinct serological relationship exists between X 9 and the haemolytic urinary organism Dow, in that all faecal strains agglutinated by X 9 were also agglutinated by Dow serum. Conversely all haemolytic urinary strains, except the two inagglutinable already mentioned, were also agglutinated by X 9 and not by X 6 serum.

Treatment by vaccines. Three cases, in the faeces of whom large numbers of haemolytic coli were present, were treated with vaccines. In each case their haemolytic organism was agglutinated by X 6 serum. Accordingly, a vaccine was prepared from this organism. These three patients were suffering from chronic colitis, colitis following removal of the colon for dysenteric ulceration, and coeliac disease, the latter being an infant under two years of age. In every case very marked improvement followed. The disease in each case was of long duration and a variety of treatments had been tried previously.

It is interesting to note that in one of the above cases the faeces were again examined after the colitis had subsided when no haemolytic coli were found.

Examination of human appendices for the presence of haemolytic strains of B. coli. While the above investigations were in progress the opportunity was taken to see if haemolytic strains of *B. coli* were of common occurrence in the appendix in appendicitis.

The appendix removed at operation was incised and a quantity of material from the lumen inoculated on to a MacConkey plate. From the resulting growth four colonies were taken and tested for evidence of haemolysis, in no instance was haemolysis observed. In all 20 appendices were examined in this manner, of which seven were necrotic, eight were acutely inflamed, and five showed no naked eye evidence of inflammation.

Examination of water contaminated by sewage for haemolytic colon bacilli. Through the kindness of Sir Alexander Houston, K.B.E., a number of cultures of the colon bacillus obtained from river water at its raw source of supply were examined for haemolytic properties. In all 22 strains were examined. In no case did these organisms have haemolytic properties.

SECTION IV.

Examination of the sera of normal individuals and others was made for the presence of *B. coli* agglutinins.

(*a*) The serum of "normal" individuals, by which is meant those who there was no reason to suppose were suffering from a *B. coli* infection, was tested with our standard antigens. While we fully realise that this is an extremely difficult question to decide, yet a similar objection applies to the definition "normal" throughout medical practice. The normal sera were mostly obtained from patients whose blood was being examined for the Wassermann or some other reaction, and such samples of sera were tested with our *B. coli* antigens, more especially "Dow" the haemolytic urinary strain. Up to the present only a very limited number of such examinations have been made—66 in all; but of this number 61 were negative in a dilution of 1 in 50 to these antigens with a 1 in 10,000 end-point, and to the non-haemolytic antigen which acted with its immune serum to 1 in 800. In five instances a positive reaction occurred.

The results are expressed as follows:

Table VII. Normal sera.

No. examined	Negative	Positive	
	Dil. 1 in 50	1 in 50	1 in 400
66	61	2	3

Of the positive cases (5) the urine was examined in two and found to be sterile.

(*b*) Sera from patients with infection of the urinary tract due to *B. coli*.

Fourteen such cases were examined of which six showed a reaction varying from 1 in 50 to 1 in 400.

Table VII *a*.

				Positive. Antigens				
				Haemolytic			Non-haemolytic	
No. examined	Negative	Type of infection		1 in 400	1 in 100	1 in 50	1 in 100	1 in 50
14	8	Haemolytic	5	4*	1	—	1*	—
		Non-haemolytic	1	—	—	1	—	—

It will be seen in the above table that the serum of one case (*) due to a haemolytic strain of *B. coli* agglutinated the haemolytic standard antigen as well as the non-haemolytic. This patient had had "cystitis" for 15 years.

(*c*) Ten cases were examined which were grouped as intestinal, and in four instances the serum of these patients agglutinated haemolytic coli antigens in dilutions varying from 1 in 50 to 1 in 200.

(*d*) Five cases, any of which may have had a *B. coli* infection, although it was not proved, reacted with one of the haemolytic antigens in dilutions varying from 1 in 100 to 400. In one case, the reaction on the first occasion was negative, but 14 days later, as the pyrexia continued, the blood was re-tested and the reaction was then 1 in 400.

(*e*) The blood of ten cases which had received a course of *B. coli* vaccines in doses from 50–1000 million were re-examined, but in only three instances a definite rise in the agglutinin content of the serum had occurred.

In all these observations the standard antigens employed by us were a haemolytic urinary antigen "Dow," two haemolytic faecal strains X 6 and X 9 and one non-haemolytic urinary antigen 4869.

Section V.

On the Immunisation of Rabbits.

When it was found that *B. coli* isolated from the urine could be separated into two groups (1) haemolytic; (2) non-haemolytic, experiments were commenced for the preparation of immune anti-sera.

At first rabbits were injected I.V. with formol-killed vaccines, but it was evident at an early date that immunisation with our non-haemolytic strains

was abnormally difficult to effect. For this reason it is necessary to refer concisely, but briefly, to the methods which we adopted in our immunisation experiments.

Methods: (*a*) *Vaccines.*

(1) *Formol-killed.* Young agar cultures (24 hours) were emulsified in normal saline (0·85 per cent.) containing 0·1 to 0·14 per cent. formaldehyde, filtered through sterile linen, and stored in the ice safe until they were dead, when suitable dilutions were made with saline containing 0·25 per cent. phenol.

(2) *Heat-killed.* Young agar cultures were employed also for these experiments, but attempts were made to reduce the temperature of the water and the time of exposure to the lowest and shortest possible. Numerous experiments were made on these lines (Table VIII), but we failed to satisfy ourselves that the minimum period and lowest temperature produced any special advantages over the time and temperature limit which we employ as a routine procedure.

Thermal death point. Cultures of *B. coli* on agar were 24 hours old. Saline emulsions were made which were put in the water bath for the required period, incubated at 37° C. for 24 hours, and subcultured on agar. These subcultures were left for varying periods at 37° C. as stated below. The amount of vaccine in each tube was about 3 c.c. and the diameter of the test tubes employed was $\frac{5}{8}$ inch.

Table VIII.

Haemolytic strains isolated from the urinary tract.

Name or No. of strain	Strength	Temp. of water bath	Time of exposure	Result
Gooch	1000 million	59° C.	40 mins.	Sterile in 5 days
Dun	1000 / 5000 ,,	,,	,,	Sterile in 48 hours
4442	1000 / 5000 ,,	,,	,,	Sterile in 72 hours
Peters	5000 / 1000 ,,	,,	,,	,,
Dow	1000 / 5000 ,,	,,	,,	,,
Dun	2000 / 2000 ,,	,,	30 mins. / 15 mins.	,,

Non-haemolytic strains of *B. coli* isolated from the urinary tract gave similar results. At the lower temperatures, such as 57° C. or 55° C., very variable results were obtained, whilst at 59° C. if the period of exposure was reduced to 15, 20 or 30 minutes equally variable results occurred.

(*b*) *Live cultures.* Young agar cultures emulsified in sterile saline, and stored in the ice safe during the course of our experiments, which varied from one to two months, were also employed.

(*c*) *Animal inoculation.* The rabbits were injected intravenously, intramuscularly or subcutaneously, and to a much less extent intraperitoneally.

Lepper (1921) made certain observations on the production of agglutinins in rabbits after the injection of various strains of *B. coli* and found that high titre agglutinins are produced only for the homologous strain, while group agglutination based on the fermentation of cane sugar and dulcitol does not occur, although members of the same group would agglutinate to a limited degree.

Herrold and Culver (1919) investigated 43 cases of "renal infections" caused by *B. coli*. They prepared four immune sera from strains separated by cane sugar—dulcitol reactions (CS + D +; CS − D −; CS + D −; CS − D +). The end point of each serum was 1 in 1280 for the autogenous strain, while the highest titres obtained when cross agglutination was attempted were 1 in 640 and 1 in 320. Apart from these two results no agglutination was observed between various strains of the same group or any other group.

On the results of intravenous inoculation. Our investigations were undertaken for the purpose of studying some of the many problems associated with the bacteriology and pathology of coli infections of the urinary tract, but we found it necessary to extend our observations to the intestinal tract. Bacteriological investigations of the faeces resulted in our finding haemolytic strains of *B. coli* under varying conditions. It was thus obvious that attempts to immunise animals with haemolytic strains (group 1) and non-haemolytic (group 2) isolated from the urine, and haemolytic strains obtained from the faeces (group 3), were essential, so as to compare if any serological affinity, based on agglutination records, existed and whether "grouping" could be attempted.

Rabbits were first immunised I.V. with formol-killed vaccines of the haemolytic urinary coli (Dow). This was readily accomplished as six injections of 100–1000 million bacilli at weekly intervals resulted in an anti-serum with an end point of 1 in 10,000 to the specific bacillus; in each instance rabbits rapidly responded to I.V. injections with this organism. The anti-serum (Dow) has proved of the utmost value in grouping colon bacilli isolated from the urine, and, as shown elsewhere, in agglutinating some strains of haemolytic coli isolated from the faeces.

Other rabbits were inoculated with other haemolytic strains of coli obtained from the urine, faeces and blood stream; the injections were made with vaccines and live cultures.

Injections of 10,000 million were employed in some instances for the immunisation, as massive doses produced no ill effects, but no special advantage was obtained. A high titred serum was produced in every instance by injecting rabbits with a haemolytic colon bacillus I.V. In Table IX are included various data which illustrate the results of the immunisation of rabbits with two of these haemolytic coli (groups 1 and 3), isolated from urine and faeces. The agglutinins formed in each rabbit were found to be strictly specific; the animals were never ill during any stage of the experiments, although the injections were made with live bacilli.

Table IX.

Rabbits (10 and 11).

10. Haemolytic *B. coli* from urine (Dow).
11. ,, ,, faeces (X 6).

Immunisation methods. These rabbits were injected I.V. with the live bacilli. Rabbit 11 received *B. coli* (Dow), and rabbit 10 *B. coli* (X 6). The inoculations were carried out in an identical manner and the agglutinin content of the serum of each rabbit is recorded for its specific bacillus.

Date	Number of bacteria injected	Agglutination titre. *B. coli* antigens X 6	Dow
11. x. 20	50 million I.V.	0	0
15. x. 20	100 ,,	50 10,000	50 10,000
22. x. 20	200 ,,	200 10,000	400 10,000
29. x. 20	500 ,,	5000 10,000	5000 10,000
3. xi. 20	—	10,000 10,000	10,000 10,000

Though the results referred to were obtained without difficulty when the haemolytic strains were employed, yet with the non-haemolytic coli isolated by us from the urine only relatively feeble immunisation was effected, whether vaccines heat or formol-killed, or live cultures were employed[1]. Numerous strains were tried with very similar results—in fact, there was only one non-haemolytic culture of *B. coli* (4869) isolated from the urine which excited the formation of agglutinins in the rabbit, and then but relatively feebly. The end point of the various anti-sera obtained with this organism failed to exceed 1–800 with the appropriate antigen[2]. Various methods were employed, but whether doses of 100 or 10,000 million bacilli were injected no greater response was effected, and I.V. injections of *Staphylococcus aureus*, typhoid, paratyphoid, or haemolytic coli vaccines were ineffective.

This result is at variance with that obtained by Dreyer and Ainley Walker (1910) with *B. coli*, who found that "the injection of various heterologous bacteria increases the production of special agglutinins in an immunised animal so long as it retains any measurable degree of acquired immunity." In Table X is illustrated the effect of I.V. injection of a haemolytic and two non-haemolytic urinary strains of *B. coli*. The rapid immunisation to the haemolytic strain, and the absence of response with one of the non-haemolytic and the comparatively feeble response with the other is fully emphasised, while the failure of a typhoid and other vaccines employed to produce the formation of agglutinins for the non-haemolytic strain is fully illustrated.

[1] The sera of the inoculated rabbits produced clumping of the appropriate antigens, but only in low dilutions and with unsatisfactory clumps.

[2] We subsequently, after many trials, have obtained two active immune sera in rabbits.

Table X.

Rabbit 2. (1) Non-haemolytic *B. coli* from urine (4657).
(2) *B. typhosus*.
(3) Haemolytic *B. coli* (Dow).
(4) Non-haemolytic *B. coli* (4869).

Immunisation methods. This rabbit received I.V. three strains of *B. coli*, as stated above, which were isolated from the urine, and a vaccine of *B. typhosus*. Three of the vaccines were formol killed, while the non-haemolytic strain (4869) of *B. coli* was injected I.V. as the living organism.

Date	Number of bacteria injected			Agglutination titre: *B. coli* Dow	*B. coli* 4657	*B. coli* 4869	*B. typhosus*
15. iv. 20	100 million		4657	—	—	—	—
19. iv. 20	500	,,	,,	—	0	—	—
23. iv. 20	500	,,	,,	—	0	—	—
3. v. 20	1000	,,	,,	—	0	—	—
11. v. 20	1000	,,	,,	—	0	—	—
18. v. 20	200	,,	*B. typhosus*	—	0	—	0
27. v. 20	500	,,	,,	—	0	—	200 8000
28. v. 20	1500	,,	4657	—	—	—	—
1. vi. 20	100	,,	*B.* Dow	0	0	—	1000 8000
7. vi. 20	250	,,	,,	100 10,000	0	—	1000 8000
14. vi. 20	500	,,	,,	1000 10,000	0	—	—
21. vi. 20	1000	,,	,,	2000 10,000	0	—	—
28. vi. 20	1250	,,	,,	7500 10,000	0	—	—
5. vii. 20	2000	,,	,,	—	—	—	—
16. vii. 20	50	,,	*Live* 4869	—	—	0	—
23. vii. 20	200	,,	,,	—	—	—	—
6. viii. 20	400	,,	,,	10,000 10,000	0	1 in 100	—
12. viii. 20	400	,,	,,	—	—	1 in 200	—
19. viii. 20	800	,,	,,	—	—	—	—
25. ix. 20	2000	,,	,,	10,000 10,000	0	1 in 400	—
11. x. 20		—		10,000 10,000	0	1 in 800	—

Intramuscular and subcutaneous inoculation. It has been said already that the immunisation of rabbits I.V. with haemolytic urinary and intestinal colon bacilli, and the feeble effect provoked with the non-haemolytic strains, were constant. By the above methods of inoculation, whether live, heat and formol killed vaccines are employed, and whatever dosage is administered, non-haemolytic strains are similarly inactive, while the haemolytic strain may not

be nearly so effective. It is therefore unnecessary to refer in detail to the rabbit experiments with non-haemolytic strains and confine our remarks to the haemolytic cultures of *B. coli*.

The result of very numerous experiments with these haemolytic urinary and faecal strains of *B. coli* (whether vaccines or live cultures, employed in doses varying from 100–2000 million bacilli) has demonstrated that the subcutaneous route for immunisation of animals cannot be relied upon as far as production of agglutinins in the blood of rabbits is the main object. An antiserum with a titre of $\frac{5000}{10{,}000}$ may be produced, but in many instances five and six inoculations with doses varying from 100–2000 million bacilli resulted in a serum with an end point of $\frac{400}{10{,}000}$. If, however, a typhoid vaccine was injected subcutaneously rapid immunisation occurred, or if a haemolytic colon bacillus was subsequently inoculated I.V. a similar active formation of agglutinins occurred, but without effect on the agglutinin content of the serum for the bacillus injected subcutaneously.

In Table XI are illustrated the varying results which followed when subcutaneous immunisation of rabbits was employed with heat killed vaccines followed by live cultures.

Table XI.

	A	B	C
Organism employed	Haemolytic urinary coli (Dow)	Two strains from faeces. X 6; X 9	Two haemolytic urinary coli cultures. (1) Dun; (2) Smith
Duration of experiment	6 weeks	3 months	5 weeks
No. of injections	7	14	8
No. of bacteria injected	5200 million	7700 million X 6 4100 „ X 9	5100 million Dun 1600 „ Smith
Titre of serum	$\frac{5000}{10{,}000}$ Dow	$\frac{200}{10{,}000}$ X 6 $\frac{400}{10{,}000}$ X 9	$\frac{200}{10{,}000}$ Dun $\frac{50}{10{,}000}$ Smith

On the inoculation and feeding of rabbits with live cultures of B. coli. Lepper injected rabbits with living cultures of *B. coli* and produced a transitory bacteraemia with large doses (4000 mills.). Of these rabbits one was killed within 24 hours as it was very ill; inoculated in this manner, the *B. coli* was recovered from 0·01 c.c. of blood at the autopsy. The other two gave positive blood cultures at end of second day, but the blood was sterile by the fourth day.

Doses of 1000 million bacilli were injected into five rabbits, but a proved blood infection was produced in one instance only, while in a further series of experiments with 15 rabbits, kidney lesions due to coliform infections occurred in three.

Many rabbits were injected by us with live cultures of haemolytic urinary and faecal coli and non-haemolytic urinary coli, but without effect, apart from

a local abscess which formed in a few instances at the seat of injection. Two experiments are referred to in detail to emphasise the fact.

Experiments X. Rabbit 5 had just previously received seven injections of a vaccine of a non-haemolytic strain of *B. coli* (4657), isolated from the urine. The injections had been administered I.V. and the last injection was a fortnight before the present experiments commenced. This rabbit had failed to form agglutinins. It now received I.V. injections of the living bacillus (4657) in doses of 50–200 and 400 millions at weekly intervals. One week after the last injection it was killed under chloroform. A complete post-mortem examination was made immediately after death. There was nothing abnormal to the naked eye.

Cultures were made from the heart blood, the urine, muscles, spleen, kidneys and from the bile. All cultures remained sterile. The urine was clear and showed no deposit.

Experiments Y. Rabbit 17 had received during a period of two months six injections I.V. of a living culture of a haemolytic coli isolated from the urinary tract (Smith) and two inoculations subcutaneously of the same organism.

The animal was killed one week after receiving 500 million of the live colon bacillus subcutaneously.

Post-mortem examination was made immediately after death (chloroform anaesthesia). There was slight redness of the tissues at the seat of the last injection of 500 million of living coli one week previously, but no other changes were observed.

Cultures were made from the heart blood, muscle at seat of redness, urine, bile, liver, spleen and kidneys.

All cultures remained sterile.

On the feeding of rabbits. Three rabbits were used for feeding experiments with live cultures of *B. coli* (X 6). Rabbit 1, which had been used for immunisation, with a living culture of X 6, was fed with same haemolytic faecal strain (X 6). The second had been injected in a similar manner with Dow. The third was a normal healthy rabbit on which no experiments had been performed.

The method employed was to saturate sliced up carrots with a thick emulsion of the haemolytic faecal colon bacillus (X 6). A daily feed was given to these three rabbits over a period of 18 days, but in each case the rabbits remained in perfect health. They were killed at the end of the period mentioned, but no lesions were apparent on naked eye examination of any of the viscera at the autopsy.

During the last ten days of these experiments in addition to the feed of bacilli and carrots, 1 c.c. of a 2 per cent. solution of bile salt with 0·5 c.c. of a 10 per cent. solution of calcium chloride was added, with the idea that some irritation of the bowel might be produced and thus enhance any effect of the live culture. This procedure made no difference to the health of the rabbits, who showed no signs of any intestinal disturbance and, as has been said, remained in perfect health throughout. At the post-mortem examinations

cultures were made from the heart's blood, liver, kidney, muscle, bile, and urine from all three rabbits, but in every case the cultures were sterile.

During the course of these experiments the animals were bled every six days, and their sera tested on an X 6 antigen for evidence of agglutination. In one case the serum of the control rabbit showed that agglutinins had been formed as the result of these feeding experiments.

Table XII.

End point agglutination of the rabbits' sera with a standard antigen X 6.

Rabbit	Before feeding	After feeding
X 6 live	$\frac{2000}{10{,}000}$	$\frac{2000}{10{,}000}$
Dow live	$\frac{0}{10{,}000}$	$\frac{0}{10{,}000}$
Control	$\frac{0}{10{,}000}$	$\frac{400}{10{,}000}$

The conclusions derived from these experiments on the immunisation of rabbits with haemolytic coli cultures isolated by us from the urine, faeces and blood, and with non-haemolytic coli from the urine are as follows:

(1) There was considerable difference in the immunising effect induced by haemolytic and non-haemolytic strains of *B. coli*. All our haemolytic strains when injected I.V., isolated from urine and faeces, excited an active formation of agglutinins in the blood of rabbits, while with non-haemolytic urinary strains this did not occur.

(2) Only one non-haemolytic urinary strain provoked agglutinin formation in the rabbit and then relatively feebly as compared with the haemolytic[1].

(3) Agglutinins were readily formed in the blood of rabbits with the haemolytic strains when the injections were made I.V. with heat or formol killed vaccines, or live cultures.

(4) If the injections were made subcutaneously with living haemolytic cultures, or vaccines, agglutinins would be formed, but as a rule much less effectively than by the I.V. route.

(5) No advantage was gained in the immunisation of rabbits with these various strains of haemolytic coli if a minimum time period and a low temperature were employed in the preparation of the heat-killed vaccines.

(6) Injection of typhoid, paratyphoid, haemolytic coli, and staphylococcal vaccines had no effect on the production of non-haemolytic coli agglutinins, if injected with the specific vaccines.

(7) The injection of live coli cultures into rabbits intravenously, intramuscularly or subcutaneously, was not associated with harmful effects apart from abscess formation at the site of injection.

(8) Our feeding experiments with living cultures of a haemolytic *B. coli* isolated from the faeces failed to produce any effect, on clinical or pathological evidence, on the rabbits, except in the formation of agglutinins referred to above.

[1] See footnote 2, page 153.

Section VI.

Bacillus coli Agglutinins.

1. *Methods employed.* Numerous methods were studied for determining the presence of coli agglutinins in a sample of serum, for estimating the titre, and for ascertaining the agglutinability of various strains of urinary and haemolytic faecal coli. Many of the details employed by us are based on the work of Dreyer and his colleagues on typhoid and dysentery agglutinins. We found, after extensive trials, that the most satisfactory temperature for the reaction is 50–55°C. for a period of five hours; the reaction should be read after the Dreyer tubes have stood for one hour at room temperature. Coli "clumps" are small and show less tendency to fall than the typhoid, while there is a strong tendency for a "trace of agglutination" to occur over a wide limit of dilutions. The sharp gradation which is so characteristic in the typhoid-paratyphoid reaction is uncommon. "Zonular agglutination" is not infrequently met with. Strains of *B. coli* isolated by us were tested with the sample of anti-sera in a somewhat similar manner to the true Weil-Felix reaction. Young agar cultures were employed, the growth was emulsified in 2 c.c. of normal saline and then one drop of the thick emulsion was added to tubes containing 1 c.c. of diluted anti-serum; the whole was incubated in a water bath for five hours at 50–55° C. as already stated and the results duly recorded.

2. *Preparation of antigens.* Standardised antigens were employed with some of our haemolytic urinary strains, of which "Dow" and "Dun" were most frequently made use of, a haemolytic strain from the blood "Hyde," and haemolytic strains from the faeces X 6 and X 9, while a non-haemolytic urinary strain most commonly employed was No. 4869. The bacilli were subcultured daily for at least ten days on agar, then planted on agar in Roux flasks or flat whisky bottles, so as to obtain a massive surface growth which was emulsified in normal saline containing 0·1–0·12 per cent. formaldehyde, and stored in the ice safe until proved to be sterile. Dilution of the antigen was made with normal saline (0·85 per cent.) containing 0·25 per cent. phenol, until a strength of 1000 million bacilli per c.c. was obtained. Antigens prepared in this way were generally found to be very satisfactory, but occasionally they had to be discarded as the sensitiveness was insufficient. Thin antigens, owing to the very fine clumps formed, were found unsuitable, but antigens of 1000 millions proved most satisfactory.

We had made various trials with beef broth antigens, but the results were generally less satisfactory than the agar antigens. Still we found from experience that it was necessary when testing strains of *B. coli* grown on agar which were inagglutinable with stock anti-sera, to re-examine the organisms grown in beef broth, and one of our standard antigens was made in beef broth in preference to agar. Experiments with veal broth antigens are in progress.

The antigens were tested with our stock anti-sera when first made, and at subsequent intervals, so as to correct any alterations that might occur, but after a period of six weeks from the time of the preparation a constant "end point" was obtained, as freshly made antigens were generally found to be most sensitive and then to fall to a constant level.

Gardner (1918) had shown from elaborate experiments with cultures of *B. dysenteriae* that a period of two months must be allowed to elapse before the emulsions can be used as standard, because of the action of the formalin.

The readings were taken in the dark against a black background and mostly by one of us (L. S. D.) with the aid of a hand lens which was found to be essential. The results were expressed as (i) "Complete" (clear tube with solid deposit of bacteria); (ii) "Incomplete" (few clumps suspended); (iii) "Marked" (numerous suspended clumps and a deposit); and (iv) "Trace" = end point (no deposit; fine clumps suspended in the fluid) is recorded by giving the end point either of the serum or antigen tested with standard antigen or stock anti-serum over the end point of the stock anti-serum and standard antigen as follows:

$$(1)\quad \frac{600}{10{,}000} = \frac{\text{"Test serum"} + \text{standard antigen}}{\text{Stock anti-serum} + \text{standard antigen}}$$

or

$$(2)\quad \frac{800}{10{,}000} = \frac{\text{"Test antigen"} + \text{stock anti-serum}}{\text{Standard antigen} + \text{stock anti-serum}}.$$

This is the same method as employed by one of us (Dudgeon, 1919) for the records on dysentery agglutinins.

3. *Saturation experiments.* The saturation experiments were carried out on similar lines to those employed by one of us (Dudgeon, 1919) for dysentery bacilli. Massive growths from agar cultures 24 hours old were emulsified in a minimum quantity of normal saline. The "saturation" was attempted by the fractional method; to a measured amount of anti-serum an equal quantity of bacillary suspension was added in fractions $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$ during the 20–24 hours the experiment was in progress, as shown in Table XIII.

Table XIII.

Anti-serum	Bacillary suspension	Time	Temperature
1 c.c.	0·5 c.c.	11 a.m.	52° C.
—	0·25 c.c.	2 p.m.	52° C.
—	0·25 c.c.	4 p.m.	52° C.
—	—	7 p.m.	Ice
		11 a.m.	(Experiment completed)

The final mixture was then centrifugalised at high speed until "cleared from bacilli," when it was ready for testing. Some experiments, however, all but the first stage which took place at 50–55° C. were completed in the ice safe. No obvious difference resulted from this technical alteration.

Experiment 1. *Anti-serum* Dow (haemolytic urinary type).

This serum was tested on the following strains of *B. coli* with the results recorded in Table XIV.

Table XIV.

Organism	Source	Type	Agglutination titre (anti-serum Dow)
1. Dow	Urine	Haemolytic	1 in 10,000
2. Dun	,,	,,	1 in 8000
3. 4657	,,	Non-haemolytic	0
4. 4869	,,	,,	0
5. X 6	Faeces	Haemolytic	0
6. X 9	,,	,,	1 in 400
7. 4442	Urine	,,	1 in 400
8. 4879	,,	,,	1 in 400

The anti-serum Dow was then saturated with the same eight strains of *B. coli* referred to above with the following results recorded in Table XV.

Table XV.

Organism	Strength of emulsion	Treated Dow serum on Dow antigen
1. Dow	80,000 million per c.c	$\frac{400}{10,000}$
2. Dun	40,000 ,,	$\frac{400}{10,000}$
3. 4657	40,000 ,,	$\frac{10,000}{10,000}$
4. 4869	50,000 ,,	$\frac{10,000}{10,000}$
5. X 6	80,000 ,,	$\frac{10,000}{10,000}$
6. X 9	70,000 ,,	$\frac{1000}{10,000}$
7. 4442	40,000 ,,	$\frac{5000}{10,000}$
8. 4879	40,000 ,,	$\frac{5000}{10,000}$

Experiment 2. *Anti-serum* 4869 (non-haemolytic urinary type).

This serum was tested on the following strains of *B. coli* with the results recorded in Table XVI.

Table XVI.

Organism	Source	Type	Agglutination titre (anti-serum 4869)
1. 4869	Urine	Non-haemolytic	1 in 800
2. Dow	,,	Haemolytic	0
3. X 6	Faeces	,,	0

The anti-serum was then saturated with the same three strains of *B. coli* with the following results recorded in Table XVII.

Table XVII.

Organism	Strength of emulsion	Treated 4869 serum on 4869 antigen
1. 4869	50,000 million per c.c.	50 800
2. Dow	80,000 ,,	800 800
3. X 6	80,000 ,,	800 800

Experiment 3. *Anti-serum* X 9 (haemolytic faecal type).

This serum was tested on the following strains of *B. coli*, and the results are recorded in Table XVIII.

Table XVIII.

Organism	Source	Type	Agglutination titre (anti-serum X 9)
1. X 9	Faeces	Haemolytic	1 in 10,000
2. Dow	Urine	,,	1 in 800
3. X 6	Faeces	,,	0
4. 4442	Urine	,,	1 in 1000
5. 4864	,,	,,	1 in 1000
6. 4869	,,	Non-haemolytic	0

The anti-serum X 9 was then saturated with the same six strains of *B. coli* referred to in the above table with the results recorded in Table XIX.

Table XIX.

Organism	Strength of emulsion	Treated X 9 serum on X 9 antigen
1. X 9	70,000 million per c.c.	50 10,000
2. Dow	70,000 ,,	1000 10,000
3. X 6	65,000 ,,	8000 10,000
4. 4442	90,000 ,,	1000 10,000
5. 4864	70,000 ,,	1000 10,000
6. 4869	50,000 ,,	10,000 10,000

Experiment 4. *Anti-serum Smith* (haemolytic urinary strain).

This serum was prepared from a haemolytic urinary strain of *B. coli* (Smith) which did not agglutinate with any of our coli anti-sera. The following strains of *B. coli* were tested with this anti-serum with the results recorded below.

Table XX.

Organism	Source	Type	Agglutination titre (anti-serum Smith)
1. Smith	Urine	Haemolytic	1 in 10,000
2. X 9	Faeces	,,	0
3. X 6	,,	,,	0
4. Dow	Urine	,,	0
5. 4869	,,	Non-haemolytic	0

The anti-serum Smith was then saturated with the same five strains of *B. coli* referred to above with the following results recorded in Table XXI.

Table XXI.

Organism	Strength of emulsion	Treated Smith serum on Smith antigen
1. Smith	50,000 million per c.c.	$\frac{50}{10,000}$
2. X 9	70,000 ,,	8000 / 10,000
3. X 6	80,000 ,,	
4. Dow	80,000 ,,	
5. 4869	50,000 ,,	$\frac{10,000}{10,000}$

The experiments illustrated in Tables XV, XVII, XIX, XXI were selected because they serve to show the effect of "saturating" four types of anti-coli serum: (i) common haemolytic urinary "Dow"; (ii) non-haemolytic urinary "4869"; (iii) haemolytic faecal "X 9" which has *definite* serological affinity with "Dow"; (iv) haemolytic urinary (Smith) which is up to date a solitary strain of haemolytic *B. coli* isolated from urine without serological affinity for any other colon bacillus which we have investigated so far. These tables simply serve to show some of the results which we have obtained in this early stage of our investigation, but in some instances it was found that considerable de-saturation of an anti-serum was effected by a coli strain which had little "affinity" as judged by the agglutination results. Our research on this difficult question is not sufficiently advanced to permit us to enter into the numerous complications surrounding this branch of the subject.

The main conclusions which are warranted from our saturation results up to date are as follows:

(1) The common type of haemolytic urinary colon bacillus which is represented by "Dow" furnishes an anti-serum which is partially or completely de-saturated by the majority of other haemolytic urinary strains.

(2) Non-haemolytic urinary strains do not, as far as our investigations permit us to affirm, de-saturate an anti-coli serum prepared from a haemolytic urinary colon bacillus. The converse is equally true.

(3) We have cultivated haemolytic colon bacilli from the faeces which have definite serological affinity for the standard haemolytic urinary coli anti-serum "Dow" as shown by agglutination and saturation experiments.

CONCLUSIONS.

(1) *Bacillus coli* in infected urine can be divided into two groups: (i) haemolytic; (ii) non-haemolytic.

(2) The haemolytic group is the common type in the infection in men and the non-haemolytic in women.

(3) Rabbits can be readily immunised with the haemolytic strains, but this is not so with the non-haemolytic which we investigated.

(4) Haemolytic *B. coli* occur in the normal faeces of adults and infants, but with increased frequency in cases of diarrhoea and colitis.

(5) A serological relationship exists between some haemolytic urinary and faecal strains of *B. coli*.

(6) All haemolytic urinary strains of *B. coli*, with two exceptions, were agglutinated by an anti-serum prepared from one strain.

(7) Non-haemolytic strains are not agglutinated by one anti-serum as is the case with the haemolytic.

(8) I.V. inoculation in animals with haemolytic strains of *B. coli* leads to the rapid formation of agglutinins; when vaccines or live cultures are injected subcutaneously or intramuscularly in man and animals, feeble response may occur.

(9) The only harmful effect of injecting intravenously, intramuscularly or subcutaneously live cultures of haemolytic or non-haemolytic strains of urinary *B. coli*, or haemolytic faecal strains, has been the formation of *local abscesses*.

(10) Feeding experiments with live cultures of haemolytic colon bacilli failed to produce a morbid process.

(11) The "sugar" reactions have proved of no value in the grouping of colon bacilli.

(12) Saturation of anti-coli sera with specific *B. coli* emulsions and other colon bacillus emulsions has assisted in the grouping of these organisms.

(13) We failed to observe any advantage in "massive doses" of *B. coli* in the immunisation of animals.

(14) In vaccine treatment in the human subject, it is necessary to investigate the organism isolated from the infected urine, and the blood serum of the patient, along the lines indicated by us. The methods for the administration of the vaccine must also be considered.

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