BACTERIOLOGICAL OBSERVATIONS ON COLON BACILLI INFECTING THE URINARY TRACT, WITH SPECIAL REMARKS ON CERTAIN COLON BACILLI OF THE "ANAEROGENES" CLASS.

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Whilst the studies of A. C. Houston and MacConkey have extended our knowledge of the characters of the bacilli found in faeces, and those of Dudgeon and Sargent have done the same with regard to colon bacilli met with in cases of appendicitis, the fermentative activities of pathogenic colon bacilli occurring in urine have not been investigated to any great extent.

In this paper I give the results obtained by the investigation of 50 colon bacilli which were isolated from the urine of patients suffering from such conditions as cystitis and pyelitis and which appeared to be responsible for the patients' illness.

Most of the organisms isolated would be classed in the *B. coli communis* group, but six (of which two were isolated by Dr Mair) differed strikingly from the others in the fact that they produced no gas in media containing glucose. With the characters of these latter bacilli I shall deal in the second part of this paper, in the meantime confining my remarks to the gas-producers.

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Part I.

Character of the Urine.

In most of the cases the urine was turbid, a deposit of pus cells occurring on standing. The reaction was almost invariably acid; in one case it was alkaline although the *B. coli* was the only organism present in it.

Microscopic examination of the deposit showed the presence of pus cells and colon bacilli, the latter being almost invariably extra-cellular.

In most cases the colon bacilli appeared as short rods or threads, but in some cases dense feltworks of long filaments were found. The assumption of the filamentous form by the bacteria was doubtless due to the action of the urea and other salts present, since, as was shown by me in a former paper, the addition of urea to a medium has this effect on the colon bacilli grown on it.

Characters of organisms.

All these organisms had the following characters in common—short Gram-negative non-sporing bacilli with rounded ends—greyish-white layer of growth on agar—gelatine never liquefied. Motility was tested for in hanging drops made from 18 hours old agar and peptone water cultures with the result that only six out of 44 were found to exhibit movement of transposition; many exhibited active Brownian movement. This absence of motility is rather remarkable and perhaps if special methods had been adopted a larger percentage of positive results would have been obtained. We may remark in passing that Dudgeon and Sargent found motility present in only 19 out of 74 colon bacilli isolated from cases of peritonitis.

Fermentative reactions produced by 44 gas-producing urinary bacilli.

The action of the bacilli on the following substances was investigated—glucose, lactose, saccharose, dulcite and mannite. One per cent. of these substances was added to peptone water tinted with litmus solution, and the medium distributed into Durham's fermentation tubes and sterilised in the steamer for ten minutes on each of two days, great care being taken not to overheat the medium.

It is evident from Table I that according to their action on these substances these organisms may be divided into seven groups.

TABLE I.

No. of organisms	Glucose	Lactose	Saccharose	Dulcite	Mannite
14	+	+	+	+	+
19	+	+	–	+	+
4	+	+	+	–	+
2	+	+	–	–	+
2	+	–	+	+	+
2	+	–	–	+	+
1	+	–	–	–	+

In this Table + indicates formation of acid and gas.

„ „ – „ no change in medium.

It will be observed that glucose and mannite were fermented by all of these organisms, that 39 of them fermented lactose, that 37 of them fermented dulcite and 20 of them fermented saccharose.

MacConkey classifies lactose fermenting colon organisms into four groups according to their action on saccharose and dulcite. Of the above organisms we have 39 lactose fermenters and the action of these on saccharose and dulcite is seen in the following table.

TABLE II.

MacConkey's group	Organisms belonging to this group according to MacConkey	Saccharose	Dulcite	No. of urinary bacilli belonging to each group
Group I.	*B. acidi lactici* (Hüppe)	–	–	2
Group II.	*B. coli communis* (Escherich)	–	+	19
Group III.	*B. neapolitanus*			
	B. pneumoniae (Friedländer)	+	+	14
	B. coli communior (Durham)			
Group IV.	*B. lactis aerogenes*	+	–	4
	B. cloacae, etc.			

With regard to certain other characters it may be mentioned that 38 out of the 44 produced fluorescence in neutral red glucose agar shake cultures, 30 caused clotting in litmus milk, 39 produced much indol, three a trace, and two no indol.

On the Drigalski-Conradi medium the growth of some of the organisms had a peculiar jelly-like appearance, especially well marked at the edge of the line of growth.

Route by which the colon bacillus enters the urinary tract.

With regard to the manner in which the colon bacillus reaches the kidneys and bladder we are unable to dogmatise, but we favour the view that it is absorbed from the intestine through the blood capillaries or lymphatics.

The fact that cases of "coli" cystitis are commoner in women than in men has been held to support the view that the colon bacilli reach the urinary system from without, the shorter urethra in the female facilitating their entrance. But the explanation may be that women suffer more from constipation than men and that therefore there is the greater liability of the colon bacilli being absorbed from the intestine. Notes of a case in which the bacilli seem to have entered from the intestine as the result of constipation and to have been excreted by the kidney without any urinary symptoms arising, may be cited here.

A woman aet. 22 was admitted into one of Professor Lindsay's Wards, Royal Victoria Hospital, Belfast, with a history of malaise of over a week's duration. Her temperature was 103·5, pulse 114; she had also the flushed face, severe frontal headache and distended abdomen characteristic of typhoid fever. Her blood gave a negative Widal reaction with the *Bacillus typhosus*. It was found that she had been completely constipated for a fortnight. On the day after admission as the result of an enema her temperature dropped two degrees. On the following day, after a second enema, her temperature became normal and continued normal. The interesting feature of this case was that during the time the girl was in Hospital, she was excreting large numbers of *B. coli communis* in her urine although she had no symptoms of kidney or bladder trouble. The spleen was palpable and on a certain day she had a dozen typical typhoid spots on the abdomen. Her blood continued to be negative to the *Bacillus typhosus* and it had no agglutinative action on the colon bacillus isolated from her urine.

This was probably a case of "faecal fever" a condition for the occurrence of which Nothnagel cites reputable authorities.

It is possible that in many individuals colon bacilli are absorbed from the intestines and excreted in the urine and that it is only in predisposed individuals that pyelitis or cystitis results.

Dudgeon found that two out of a total of 20 habitually constipated persons examined, had colon bacilli in their urine.

Agglutination of colon bacilli.

The blood serum of a small number of the cases was investigated with regard to its agglutinative action on the bacilli isolated from their urine. In most cases the result was negative even in as low dilutions as 1 in 14. In one case there was agglutination in a dilution of 1 in 50, but it was found that four out of five normal bloods also clumped this bacillus in a dilution of 1 in 50.

Further experiments convinced me that in ordinary cases of colon cystitis there is little or no increase of agglutinins in the blood of the patients. "Clumping" occurring in comparatively high dilutions is more often due to the easily agglutinable nature of the particular strain of colon bacillus used than to any absolute increase of agglutinins in patient's blood.

PART II.

Bacilli of the "anaerogenes" class.

Of the six bacilli which formed no gas (anaerogenic) in glucose media one differed from the others in being utterly devoid of fermentative action on carbohydrates and in causing liquefaction of gelatine. It was associated with a non-lactose fermenting colon bacillus. That it was probably infecting the patient seemed to be indicated by the fact that the patient's blood in 1 in 50 and in 1 in 100 dilutions agglutinated it in one hour whilst normal blood under the same conditions had no effect on it. Moreover it was isolated on two occasions, an interval of two months elapsing between the examinations. The remaining five are members of the same group, but before considering their characters in detail we will preface our remarks with a short account of our knowledge of this class of micro-organisms.

Lembke in the faeces of a dog found a colon-like bacillus which was non-motile, produced indol, acidified and clotted milk but produced only acid in glucose and lactose bouillon. To this organism he gave the name *Bacillus coli anaerogenes.*

Mair isolated two organisms of this class from cases of cystitis and described the cultural characters of one of them. I have obtained cultures from Mair of his bacilli and have compared them with the three isolated by myself.

Dudgeon found a pathogenic organism of this class in an enlarged prostate gland. He gave a careful description of his bacillus and alluded to the fact that A. C. Houston had described somewhat similar organisms.

Cathcart described such bacilli among the flora of "blown" tins of preserved food.

Castellani in four cases of continued fever occurring in Ceylon found the causative organisms to belong to this class.

It would be perhaps well to give some such distinctive name as the *B. coli anaerogenes* to this group of micro-organisms. The term "coli"

would indicate not only their relationship to the *B. coli communis* but also the habitat of some at least of them, whilst the term anaerogenes would serve to distinguish them from the intermediate typhoid-colon gas-producing group to which such organisms as the paratyphoid bacilli, Gaertner's bacillus and the Aertryck bacillus, belong.

Characters of the Belfast anaerogenic bacilli.

The Belfast anaerogenic bacilli had the following characters in common:—Gram-negative, non-sporing bacilli—greyish-white growth on agar—uniform turbidity in broth—no liquefaction of gelatine—abundant red growth on the Drigalski-Conradi medium. The fermentative action of the Belfast bacilli was investigated and the result is given in Table III along with certain other characters of the organisms. For contrast we give the corresponding properties possessed by the *B. typhosus.*

We may observe that the fermentative changes produced were very constant.

An analysis of Table III shows:

(1) that two of the cultures were feebly motile, the other three being non-motile,

(2) that all five produced acid but no gas from glucose, laevulose, lactose, arabinose and rhamnose,

(3) that none of them had any action on inulin, erythrite and adonite,

(4) that all five fermented mannite but that *Belfastiensis I* differed from the others in producing not only acid but gas also,

(5) that they differed from each other in their respective capacities for fermenting such substances as maltose, saccharose, glycol, glycerine, dulcit, salicin, sorbit and dextrin,

(6) that no two of the five were identical in their fermentative characters,

(7) that four of the five produced much indol, the fifth being negative in this respect,

(8) that all five produced permanent acidity in litmus milk: three of them also caused clotting,

(9) that none of them produced gas or fluorescence in glucose neutral red agar shake cultures,

(10) that in their growth on potato and gelatine some possessed colon-like, others typhoidal characters.

TABLE III.

Name organi	Motility	Glucose	Laevulose	Maltose	Lactose	Saccharose	Dulcit	Mannite	Glycol	Glycerine	Erythrite	Adonite	Arabinose	Raffinose	Salicin	Sorbit	Rhamnose	Dextrin	Inulin	Litmus milk: Acid	Litmus milk: Clot	Indol	Glucose neutral red agar shake cultures	Potato		ce colonies on atine plates
Belfastien (Mair)	–	Ac	Ac	Ac	Ac	–	–	AcG	–	–	–	–	Ac	–	Ac	AcG	Ac	Ac	–	+	+	+	no gas, no fluorescence	brown growth	T	rent "vine-leaf"
Belfastien (Mair)	+	Ac	Ac	Ac	Ac	Ac	–	Ac	–	Ac	–	–	Ac	Ac	–	Ac	Ac	–	–	+	–	+	,,	moist invisible growth	B	h colonies, short ning branches wth
Belfastien (Wilsor	–	Ac	Ac	Ac	Ac	–	Ac	Ac	Ac	Ac	–	–	Ac	–	–	Ac	Ac	Ac	–	+	+	+	,,	brown growth	F	brownish circu- onies, even edge
Belfastien (Wilsor	–	Ac	Ac	–	Ac	–	–	Ac	–	Ac	–	–	Ac	–	Ac	Ac	Ac	–	–	+	–	+	,,	moist invisible growth	B	h colonies, short ning branches wth
Belfastien	+	Ac	Ac	Ac	Ac	Ac	–	Ac	–	–	–	–	Ac	Ac	Ac	–	Ac	Ac	–	+	+	–	,,	brown growth	B	h colonies, y crenated
B. typhos	+	Ac	Ac	Ac	–	–	–	Ac	–	...	–	–	–	–	–	Ac	–	Ac	–	+	–	–	,,	moist invisible growth	T	cent "vine-leaf" es

In this table Ac indicates production of acid.

Ac G ,, ,, ,, and gas.

– ,, a negative result.

TABLE IV.

Name of organism	Motility	Bouillon	Glucose	Maltose	Saccharose	Lactose	Raffinose	Arabinose	Mannite	Dulcit	Salicin	Dextrin	Sorbit	Litmus milk	Indol	Neutral red shake cultures
B. coli communis	+	G T	Ac G	Ac G	–	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac C	+	fluorescence
B. enteritidis (Gaertner)	+	G T	Ac G	Ac G	–	–	–	Ac G	Ac G	Ac G	–	Ac G	Ac G	Ac then alk.	–	,,
B. Paratyphosus B.	+	G T	Ac G	Ac G	–	–	–	Ac G	Ac G	Ac G	–	Ac G	Ac G	Ac then alk.	–	,,
B. Paratyphosus A.	+	G T	Ac G	Ac G	–	–	–	Ac G	Ac G	Ac G	–	Ac G	Ac G	Ac	–	,,
B. Belfastiensis III.	–	G T	Ac	Ac	–	Ac	–	Ac	Ac	Ac	–	Ac	Ac	Ac C	+	no fluorescence

TABLE V.

Name of organism	Motility	Bouillon	Glucose	Mannite	Lactose	Saccharose	Dulcite	Litmus milk	Indol
B. pyogenes foetidus	–	G T	Ac	Ac	Ac	Ac	Ac	Ac C	+
B. ceylanensis B. (Castellani)	–	G T P	Ac	Ac	Ac	Ac	Ac	Ac C	+
Cathcart's bacilli, *g*	...	...	Ac	Ac	Ac	Ac	–	Ac	+
f	...	...	Ac	Ac	–	Ac	–	Ac C	trace
b	...	...	Ac	Ac	Ac	Ac	–	Ac C	trace
c	...	...	Ac	–	Ac	–	–	Ac C	trace
VI.	...	...	Ac	Ac	–	–	–	Ac	+
VIII.	...	...	Ac	Ac G	–	–	–	Ac C	+
Dudgeon's bacillus	+	G T	Ac	Ac	Ac	–	–	Ac	+
Belfastiensis I.	–	G T	Ac	Ac G	Ac	–	–	Ac C	+
II.	+	G T	Ac	Ac	Ac	Ac	–	Ac	+
III.	–	G T	Ac	Ac	Ac	–	Ac	Ac C	+
IV.	–	G T	Ac	Ac	Ac	–	–	Ac	+
V.	+	G T	Ac	Ac	Ac	Ac	–	Ac C	–
B. ceylanensis A. (Castellani)	–	G T P	Ac	–	–	–	–	Ac C	–
B. typhosus	+	G T	Ac	Ac	–	–	–	Ac	–
B. dysenteriae (Flexner)	–	G T	Ac	Ac	–	–	–	Ac then alkaline	–
B. dysenteriae (Shiga)	–	G T	Ac	–	–	–	–	Ac then slightly alkaline	–

In these tables + indicates a positive reaction.
– indicates a negative reaction.
Ac indicates production of acid.
Ac G indicates production of acid and gas.
Ac C indicates production of acid and clot.
G T indicates general turbidity
G T P indicates general turbidity and formation of a pellicle.

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In Table IV we have compared the fermentative changes produced by certain well-known intestinal organisms with those produced by *Bacillus Belfastiensis III.*

And in Table V from the data at our disposal we have contrasted the Belfast organisms with those of Castellani, Dudgeon, and Cathcart as well as with the *B. pyogenes foetidus*, the *B. dysenteriae* and the *B. typhosus.*

A glance at Table V shows the great amount of variation there is amongst micro-organisms of this class.

Belfastiensis I (Mair) and Cathcart's bacillus VIII resemble each other in forming acid and gas in mannite broth.

One notes a progressive decrease in fermentative activity in passing down the Table, the *B. pyogenes foetidus* and *B. ceylanensis B.* fermenting all the sugars and alcohols in the list, whilst the *B. dysenteriae* of Shiga is able to ferment glucose only.

Dudgeon's bacillus and *B. Belfastiensis IV* give identical fermentative reactions with the substances mentioned in the Table.

B. Belfastiensis IV, *B. Belfastiensis V* and Cathcart's bacillus *b* differ from each other in regard to indol formation and clotting of milk but in other respects resemble each other.

We thus see that these "anaerogenic" colon bacilli have characters in which they resemble the *B. coli communis*; such are the feeble motility, the fermentation of lactose, the production of indol, the formation of acid and clot in litmus milk, the brown growth on potato. On the other hand in their absence of gas forming powers they resemble the *Bacillus typhosus* and the *B. dysenteriae.*

Belfastiensis I in producing gas from mannite and sorbit forms a connecting link between the gas-producing (aerogenic) and the non gas-producing (anaerogenic) bacilli.

The patients from whom these anaerogenic bacilli were isolated presented on four occasions the signs and symptoms of cystitis, in the fifth case those of pyelitis.

In three of the cases the bacilli were isolated on two occasions, an interval of two months elapsing between the examinations.

In all cases the bacilli were obtained in practically pure culture.

In conclusion I desire to acknowledge my indebtedness to Professor Symmers for affording me every facility for carrying out this investigation in his Laboratory, and also to those medical gentlemen who supplied me with the necessary material.

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