ON THE OCCURRENCE OF DYSENTERY-LIKE ORGANISMS IN THE URINARY TRACT OF MAN IN MAURITIUS

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Not infrequently, organisms culturally resembling dysentery bacilli have been found associated with pyrexial reactions suggesting typhoid or paratyphoid fevers, or in cases of pyelitis, cystitis, colitis and even acute dysentery.

Prominent amongst them, are the metadysentery bacilli of Castellani which, in a recent classification of the dysentery organisms, he (1927) describes as: Organisms, which as is the case with the true dysentery bacilli, do not produce gas in any sugar, but either ferment lactose (acid only) and clot milk or ferment lactose (acid only) without clotting milk, or clot milk without fermenting lactose (organisms of the genus *Dysenteroides* and *Lankoides*).

Nabarro, quoted by Topley & Wilson (1931) "finds certain affinities between Castellani's organisms and the other lactose-fermenting types of dysentery bacilli with the organisms which have from time to time been described as *B. coli anaerogenes*". This view is shared by Bamforth (1934) and Mackie & McCartney (1934).

Topley & Wilson themselves (1931) find also possible affinities between the organisms referred to above and the atypical *B. coli* strains of Dudgeon. This group of bacilli, isolated by Dudgeon and his colleague (1924-7) from the human urinary tract, in cases of enteric-like fevers, cystitis and pyelitis, including three fatal cases, is characterized by its slow action on lactose, which is fermented in many cases without gas formation, and its marked haemolytic power for human red blood corpuscles.

Referring to the pathogenicity of the slow lactose-fermenting bacilli, Topley & Wilson (1931) state that "they appear to occupy a position intermediate between the lactose fermenters and the non-lactose-fermenting bacilli which make up the typhoid, paratyphoid and dysentery groups. They may be associated with acute urinary infections or with infection of the enteric or dysenteric type."

No doubt, such febrile illness of unknown origin is not uncommon in Mauritius; the best known is usually referred to as "Fièvre letchee", the majority of cases coinciding with the ripening of the fruits of *Letchie sinensis*, which occurs by midsummer.

This paper deals with eleven strains of dysentery-like organisms recovered from the urinary tract in cases of enteric-like fevers, pyelitis and cystitis, with the record of a fatal case.

Observation No. 1. R. A. B., a man of about 38 years, had a feeling of uneasiness in the kidneys, with occasional acute pains occurring at irregular intervals, on an average every 2 months and lasting for 3–4 days. This acute stage of the affection was accompanied by a slight rise of temperature (maximum 37·5° C.) and a mild diarrhoea. His urine was highly coloured, but albumen free; a few pus cells and red blood corpuscles, both in clumps, with scanty hyaline, granular and leucocyte casts with much mucus, composed the sediment after spinning. A pure growth of a non-motile organism was obtained on Endo's medium, the biochemical characters of which are detailed in Table I. This organism was not agglutinated by *Bact. shigae*, *flexneri*, *newcastle*, *sonne* and *typhosum* standard agglutinating sera, but was agglutinated by the patient's own blood serum in a dilution of 1 in 125.

Observation No. 2. P. R., a man of 30 years, suffered from a typhoid-like fever which lasted for about 3 weeks; his temperature oscillated between 38° C. in the morning and 39·5° C. in the evening. A blood culture was negative. No agglutination to significant titre was obtained with his serum tested against the standard *Bact. typhosum* "O" emulsion, whilst a titre of 1 in 250 (R.T.=31) was reached with the "H" emulsion. This patient had some 12 years ago been inoculated with a typhoid prophylactic vaccine, followed since by Besredka's oral bilivaccine. His urine yielded an organism differing but only slightly from that of R. A. B. Again this organism was inagglutinable by any of the standard enteric or dysenteric sera, but was agglutinated by his own serum in a dilution of 1 in 50. His faeces seeded on Endo yielded none of these organisms.

Observation No. 3. N. W., a woman of 60 years, had some 3–4 years ago aching pains in the loins, resembling those of R. A. B.; her urinary picture also agreed with that of the latter. Her urine yielded an organism identical to that of P. R.; it was agglutinated by her own blood serum in a dilution of 1 in 50. All pains subsided after vaccine treatment. Recently her serum was tested against R. A. B. organisms and an agglutination titre of 1 in 250 obtained, but her urine was found to contain none of these bacilli. More recently her pains recurred, accompanied by a slight rise of temperature and 5–6 loose motions daily. A new culture yielded organisms identical to those previously found. Her faeces were free from these bacilli.

Observation No. 4. K. B., a man of 21 years. A sample of his urine was received at the laboratory for chemical and microscopical examinations; no clinical data accompanied the specimen. His urinary picture being found similar to that of the above-mentioned cases, a bacteriological examination was tried and a more or less similar organism obtained. The medical practitioner was informed accordingly, and the following clinical notes received: "In the past 2 years, there have been slight pains in both loins, radiating towards the abdomen, colicky in nature. No diarrhoea, no fever. Pains come on for a few days at a time, at irregular intervals, on the average every 6 weeks or 2 months. General condition good, but suffered from mild anxiety neurosis." A new sample of his urine aseptically taken, was cultured and the same organisms obtained; they were agglutinated by patient's serum in a dilution of 1 in 250.

Observation No. 5. A. D., a male adult, presented symptoms of cystitis; his urine showed large amount of pus, scanty red blood corpuscles and numerous bacilli culturally identical to those of R. A. B.; his blood was not available for agglutination tests.

Observation No. 6. N. G., a girl 17 years, had a hyperpyrexia of unknown origin, for about 1 month, which suggested paratyphoid fever. Her blood serum did not agglutinate either the T.A.B.C. or enteridis emulsions to significant titre, and a bacteriological examination of her blood was negative. The same type of organism was recovered from her urine; it was agglutinated, by her own serum, in a dilution of 1 in 250, but was inagglutinable by any of the standard specific sera, including the *typhosum* and *paratyphosum*.

Observation No. 7. C., a child aged 2 years, a fatal case from which this kind of bacterium was recovered. The patient had a severe febrile attack which lasted for a fortnight and was supposed to be due to dental trouble. Pus was seen in her urine together with a few red

blood corpuscles and granular casts. An actively motile organism, biochemically identical to that of P. R., grew from her urine.

Observation No. 8. J. E., a child aged 2 years, had a cystitis. Pus and red blood corpuscles were present in her urine. Bacilli differing slightly from those of K. B. grew on Endo. Cultured on three occasions, this same type of organism was obtained.

Observation No. 9. Mrs R. A. B., wife of patient No. 1, presented symptoms of kidney trouble, resembling those of her husband. The same type of organism was obtained from her urine which was free of blood or pus; the bacterium was not agglutinated by her own serum.

Observation No. 10. Ph. P., a man aged 24 years, suffered from kidney trouble of the irregular type. His urine which was full of pus when the pains were acute, yielded P. R. type of organisms. His serum gave an agglutination titre of 1 in 25.

Observation No. 11. H. M., a man of about 28 years, suffered from continued hyperpyrexia of over 6 weeks' duration; he had a slight swelling of the right wrist, which led to a possible diagnosis of rheumatic fever. Blood cultures and agglutination tests with the enteric emulsions were completely negative. His urine showed some pus cells, red blood corpuscles and hyaline, granular and leucocyte casts; all of which increased appreciably as the illness progressed. The organisms of P. R. type scanty at the onset were swarming at the close of the disease. It is interesting to note that this patient had lost two elderly members of his family about 1 month previous to his illness, at an interval of roughly 1 month with acute febrile symptoms thought to be of malarial origin. He looked after his uncle, until the latter's death; 2 or 3 days later his temperature was found abnormally high, when he took to bed.

BIOCHEMICAL REACTIONS

The sugar media were prepared according to McCartney's technique: 1 per cent peptone water containing 0.5 per cent sodium chloride and 1 per cent Andrade's indicator is sterilized for 30 min. under 5 lb. pressure; when cool, 1 per cent of the appropriate sugar is added—the sugars are stocked in 10 per cent aqueous solution, after previous sterilization by filtration through candle. Table I shows the biochemical activities of the organisms recovered from the urinary tract of the above-mentioned patients. No sugar was accepted as non-fermented unless no acidity was formed after 3 weeks' incubation at 37° C.

Table I

Observation No.	Name	Glucose	Lactose	Mannite	Saccharose	Maltose	Xylose	Arabinose	Laevulose	Dulcite	Litmus milk	Indol	Motility
1	R. A. B.	A	-/A	-	A	-	A	A	A	-	AC	-	-
2	P. R.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-
3	N. W.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-
4	K. B.	A	-/A	-	A	-	A	A	A	-	A/alk	-	-
		A	-	A	A	-	A	A	A	-	A/alk	-	-
5	A. D.	A	-/A	-	A	-	A	A	A	-	AC	-	-
6	N. G.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-
		A	-/A	A	-	-	A	A	A	-	A/alk	-	-
7	Baby C.	A	-	-	-	-	A	A	A	-	A/alk	-	+
8	J. E.	A	-	At	-/A	-	A	A	A	-	A/alk	-	-
9	Mrs R. A. B.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-
10	Ph. P.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-
11	H. M.	A	-/A	-	-	-	A	A	A	-	A/alk	-	-

A = acidity; At = transient acidity; AC = acid and clot; alk = alkaline; + = positive; - = negative.

AGGLUTINATION TESTS

Dreyer's macroscopical technique was adopted; the mixtures were incubated at 52–55° C. overnight and the results recorded after standing for about 15 min. at room temperature (average 25° C.).

Patients' serum. Results obtained are recorded in Table II. Except in one case, all the sera tested had agglutinins in dilutions varying from 1 in 25 to 1 in 250 for the homologous organism and in most cases for R. A. B. (♂) organisms as well. It will be noted that some sera agglutinated R. A. B. organisms to a titre even higher than when the homologous organism was used; this is better shown by Mrs R. A. B.'s serum which did not agglutinate her own organisms but agglutinated her husband's to exactly the same titre as was reached when his serum was used.

Table II

Patient's serum	Agglutination titre; patient's own bacilli	Agglutination titre; R. A. B. bacilli
R. A. B. (♂)	1 in 125	1 in 125
P. R.	1 in 50	1 in 50
N. W.	1 in 50	1 in 250
K. B.	1 in 250	1 in 50
R. A. B. (♀)	Nil	1 in 125
N. G.	1 in 250	Nil
Ph. P.	1 in 25	1 in 25

Immune rabbit serum. Three to four intravenous inoculations, made at weekly intervals, were usually sufficient to bring the agglutinating power of the rabbit serum to a high titre. The results obtained are recorded in Table III.

Table III

Bact. emulsion	R. A. B. rabbit serum	K. B. rabbit serum
R. A. B. (♂)	1 in 5,000	1 in 125
P. R.	1 in 5,000	1 in 125
N. W.	1 in 12,500	1 in 125
K. B.	Nil	1 in 25,000
A. D.	Nil	1 in 12,500
N. G.	1 in 250	1 in 25
Baby C.	Nil	1 in 25,000
J. E.	1 in 250	1 in 25
R. A. B. (♀)	1 in 5,000	1 in 25
Ph. P.	1 in 125	Nil
H. M.	Nil	—

ABSORPTION TEST

To 1 c.c. of a 1 in 5 dilution of the immune serum was added 1 c.c. of a very thick emulsion of a 24 hours old culture, on three agar plates, of our bacilli suspended in 0.75 per cent saline; the mixture was incubated overnight at 37° C., then centrifuged at high speed; the supernatant fluid was finally tested for its agglutinating properties against the organism under study and the homologous one. Results arrived at are recorded in Table IV.

Table IV

Immune sera	Emulsion used for saturation	Antigen used for agglutination test	Agglutination titre*
R. A. B.	R. A. B. (♂)	R. A. B. (♂)	0/ 5,000
"	"	P. R.	0/ 5,000
"	"	N. W.	500/12,500
"	"	R. A. B. (♀)	0/ 5,000
"	"	Ph. P.	0/ 125
"	P. R.	R. A. B. (♂)	250/ 5,000
"	"	P. R.	250/ 5,000
"	N. W.	R. A. B. (♂)	0/ 5,000
"	"	N. W.	250/12,500
"	R. A. B. (♀)	R. A. B. (♂)	0/ 5,000
"	"	R. A. B. (♀)	0/ 5,000
"	Ph. P.	R. A. B. (♂)	125/ 5,000
"	"	Ph. P.	0/ 125
K. B.	K. B.	K. B.	500/25,000
"	"	A. D.	0/12,500
"	"	Baby C.	0/ 2,500
"	A. D.	K. B.	1,250/25,000
"	"	A. D.	250/12,500

* Results expressed as a fraction; numerator represents titre after absorption and denominator titre before absorption.

HAEMOLYSIS

Following the remarkable work of Dudgeon and his co-workers (1924-7) on the haemolytic *B. coli* of the urinary tract, an attempt was made to find whether these organisms possessed similar dissolving action on red blood corpuscles.

The technique adopted was that described by Dudgeon & Pulvertaft (1927). Table V shows the amount of haemolysis obtained with the local urinary organisms, in Dudgeon's blood medium, after 24 hours' incubation at 37° C.

Table V*

Organisms tested	Medium + 0.5 % sodium chloride	Medium + 0.85 % sodium chloride
R. A. B. (♂)	I.C.	I.C.
P. R.	I.C.	I.C.
N. W.	I.C.	I.C.
K. B.	C.	I.C.
A. D.	C.	C.
N. G.	I.C.	I.C.
Baby C.	I.C.	C.
J. E.	I.C.	T.
R. A. B. (♀)	I.C.	0
Ph. P.	I.C.	I.C.
H. M.	I.C.	I.C.

* C.=complete haemolysis; I.C.=incomplete haemolysis; T.=trace of haemolysis; 0=no haemolysis.

ANIMAL EXPERIMENTS

White mice (a). Inoculated intraperitoneally, an emulsion of these organisms was invariably fatal to these small animals. Suspensions containing from 2.5 to 10×10^9 organisms, grown for 24 hours on plain agar, and emulsified in 0.75 per cent saline were used. Generally 2 hours after the injection the

mouse was found crouched in a corner of its cage, with bent head, closed eyes and hairs upright; a marked and progressive dyspnoea set in, which lasted till death. Autopsy showed a marked subcutaneous haemorrhage, with congested and slightly haemorrhagic intestines. In some cases the lungs too, were highly coloured.

White mice (b). Inoculated subcutaneously, these bacilli proved less virulent; each mouse was inoculated with $5-20 \times 10^9$ organisms injected into a hind leg. Usually after a few days, a small ulcer developed at the site of the inoculation, which widened gradually to reach a diameter of roughly 1 cm.; this was in most cases followed by a big abscess which did not in any case burst, but receded gradually, so that after 2-3 weeks no outward trace of the infection was visible. None of these animals died. Killed within the first weeks, a marked congestion of the intestines was seen, the contents of which seeded on Endo, yielded organisms identical with those inoculated. During the first week after inoculation, positive urine cultures were obtained, and a transitory bacteraemia occurred.

Guinea-pigs are also susceptible to infection with these organisms either by the peritoneal or subcutaneous route. The clinical picture and autopsy appearances were more or less similar in guinea-pigs and mice.

Rabbits. Suspensions containing $20-40 \times 10^9$ organisms when injected either intravenously or intraperitoneally produced no apparent ill-effects on rabbits.

THE TOXIN

The rapidity with which our small laboratory animals died after intraperitoneal inoculations of these organisms suggested a study of the nature of the toxin they produce. With this object, a plain broth culture 1 week old was employed, 2 c.c. of the filtrate thereof being injected intraperitoneally into a mouse (A); a second mouse (B) received 0.25 c.c. of an emulsion in 0.75 per cent saline of the bacterial cells obtained, by spinning at high speed, 2 c.c. of the same broth culture. Mouse (A) remained unaffected whilst mouse (B) died within 8 hours of the injection.

Therefore (A) the bacilli do not appear to give off a soluble toxin and (B) by their rapid and massive destruction in the peritoneal cavity, set free an endotoxin which is lethal to small animals.

DISCUSSION

The foregoing account deals with eleven strains of a bacillus, culturally identical, but serologically different from the dysentery group of organisms. The strains were recovered from the urinary tract of man in certain pathological conditions. The strains differ amongst themselves in some minor biochemical characters; serologically they differ more, but two main groups stand out.

On the whole the strains agree with Castellani's recent definition of the metadysentery bacilli which are regarded by several workers as synonymous

with *B. coli anaerogenes* Lembke (1896) and possibly Dudgeon's slow lactose-fermenting bacilli of the urinary tract.

This view is rendered more apparent by the following characters common to both Dudgeon's organisms and the local ones: (1) a marked lytic action on red blood corpuscles, (2) their absence from the blood stream and the intestinal contents, (3) their rapid disappearance from urine samples once the clinical symptoms subside, and (4) the similarity of the affections in which they have been met.

All the human sera tested, except one, contained agglutinins for the homologous organism sometimes to a relatively high titre; if, concurrently with the results of these agglutination tests, we consider (*a*) the purulent effusion in the human urinary tract, (*b*) the high toxicity of the bacilli for small laboratory animals when injected intraperitoneally, and (*c*) the ease with which they are recovered from the intestinal contents of these same animals long after subcutaneous inoculation, we feel justified in regarding these bacilli as the causal agents of the pathological conditions described in our case histories.

It should be remembered that Dudgeon & Pulvertaft (1927) attribute the acute febrile illness produced in man by the slow lactose-fermenting bacilli, to an inflammation of the urinary passages causing partial or complete obstruction to the outflow of urine; they hold that in all cases of clinical enteric-like fevers and septicaemic affections in women, following confinement, and not confirmed by laboratory methods, a bacteriological examination of the urine should be resorted to. Our results, in this respect, fully agree with the views expressed by these workers.

SUMMARY

1. Organisms biochemically resembling the metadysentery bacilli of Castellani (1927) or sensu largo *B. coli anaerogenes* of Lembke (1896) have been recovered locally from the urinary passages of man.

2. These organisms were found in cases of enteric-like fevers, pyelitis and cystitis.

3. Similar to Dudgeon's slow lactose-fermenting bacilli of the urinary tract, they have been shown to possess marked haemolytic action for red blood corpuscles.

4. Serologically, they appear to fall into at least two groups of bacilli.

5. They are believed to be pathogenic.

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REFERENCES

- BAMFORTH, J. (1934). An enquiry into the coli anaerogenes bacteria. *J. Hygiene*, **34**, 69–80.
- CASTELLANI, A. (1927). The classification of bacterial dysenteries and of dysentery bacilli. *Amer. J. Trop. Med.* **7**, 199–216.
- DUDGEON, L. S. (1924). Acute infection of the urinary tract due to a special group of haemolytic bacilli. *J. Hygiene*, **22**, 348–54.
- DUDGEON, L. S. & PULVERTAFT, R. J. V. (1927). On slow lactose-fermenting *B. coli* in urinary and intestinal infections. *Ibid.* **26**, 285–304.
- MACKIE, T. J. & MCCARTNEY, J. E. (1934). *An Introduction to Practical Bacteriology*, 4th ed., London.
- TOPLEY, W. W. C. & WILSON, G. S. (1931). *The Principles of Bacteriology and Immunity*, 2nd imp., London.

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