

ACUTE INFECTION OF THE URINARY TRACT DUE TO A SPECIAL GROUP OF HAEMOLYTIC BACILLI.

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(With 2 Charts.)

IN a paper on *Bacillus coli* infections of the urinary tract, Dudgeon, Wordley and Bawtree (1922)¹ refer to two cases of acute pyelo-cystitis, one of which terminated fatally, due to slow lactose fermenting bacilli which were strongly haemolytic. The diagnosis of paratyphoid fever was considered probable on clinical evidence, chiefly because of the severity of the illness and the prolonged pyrexia which was infinitely longer than occurs in typical cases of acute coli fever. An organism was isolated from each case with identical cultural, serological, and haemolytic properties. On cultural evidence both strains were distinct from true colon bacilli, as they formed blue colonies on litmus-lactose-agar which were similar in appearance to typhoid-paratyphoid colonies, and slowly fermented lactose-broth.

Since this paper was published, I have met with 49 cases of infection of the urinary tract due to this group of bacilli, all of which have run an acute course, in some instances with prolonged fever, and the clinical diagnosis of "enterica" was made in some of the cases. Owing to the appearance of the colonies on litmus-lactose-agar the clinical diagnosis may receive support at the outset, but detailed investigation of the organisms renders the bacteriological diagnosis relatively simple. Some of these cases have occurred in women recently confined, and the same organism has been recovered from the urine and lochia. The urinary symptoms may be masked by the severity of the general reaction which as already stated has led to the clinical diagnosis of "enterica." Two temperature charts are figured to illustrate the types of fever which may occur.

Chart I is from a medical man whose illness ran an acute course for three weeks, while convalescence lasted for about one month. The chief symptoms were pain on passing water, pain in the lumbar region with tenderness on both sides on deep pressure, and severe malaise. There was no doubt that the malaise and general weakness were infinitely greater manifestations of the illness than the urinary symptoms. This patient had suffered from malaria in the Struma Valley during the Great War, but no malarial parasites were

¹ Dudgeon, L. S., Wordley, E., Bawtree, F. (7. XII. 1922). On *Bacillus coli* infection of the urinary tract especially in relation to haemolytic organisms. *Journ. Hygiene*, XXI. 168. (2nd communication.)

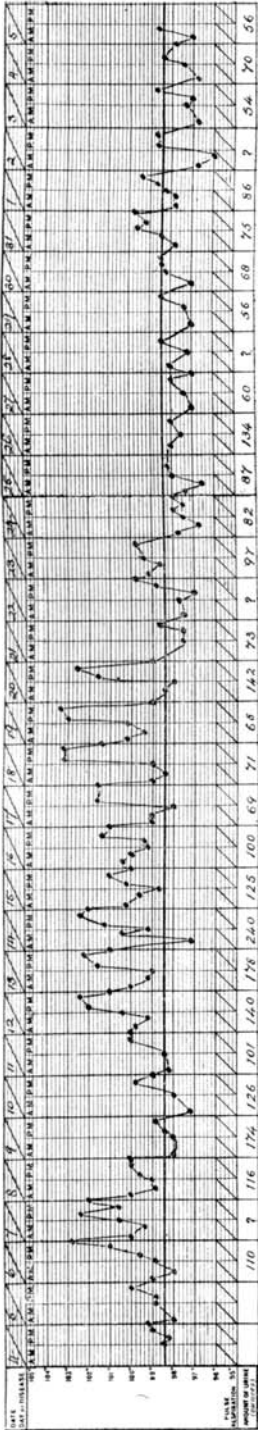


Chart I

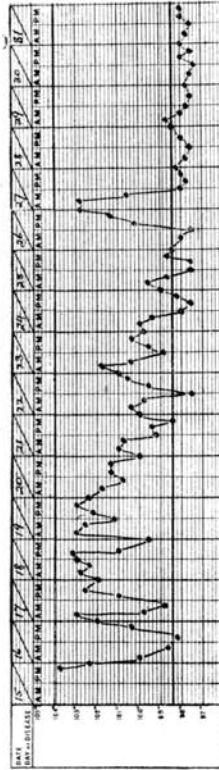


Chart II

found in his blood on this occasion, his spleen was not enlarged, and quinine was ineffective.

The patient's urine contained a thick deposit of pus and bacilli. The serum gave no reaction with the paratyphoid group A, B, and C, and three colon strains. There was a reaction of 1 in 50 to *B. typhosus*, which may have been due to anti-typhoid inoculation, and to his own bacillus. Blood culture was sterile.

Chart II is taken from a woman who developed a sudden acute illness following her confinement. She had twelve days of fever which reached a maximum of 103° F., and was extremely ill during this illness. Here again the general symptoms were so severe as to somewhat mask the urinary infection.

There has been only one fatal case out of the total of 49. The urine from patients who have convalesced from this infection has been found to be sterile, which is in direct contrast to my experience of the usual sequence of events in pure coli cases. Patients infected with this group of organisms, as a rule, are infinitely more sensitive to vaccine therapy than coli cases. Even small doses of vaccine may have the effect of recommencing the pyrexia and the patients' symptoms; so much is this the case that it is necessary to employ considerable discretion in the treatment of these patients with specific vaccines.

Bacteriology. When the urine from these cases was plated on litmus-lactose-agar the colonies for the first 48 hours resembled those of the enterica group, but subsequently with some strains the colonies acquired a greenish-blue colour after a few days' incubation at 37° C. and showed a reddish-tinged centre by transmitted light. Other indicators employed with lactose-agar were neutral-red, phenol-red, and brom-cresol, but no advantage was obtained. In liquid media phenol-red was the only indicator employed.

All the 49 strains have produced marked haemolysis of human red corpuscles in both the stages which have been referred to elsewhere. They were gram-negative bacilli, motile, formed acid and gas in mannitol, dextrose, maltose and dulcitol, although dulcitol was subsequently rendered alkaline by some of the strains. Milk was acidified, and clotting occurred with most of the strains in 72 hours, while a thick or solid clot was formed at the end of one week at 37° C. but with three strains milk became definitely alkaline and no clotting occurred.

Lactose-lemco-broth was acidified during the first 24 hours by some of these cultures, and then became alkaline. Some did not form gas, while some fermented lactose to a slight degree in the course of 72 hours. The majority of the strains did not alter litmus-lactose-agar stab-cultures apart from changing the litmus-blue to a yellow colour. Organism 5659, however, fermented lactose in both liquid and solid media, and 6752 formed typical blue surface colonies on litmus-lactose-agar which finally had a greenish tinge, but it fermented lactose-broth in 48 hours and lactose in agar stab-cultures, and clotted milk. It was the only strain which showed these atypical characters.

Cane-sugar was rendered alkaline with every strain in 48 hours, and the

alkalinity gradually increased up to a period of ten days. A good growth was obtained on gelatine, but this medium was not liquefied.

Table I, showing the cultural reactions of those strains used for the immunisation of rabbits and for the serological tests.

Name or No.	Milk		Saccharose		Jelly	Lactose			Litmus-lactose-agar	
	1	10	1	10	10	1	6	10		
6489	1	Trace	+2	-	-1	0	-	+	-2	D
Sapsford	2	Trace	+2	-	-2	0	+	+1	-3	D
Duncan	3	Trace	-	-	-1	0	-	+	-	D
6614	4	Trace	-	-	-1	0	-	+3	+3	D
5659	5	Trace	+2	-	-1	0	0	+2	-1	+1
Winson	6	Trace	+2	-	-1	0	0	+2	-1	D
Williams	7	Trace	+2	-	-1	0	+	+1	-2	D
Ash	8	Trace	+2	-	-3	0	+	+1	-1	D
Northam	9	Trace	+2	-	-3	0	0	-1	+1	D
6752	10	+	+2	-	-2	0	0	+1	-3	+2

+ = acid.
+1 +2 +3 = acid and gas formation in varying gradations, and clotting of milk.
- = alkaline: -1, -2, -3 signifies gradations of alkalinity.
D = Decolorised.
0 = No change.

Blood cultures made into various media have been negative, even when taken at the height of the fever.

Faeces. So far I have not been able to cultivate an organism of this group from the faeces in the acute cases referred to in this paper, but very infrequently such bacilli have occurred in the faeces under other conditions.

SEROLOGICAL REACTIONS.

Patients' sera. The sera from many of the cases were tested with the haemolytic and non-haemolytic coli antigens, with one, two or three of the haemolytic slow lactose strains and with the enterica antigens. No reaction occurred with the coli, typhoid, and paratyphoid A, B, and C antigens, except in the case of those individuals who had been inoculated during the war. No reaction occurred with antigens made from members of this haemolytic group, except in three cases, but here the reaction did not exceed 1 in 400. The absence of reaction in so many cases justified the conclusion that agglutination reactions with human sera and these antigens are of little value in most instances for diagnostic purposes, but a reaction of 1 in 100, or over, is very suggestive of this infection.

The immune sera. The serum from rabbits which had been immunised with vaccines and living cultures of Nos. 5659, 6489 and "Williams," were used for all the serological tests. The rabbits were immunised with formalised vaccines, followed by intravenous injections of the living organisms, or with the living bacilli only. High titre anti-sera were obtained most readily by the intravenous inoculation of rabbits with living organisms. The injections of

live bacilli were commenced with doses of 50 million and then 100, 250 and 500 million, at intervals of one week. No ill effects occurred.

Veal-broth antigens. These were prepared in the usual way by daily sub-culture, killed with 0.1 per cent. formalin, and diluted to 1000 million per c.c. These antigens, however, were seldom satisfactory as, although the end points might remain constant, in many instances the agglutinability diminished rapidly to a considerable degree. The experience gained with these antigens is in direct contrast to that obtained with the true coli antigens which on the whole are perfectly satisfactory.

Agar antigens. These were prepared on the same lines as the veal broth, but were equally unsatisfactory.

Living agar antigens. Living agar cultures grown for 24 hours at 37° C. were found to be the most satisfactory antigens. The agar slopes were inoculated freely so as to cover the entire surface. 2 c.c. of saline were added to each tube ($6 \times \frac{5}{8}$), and the emulsion obtained was filtered when necessary through filter paper. One drop of the emulsion was added to each tube of 1 c.c. of diluted serum, and the whole incubated at 52° C. for 5 hours, and the results read after standing at room temperature for 15 minutes. The method adopted is very similar to that employed in the Weil-Felix reaction. From each strain a veal-broth antigen was prepared, but for reasons already stated it was discarded, and the living agar antigens were used with satisfactory results. Every one of the 49 strains has been found to agglutinate with the three anti-sera employed, and also with high dilutions of these three anti-sera, "Williams," 5659, 6489.

Every strain was tested with a haemolytic colon anti-serum (Dow), but only a very slight reaction was obtained in a few instances. No reaction occurred with T.A.B.C. anti-sera. A non-haemolytic colon anti-serum (5651) did, however, agglutinate some of these strains.

Saturation experiments. An anti-serum, 6489, prepared from one of the cases referred to in Table I, was saturated with one of the cultures "Duncan" referred to in the same table, and as a similar method was adopted in each saturation experiment one description of the details employed will suffice. 2 c.c. of a thick saline suspension of bacillus "Duncan," grown on agar plates, was mixed with 1 c.c. of a 1 in 5 dilution of the immune serum, 6489, and the mixture was left in the ice safe for one week. Each emulsion contained 0.25 per cent. phenol. The mixture was then centrifugalised at high speed, and the control serum and the treated serum compared. The results are expressed as a fraction, the numerator is the result of the treated serum and the denominator the control serum.

The result of the agglutination and saturation experiments point to these organisms as one group of bacilli. Anti-sera prepared from three members of the group agglutinate all strains up to date and de-saturation of these anti-sera has been effected. Anti-coli sera (except no. 5651) do not react with these bacilli except in very low dilutions.

I. I.S. 6489 saturated with bacillus "Duncan." Emulsion 70,000 million per c.c.

1. On 6489	$\frac{0^1}{15,000}$
2. On Duncan	$\frac{400}{15,000}$
3. On Williams	$\frac{0}{400}$

II. I.S. 6489 saturated with bacillus "Williams." Emulsion 90,000 million per c.c.

1. On Duncan	$\frac{2,000}{15,000}$
2. On 6614	$\frac{0}{1,000}$
3. On 5659	$\frac{400}{5,000}$
4. On 6489	$\frac{400}{15,000}$
5. On Salthouse	$\frac{0}{400}$
6. On Sapsford	$\frac{0}{400}$

III. I.S. 6489 saturated with bacillus "Sapsford." Emulsion 70,000 million per c.c.

1. On 6489	$\frac{0}{15,000}$
2. On Williams	$\frac{0}{400}$
3. On Sapsford	$\frac{0}{400}$

IV. I.S. "Williams" saturated with bacillus "Sapsford." Emulsion 70,000 million per c.c.

1. On Williams	$\frac{1,000}{10,000}$
2. On 6489	$\frac{5,000}{15,000}$
3. On Sapsford	$\frac{2,000}{10,000}$
4. On Duncan	$\frac{400}{10,000}$
5. On Salthouse	$\frac{1,000}{15,000}$
6. On 5659	$\frac{1,000}{10,000}$
7. On 6614	$\frac{5,000}{15,000}$

IV a. I.S. "Williams" saturated with bacillus "Duncan." Emulsion 70,000 million per c.c.

1. On Williams	$\frac{2,000}{10,000}$
2. On 6489	$\frac{2,000}{15,000}$
3. On Sapsford	$\frac{2,000}{10,000}$
4. On Salthouse	$\frac{2,000}{15,000}$
5. On 5659	$\frac{1,000}{10,000}$
6. On 6614	$\frac{400}{15,000}$
7. On Duncan	$\frac{0}{10,000}$

V. I.S. "Williams" (2) saturated with bacillus 6752. Emulsion 175,000 million per c.c.

1. On Williams	$\frac{0}{5,000}$
2. On 5659	$\frac{0}{400}$
3. On Sapsford	$\frac{0}{5,000}$
4. On 6489	$\frac{400}{10,000}$
5. On Winson	$\frac{0}{2,000}$
6. On 6752	$\frac{400}{8,000}$
7. On 6614	$\frac{0}{800}$

VI. I.S. 5659 (2) saturated with bacillus 6752. Emulsion 175,000 million per c.c.

1. On Williams	$\frac{0}{2,000}$
2. On 5659	$\frac{400}{2,000}$
3. On 6489	$\frac{800}{2,000}$
4. On Ash	$\frac{0}{400}$
5. On Northam	$\frac{0}{2,000}$
6. On Salthouse	$\frac{0}{400}$
7. On Struchett	$\frac{0}{400}$
8. On Duncan	$\frac{400}{1,500}$

VII. I.S. *B. coli* (Dow, Haemolytic Type). Saturated with bacillus 6752. Emulsion 175,000 million per c.c.

On Dow antigen	$\frac{1,800}{1,800}$
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¹ All these results are expressed as end point reaction which is the limit of agglutination as ascertained with a hand lens. The lowest limit employed was 1 in 400

CONCLUSIONS.

1. A severe acute infection of the genito-urinary tract can be caused by slow lactose fermenting haemolytic bacilli.
2. This infection, as contrasted with acute coli infections, is much less common, more severe, and persists for a longer period, but ultimate complete recovery and freedom from infection is much more probable. Patients suffering from this infection are very sensitive to specific vaccine therapy.
3. No case of chronic infection has occurred up to the present period.
4. Forty-nine cases have been investigated. In every instance the bacilli have been found to be actively haemolytic, serologically similar, and in plate cultures form blue colonies on the surface of litmus-lactose-agar, with delayed fermentation of lactose-broth and alkaline production in saccharose.
5. The organism has not been cultivated from the blood stream or faeces in these acute cases.

(*MS. received for publication* 1. XII. 1923.—Ed.)