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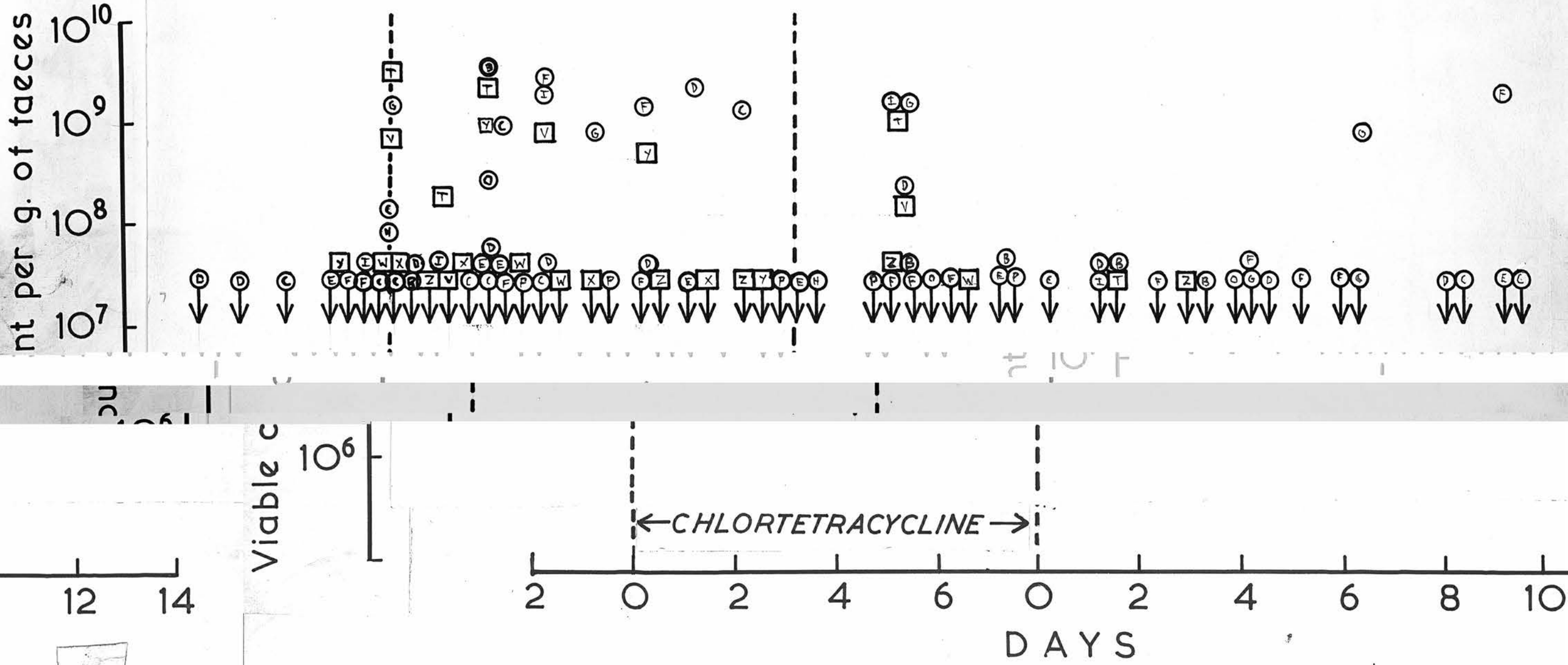

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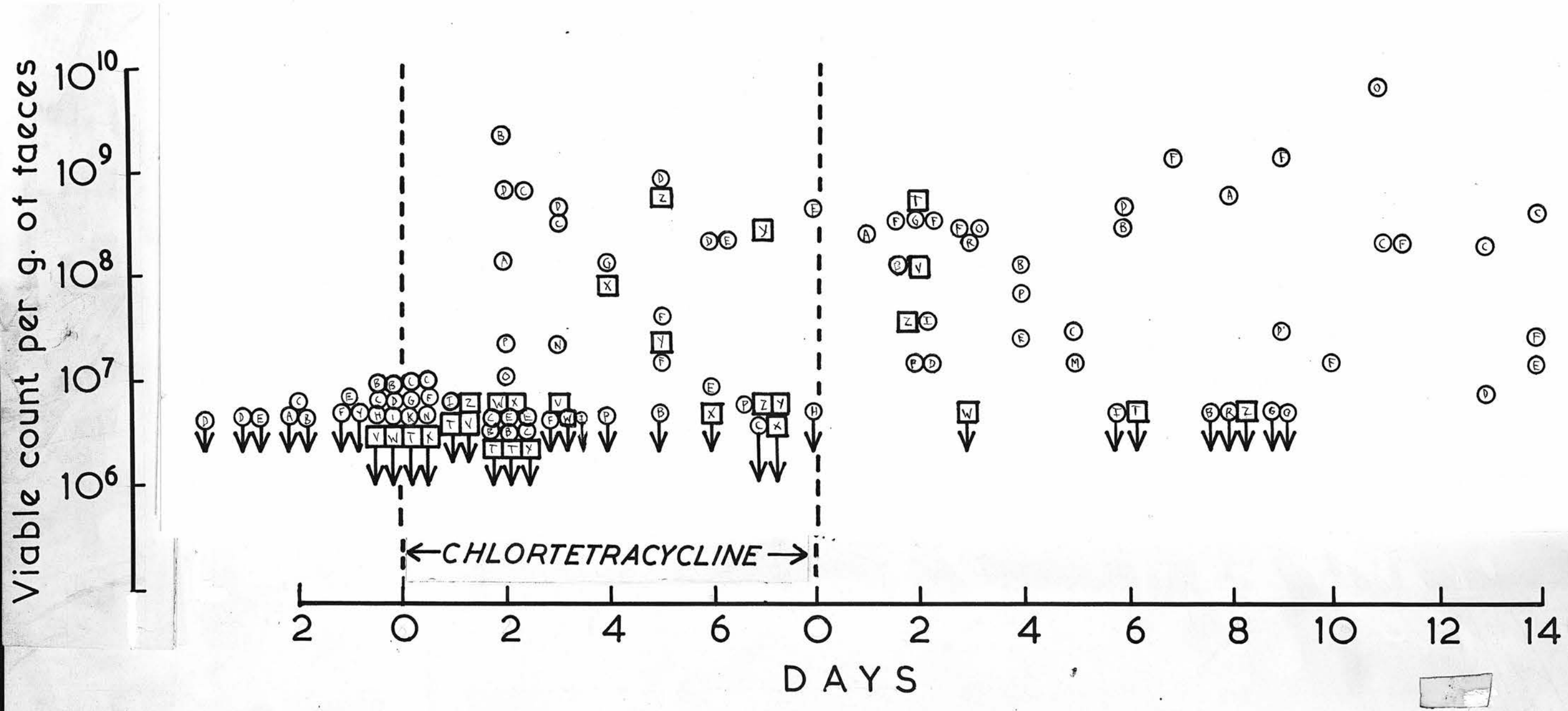

○ Patients with liver disease

□ Patients without liver disease

♀ ↓ / □ ↓ } Less than 5×10^6 orgs. per g. [lower limit of counting method.]

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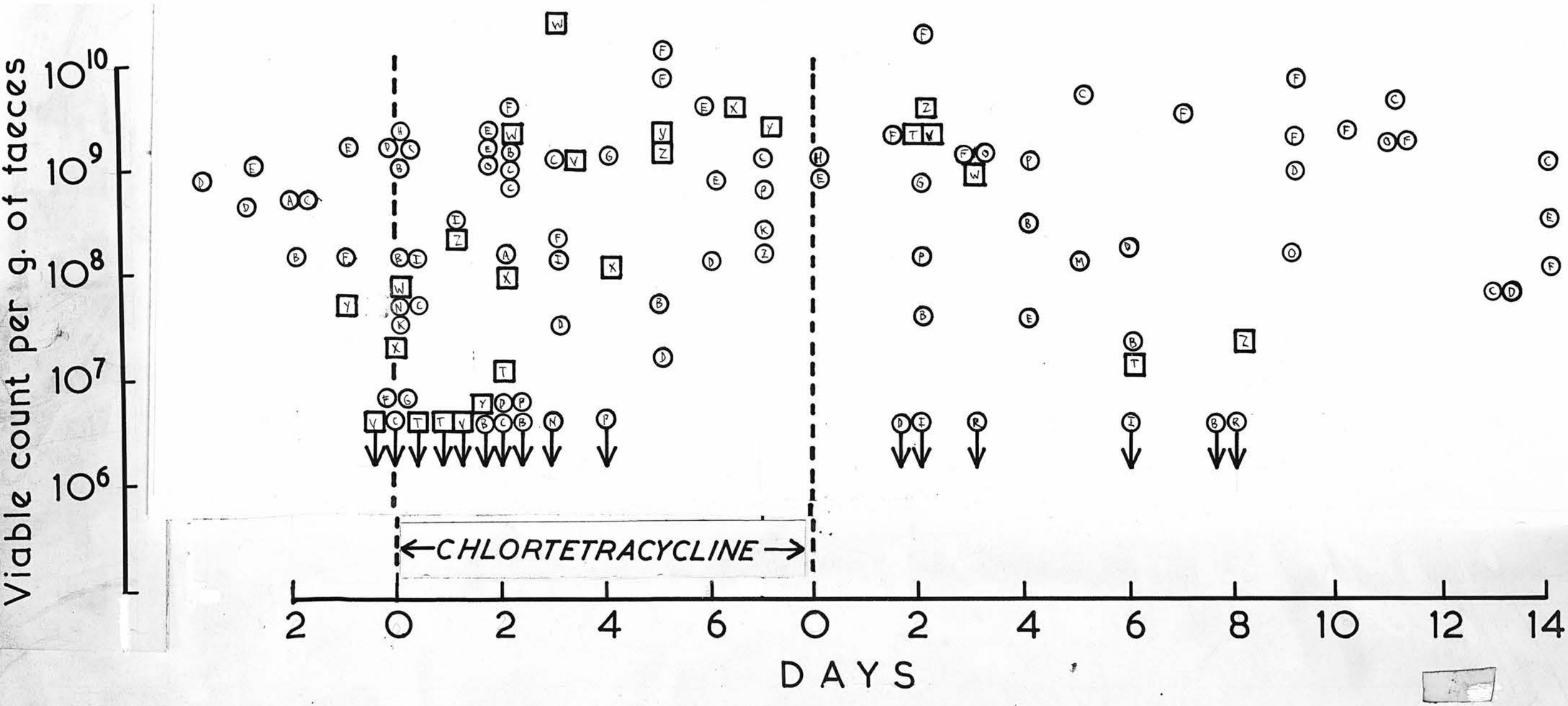
STREPTOCOCCI



○ Patients with liver disease

□ Patients without liver disease

○ / □ Less than 5×10^6 orgs. per g. [lower limit of counting method.]

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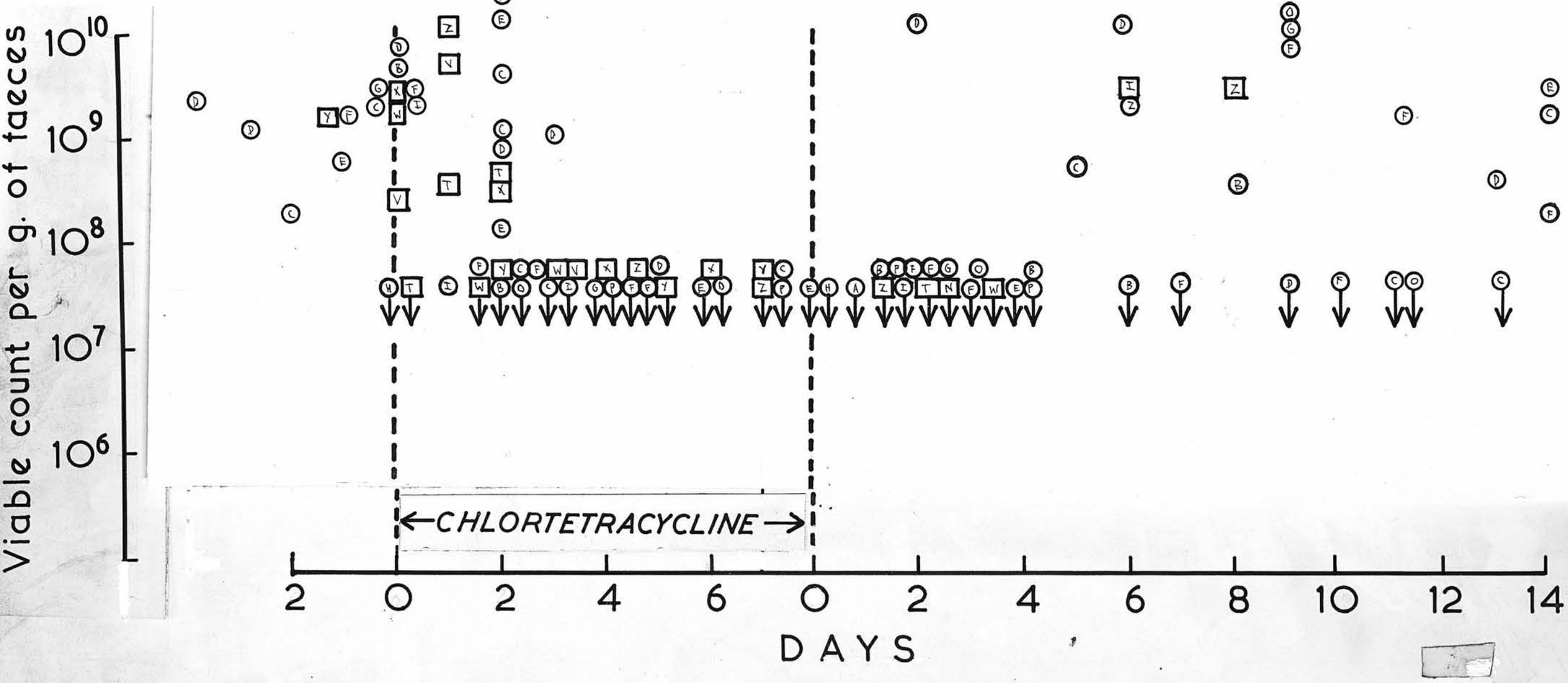
BACTEROIDES



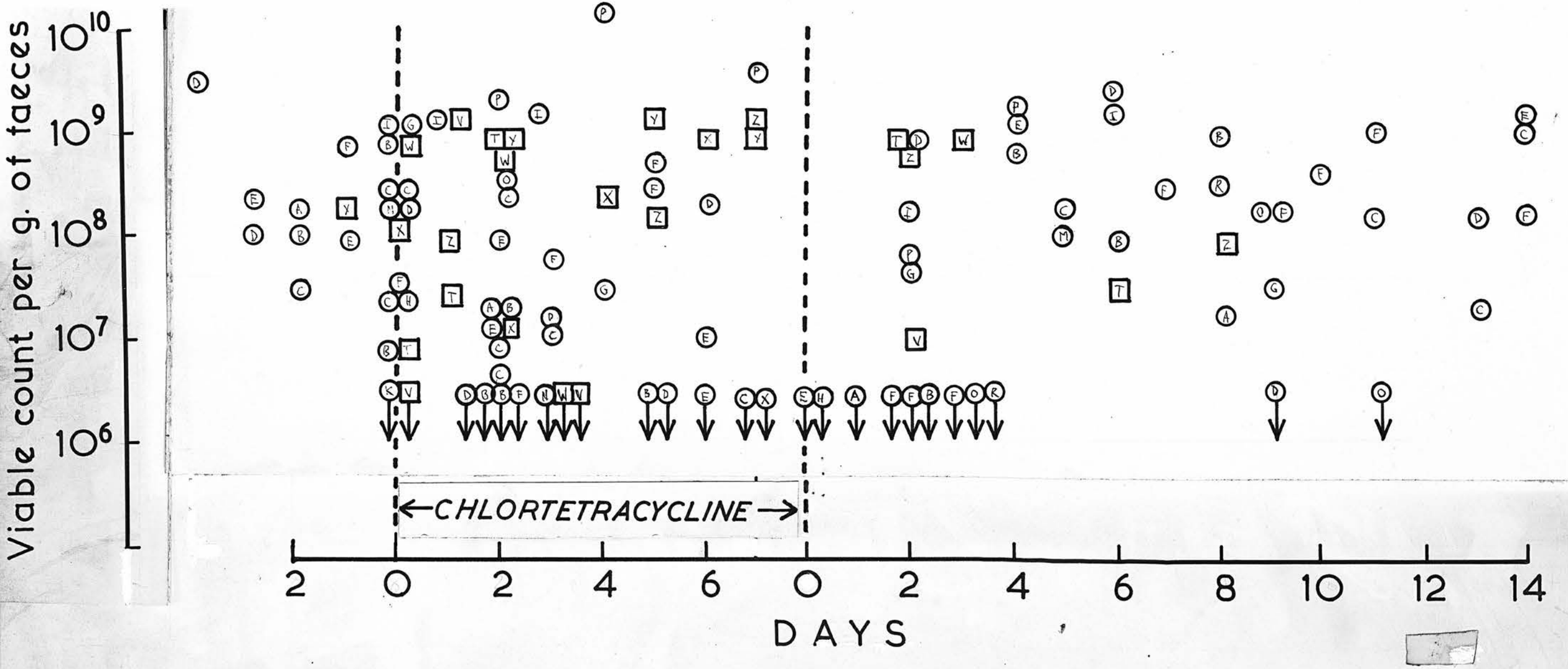
○ Patients with liver disease

□ Patients without liver disease

♀ ⎫
□ ⎬ Less than 5×10^6 orgs. per g. [lower limit of counting method.]

EX BIBL UNIV ED NEURENBERG

COLIFORM ORGANISMS



○ Patients with liver disease

□ Patients without liver disease

Less than 5×10^6 orgs. per g. [lower limit of counting method.]

EX BIBL UNIV ED NEUBURGEN

TABLE A

The intestinal flora of patients with cirrhosis and other subjects.

	DUODENUM												JEJUNUM						
	S.C.	coli	St.f.	St.v.	St.an.	Diph.	Mic.	Alk.	pyo.	P.	Bac.	Lac.	Others	S.C.	coli	St.f.	St.v.	St.an.	Diph.	Mic.
Patients with Cirrhosis																				
2 x	4.5×10^7	7.2×10^4	2.5×10^4	-	-	-	-	-	-	3×10^3	-	-	-	-	3×10^6	4.4×10^6	-	-	-	-
3 x	6.4×10^6	3×10^5		-	-	-	-	-	-	2.8×10^6	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
4	.....	.....	.....	not investigated	.....	.....	.....	.....	-	-	-	-	-	9×10^6	3.2×10^6	2.8×10^6	-	-	-	-
9	3.2×10^7	4×10^2	-	-	-	-	-	-	-	-	2.4×10^4	2×10^4	3×10^3	2×10^3	7×10^5	-	-	-	-	-
18	-	6×10^2	-	-	-	-	-	-	-	-	-	-	4×10^2	-	3×10^4	-	2×10^5	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	7×10^4	2×10^3	-	-	-	-	-	-	-
20	3.3×10^7	-	-	-	1.6×10^4	-	-	-	-	-	-	-	7×10^3	-	3.9×10^4	1.7×10^4	-	-	-	-
21	-	6.8×10^7	-	-	-	-	-	-	-	6.4×10^4	-	-	-	-	2.6×10^6	2×10^8	-	-	-	-
22	1×10^8	1.9×10^5	1.2×10^4	+	-	+	-	-	-	-	-	-	-	1.1×10^7	2.4×10^6	1.3×10^8	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4×10^4	2.5×10^4	-	-	-	-
24	-	-	1.4×10^3	+	-	-	+	-	-	-	-	-	-	1×10^6	-	6.3×10^3	1×10^6	-	-	8×10^2
25	5×10^6	-	-	-	-	-	-	-	-	-	-	-	-	5×10^6	3×10^2	-	+	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Patients with gastro-intestinal disorders																				
42	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	.....	-	-	-	-	-	6×10^2	6×10^2
43	6×10^7	-	-	1.2×10^5	-	1.7×10^4	-	-	-	6×10^2	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
44	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	.....	-	4.4×10^6	4.4×10^7	-	-	-	-
45	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	not investigated	.....
46	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	.....	-	4×10^7	-	-	-	-	-
47	-	6×10^8	-	-	-	-	-	-	1.4×10^6	-	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2×10^5	3×10^4	-	-	-	-
49	-	-	-	-	5×10^2	-	-	-	-	-	-	-	-	-	1×10^2	-	-	5.7×10^3	-	-
50	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
51 (a)	4×10^8	5.6×10^7	3.4×10^6	-	-	-	-	-	-	-	+	-	-	.....	.....	.....	.....	.....	not investigated	.....
(b)	4.1×10^7	1.6×10^7	1.6×10^6	-	-	-	-	-	-	-	+	-	-	.....	.....	.....	.....	.....	not investigated	.....
52	3×10^6	1.1×10^5	7×10^3	-	-	-	-	-	-	-	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
53	1.2×10^9	1×10^5	-	-	-	-	-	-	-	-	-	-	1×10^5	(Cl.welchii) 5.2×10^7		10^5	-	-	-	-
54	-	-	-	-	-	-	-	-	-	-	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
Healthy subjects																				
63	6×10^6	-	-	-	-	-	1.6×10^3	-	-	-	-	-	-	.....	.....	.....	.....	.....	not investigated	.....
64	-	-	-	-	±	-	-	-	-	-	-	-	-							
65	-	-	-	1.5×10^3	-	-	-	-	-	-	-	-	-	-	-	-	5×10^2	5×10^2	-	-
66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5×10^4	3.8×10^4	-
68	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	1.1×10^6	-	-	-
69	-	-	-	3.7×10^4	-	-	-	-	-	-	-	-	-	-	-	-	1.5×10^3	-	-	-

S.C. = Total smear count. Coli = coliform orgs. including E.coli., E.freundii, Klebsiella, Bact.cloacae and Paracolon bacilli.

St.f. = Strep.faecalis. St.v. = Strep viridans. Diph. = Diphtheroid organisms, Mic. = Micrococci. Alk. = Bact.alkaligenes.

Pyo. = Ps. pyocyanea. P. = Proteus. Bac. = Bacteroides. Lac. = Lactobacilli. St.an. = Strep.anaerobic.

x = specimens taken after administration of chlortetracycline. Smear counts were only done in some cases.

numbers represent orgs per ml of intestinal fluid or per gram of faeces.

± = a few colonies.

\+ = moderate numbers of organisms.

+++ = many organisms.

\- = organisms present in numbers below the upper limit of the counting method (10^2 per ml).

TAB LE A

The intestinal flora of patients with cirrhosis and other subjects.

					JEJEUNUM													
P.	Bac.	Lac.	Others		S.C.	coli	St.f.	St.v.	St.an.	Diph.	Mic.	Alk.	pyo.	P.	Bac.	Lac.	Others	
3×10^3	-	-	-		-	3×10^6	$4{\cdot}4 \times 10^6$	-	-	-	-	-	-	5×10^5	-	-	-	
$2{\cdot}8 \times 10^6$	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		9×10^6	$3{\cdot}2 \times 10^6$	$2{\cdot}8 \times 10^6$	-	-	-	-	-	-	-	-	-	-	
-	$2{\cdot}4 \times 10^4$	2×10^4	3×10^3	Candida	2×10^3	7×10^5	-	-	-	-	-	-	-	5×10^5	-	-	$6{\cdot}4 \times 10^4$	Anitratum
-	-	-	4×10^2	Anitratum	-	3×10^4	-	2×10^5	-	-	-	-	-	-	-	3×10^5	$1{\cdot}2 \times 10^3$	Staph.pyog.
-	-	7×10^4	2×10^3	Staph.pyog.	-	-	-	-	-	-	-	-	-	-	-		$1{\cdot}5 \times 10^3$	Candida
-	-	-	7×10^3	Candida	-	$3{\cdot}9 \times 10^4$	$1{\cdot}7 \times 10^4$	-	-	-	-	-	-	-	-	-	-	
-	-	-	-		-	$2{\cdot}6 \times 10^6$	2×10^8	-	-	-	-	-	-	-	-	-	-	
$6{\cdot}4 \times 10^4$	-	-	-		$1{\cdot}1 \times 10^7$	$2{\cdot}4 \times 10^6$	$1{\cdot}3 \times 10^8$	-	-	-	-	-	-	$3{\cdot}4 \times 10^5$	-	-	-	
-	-	-	-		-	$2{\cdot}4 \times 10^4$	$2{\cdot}5 \times 10^4$	-	-	-	-	-	-	-	-	-	-	
-	-	-	-		1×10^6	-	$6{\cdot}3 \times 10^3$	1×10^6	-	-	8×10^3	-	-	-	-	2×10^4	-	
-	-	-	-		5×10^3	3×10^2	-	+	-	-	-	-	$1x\ 10^2$	-	-	-	-	+ N. pharyngis
-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	+ Haemophilus
-	-	-	-		-	-	-	-	-	-	±	-	-	-	-	-	-	
.....	.....				-	-	-	-	-	6×10^2	6×10^2	-	-	-	-	-	-	
6×10^2	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
.....					-	$4{\cdot}4 \times 10^6$	$4{\cdot}4 \times 10^7$	-	-	-	-	-	-	-	-	-	-	
.					.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		-	4×10^7	-	-	-	-	-	-	-	3×10^6	-	-	-	
-	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		-	$1{\cdot}2 \times 10^5$	3×10^4	-	-	-	-	-	-	-	-	-	-	
-	-	-	-		-	1×10^2	-	-	$5{\cdot}7 \times 10^3$	-	-	$2{\cdot}1 \times 10^4$	-	-	-	-	-	
-	+	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	+	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	1×10^5	(Cl.welchii)	$5{\cdot}2 \times 10^7$	10^5	-	-	-	-	-	-	-	-	-	-	10^5	Cl.welchii.
-	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		.....	.....	.....	.....	.....	not investigated	.....	.....	.....	.....	.....	.....	.....	
-	-	-	-		-	-	-	5×10^2	5×10^2	-	-	-	-	-	-	-	-	
-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	
-	-	-	-		-	-	-	-	$1{\cdot}5 \times 10^4$	$3{\cdot}8 \times 10^4$	-	-	-	-	-	-	-	
-	-	-	-		-	-	-	$1{\cdot}1 \times 10^6$	-	-	-	-	-	-	-	5×10^5	-	
-	-	-	-		-	-	-	$1{\cdot}5 \times 10^3$	-	-	-	-	-	-	-	-	-	

t. cloacae and Paracolon bacilli.

cci. Alk. = Bact. alkaligenes.

acilli. St.an. = Strep. anaerobic.

n some cases.

TABLE A (Cont'd)

The intestinal flora of patients with cirrhosis and other subjects.

ILEUM

	S.C.	coli	St.f.	St.v.	St.an.	Diph.	Mic.	Alk.	pyo.	P.	Bac.	Lac.	Others	S.C.	coli	St.f.
Patients with cirrhosis																
2 x	4.3×10^7	9.6×10^7	1.8×10^7	-	-	-	-	-	-	1.2×10^7	-	-	-	9.5×10^{10}	4.3×10^9	9×10^8
3 x	-	1.6×10^8	8×10^6	-	-	-	-	-	-	4×10^7	2×10^8	-	-	9.5×10^{10}	1×10^9	1.5×10^8
4	-	9.4×10^7	6×10^7	-	-	-	-	-	-	-	-	-	-	9.1×10^{10}	2.4×10^9	5×10^7
9	3×10^7	2.2×10^7	-	1×10^6	-	-	-	-	-	4×10^5	+	-	-	-	-	6×10^8
18	-	2×10^5	-	-	-	-	-	-	-	-	-	-	10^5 Anitratum	-	-	-
19	-	-	-	4×10^3	-	-	-	-	-	-	-	1.2×10^5	10^4 Candida	-	6×10^7	-
20 *	-	1×10^5	1.2×10^4	-	-	-	-	-	-	-	-	-	10^4 Staph.pyo.	5×10^{10}	2.4×10^9	-
21	3.7×10^7	4.8×10^7	-	-	-	-	-	-	-	-	-	-	-	9.3×10^{10}	9×10^8	7×10^8
22	7×10^7	1×10^7	2.7×10^6	-	-	-	-	-	-	1.6×10^6	-	-	-	1.6×10^{10}	8×10^7	-
23	-	4.7×10^3	1.9×10^4	-	-	-	-	-	4×10^2	-	-	-	-	-	1×10^5	2×10^5
24	6×10^6	8×10^2	1.8×10^6	±	-	-	-	-	-	-	-	-	3×10^6 Staph.albus	.....		
25	4×10^2	-	-	1.5×10^5	-	+	-	-	1×10^2	-	-	-	-	-	1.5×10^9	-
26	-	-	3×10^2	-	-	-	-	-	-	-	-	-	-	-	6×10^7	-
Patients with gastro-intestinal disorders																
42	-	-	-	-	-	6×10^2	6×10^2	-	-	-	-	-	-	.....		
43	9.5×10^7	7×10^2	-	-	-	-	4.2×10^3	-	-	-	-	-	-	.....		
44	-	1×10^7	8×10^7	-	-	4×10^3	4×10^3	-	-	-	-	-	-	.....		
45	-	3.2×10^8	9.9×10^8	-	-	-	-	-	-	-	-	-	-	.....		
46	-	4×10^7	-	-	-	-	-	-	-	3×10^6	-	-	-	-	2.5×10^6	-
47	8×10^7	-	-	-	-	-	-	-	1.8×10^7	-	-	-	-	-	1.5×10^8	-
48	-	8.2×10^4	4.5×10^4	-	-	-	-	-	-	-	-	-	-	-	1×10^8	-
49	-	6.2×10^5	-	-	-	-	-	-	-	-	-	-	-	-	1.1×10^8	2×10^9
50	-	-	-	±	-	-	-	-	-	-	-	-	-	-	8×10^6	26×10^9
51 (a)	.....					)not investigated								.....		
(b)	.....					)not investigated								.....		
52	2.8×10^7	1.2×10^5	-	-	-	-	-	-	-	-	-	-	-	.....		
53	2×10^8	10^5	-	-	-	-	-	-	-	-	-	-	10^5 Cl.w.	.....		
54	-	2.5×10^6	-	3.5×10^6	-	-	-	-	3×10^2	-	-	-	-	.....		
Healthy subjects																
63	1.2×10^7	2×10^3	8.5×10^4	-	-	-	-	-	-	-	-	-	-	-	1.5×10^8	-
64	-	-	-	1.8×10^8	1×10^3	-	-	-	-	-	-	-	-	-	2.5×10^5	-
65	-	-	-	-	-	-	-	3×10^2	-	-	-	-	-	-	1.1×10^9	-
66	-	1.5×10^3	-	-	-	-	-	1×10^2	-	-	-	-	-	-	3×10^7	-
67	-	-	-	-	1.5×10^4	1.2×10^4	-	-	-	-	-	-	-	-	1×10^8	2×10^7
68	-	-	-	5.6×10^6	-	-	-	-	-	-	-	-	-	-	1.1×10^8	-
69	-	2×10^2	-	4.7×10^3	-	-	-	-	-	-	-	-	-	-	-	-

* = lower limit of counting

TABLE A (Cont'd)

The intestinal flora of patients with cirrhosis and other subjects.

Bac.	Lac.	Others	FAECES* S.C.	coli	St.f.	Bac.	Lac.	St.an.	Cl.w.	Others
-	-	-	9.5×10^{10}	4.3×10^{9}	9×10^{8}	5×10^{9}	-	-	-	2.5×10^{7} (P)
2×10^{8}	-	-	9.5×10^{10}	1×10^{9}	1.5×10^{8}	2.5×10^{9}	±	±	-	4×10^{8} (P)
-	-	-	9.1×10^{10}	2.4×10^{9}	5×10^{7}	4×10^{9}	-	-	-	-
+	-	-	-	-	6×10^{8}	-	-	6×10^{8}	-	-
-	-	10^{5} Anitratum	-	-	-	-	6×10^{7}	6×10^{9}	-	-
-	1.2×10^{5}	10^{4} Candida	-	6×10^{7}	-	8×10^{11}	5×10^{8}	3.2×10^{9}	-	-
-	-	10^{4} Staph. pyog.	5×10^{10}	2.4×10^{9}	-	3.2×10^{9}	-	-	-	± (P)
-	-	-	9.3×10^{10}	9×10^{8}	7×10^{8}	4.5×10^{9}	-	-	-	2.5×10^{7} (P)
-	-	-	1.6×10^{10}	8×10^{7}	-	5×10^{8}	-	±	-	-
-	-	-	-	1×10^{5}	2×10^{5}	9×10^{7}	-	-	-	3×10^{7} (Mic)
-	-	3×10^{6} Staph. albus	..... not investigated							
-	-	-	-	1.5×10^{9}	-	2.5×10^{9}	±	1×10^{9}	-	-
-	-	-	-	6×10^{7}	-	2×10^{9}	1×10^{7}	-	1×10^{8}	-
-	-	-	..... not investigated							
-	-	-	..... not investigated							
-	-	-	..... not investigated							
-	-	-	..... not investigated							
-	-	-	-	2.5×10^{6}	-	-	-	-	-	-
-	-	-	-	1.5×10^{8}	-	-	2.5×10^{8}	-	-	-
-	-	-	-	1×10^{8}	-	-	3×10^{9}	-	-	-
-	-	-	-	1.1×10^{8}	2×10^{9}	4×10^{10}	5×10^{8}	-	-	-
-	-	-	-	8×10^{6}	26×10^{9}	2.4×10^{10}	-	-	-	-
.....			..... not investigated							
.....			..... not investigated							
-	-	-	..... not investigated							
-	-	10^{5} Cl.w.	..... not investigated							
-	-	-	..... not investigated							
-	-	-	-	1.5×10^{8}	-	4×10^{8}	-	-	-	-
-	-	-	-	2.5×10^{5}	-	1×10^{6}	5×10^{9}	-	-	-
-	-	-	-	1.1×10^{9}	-	-	6×10^{8}	1.7×10^{9}	-	-
-	-	-	-	3×10^{7}	-	5×10^{8}	2×10^{9}	-	-	-
-	-	-	-	1×10^{8}	2×10^{7}	5×10^{6}	5×10^{8}	-	-	-
-	-	-	-	1.1×10^{8}	-	2.5×10^{7}	2.5×10^{9}	1×10^{9}	1.4×10^{9}	-
-	-	-	-	-	-	-	-	-	-	-

* = lower limit of counting method = 5×10^{6} for aerobes and 5×10^{7} for anaerobes.

TABLE B

A Comparison of the faecal flora of patients with cirrhosis of the liver and of patients without liver disease.

	Smear Count	coliforms	Strep.f.	Bac.	Lac.	St.an.	Cl.w.	Others
Cirrhosis								
1	$2{\cdot}5 \times 10^{10}$	$4{\cdot}6 \times 10^{8}$	5×10^{7}					
2)		$1{\cdot}2 \times 10^{6}$	$2{\cdot}2 \times 10^{8}$				$3{\cdot}9 \times 10^{8}$	
3) before chlortetracycline		$4{\cdot}5 \times 10^{7}$		1×10^{10}	2×10^{8}		2×10^{8}	$1{\cdot}2 \times 10^{9}$ (Micrococci)
4	$9{\cdot}1 \times 10^{10}$	$2{\cdot}5 \times 10^{9}$	5×10^{7}	3×10^{9}				
5		$5{\cdot}3 \times 10^{8}$	$1{\cdot}3 \times 10^{9}$	9×10^{8}			1×10^{8}	$1{\cdot}5 \times 10^{8}$ (Micrococci)
6	$2{\cdot}8 \times 10^{10}$	$7{\cdot}5 \times 10^{7}$	$1{\cdot}1 \times 10^{9}$	8×10^{9}				
7	$4{\cdot}8 \times 10^{10}$	$9{\cdot}4 \times 10^{8}$	1×10^{8}	$1{\cdot}7 \times 10^{9}$				
11		1×10^{9}	$3{\cdot}1 \times 10^{9}$					
12		1×10^{8}	8×10^{8}					$2{\cdot}4 \times 10^{9}$ (Bact.alk)
20 " "	$4{\cdot}2 \times 10^{10}$	$1{\cdot}2 \times 10^{9}$	$1{\cdot}5 \times 10^{9}$	2×10^{9}				
27	$2{\cdot}3 \times 10^{10}$	4×10^{8}	$9{\cdot}4 \times 10^{7}$	$3{\cdot}8 \times 10^{7}$			$5{\cdot}3 \times 10^{6}$	
28	$5{\cdot}2 \times 10^{10}$	7×10^{8}	1×10^{7}	7×10^{10}	1×10^{9}			4×10^{6} (Bact.alk)
29		1×10^{6}	3×10^{7}					
30		2×10^{7}	5×10^{6}					3×10^{6} (Proteus)
31		3×10^{8}	6×10^{7}					
32	$7{\cdot}5 \times 10^{10}$	$4{\cdot}4 \times 10^{9}$	$3{\cdot}5 \times 10^{9}$					
33	4×10^{10}	$1{\cdot}2 \times 10^{9}$	$9{\cdot}6 \times 10^{8}$					
34		$2{\cdot}3 \times 10^{8}$		$1{\cdot}5 \times 10^{9}$	±	±		
35		$8{\cdot}4 \times 10^{9}$		2×10^{9}				
36		$6{\cdot}5 \times 10^{8}$	5×10^{6}	±	$4{\cdot}5 \times 10^{8}$	1×10^{8}		5×10^{6} (Proteus)
37		4×10^{9}		$1{\cdot}8 \times 10^{10}$				2×10^{7} (Proteus)
41		$8{\cdot}5 \times 10^{7}$	7×10^{7}	1×10^{7}				
Other hospital patients								
55		$2{\cdot}1 \times 10^{10}$	1×10^{8}					
56		$1{\cdot}5 \times 10^{8}$	$1{\cdot}4 \times 10^{7}$	$2{\cdot}5 \times 10^{11}$		$2{\cdot}5 \times 10^{8}$		2×10^{7} (Staph.albus)
57	4×10^{10}	6×10^{7}	4×10^{8}	$5{\cdot}5 \times 10^{8}$				
58	$8{\cdot}5 \times 10^{10}$	$5{\cdot}5 \times 10^{7}$	$2{\cdot}5 \times 10^{7}$	5×10^{8}		1×10^{9}		
59		$2{\cdot}4 \times 10^{9}$	$2{\cdot}5 \times 10^{9}$					
60		$3{\cdot}3 \times 10^{8}$	$3{\cdot}5 \times 10^{7}$	3×10^{9}			5×10^{7}	$3{\cdot}2 \times 10^{8}$ (Micrococcus)
61		$2{\cdot}8 \times 10^{7}$		$2{\cdot}3 \times 10^{9}$		5×10^{7}		
62		7×10^{8}	1×10^{10}	3×10^{9}	$7{\cdot}5 \times 10^{9}$			
70		$1{\cdot}5 \times 10^{7}$	5×10^{9}					
71	$7{\cdot}4 \times 10^{10}$	8×10^{8}	8×10^{7}	$2{\cdot}5 \times 10^{9}$				
72	$3{\cdot}4 \times 10^{10}$	$2{\cdot}3 \times 10^{8}$	5×10^{6}	2×10^{8}				
73		$1{\cdot}3 \times 10^{8}$	$1{\cdot}5 \times 10^{7}$	$1{\cdot}3 \times 10^{9}$				
74		$1{\cdot}5 \times 10^{7}$	$9{\cdot}3 \times 10^{8}$	$1{\cdot}2 \times 10^{9}$				
75		$1{\cdot}1 \times 10^{10}$	$7{\cdot}5 \times 10^{8}$	1×10^{10}	5×10^{8}			
76		$1{\cdot}4 \times 10^{7}$	$4{\cdot}1 \times 10^{7}$	+ + +				
77		1×10^{8}	$2{\cdot}5 \times 10^{8}$	$9{\cdot}4 \times 10^{9}$				
78	$3{\cdot}4 \times 10^{10}$	$4{\cdot}6 \times 10^{8}$	$3{\cdot}6 \times 10^{8}$	3×10^{8}				

+++ = profuse growth (Colonies not counted)

± = a few colonies.

Smear counts done in some cases only.

TABLE C

Biochemical findings in intestinal aspirates and the effect of incubation of ileal fluid under various conditions on the pH and the contents of ammonia, methionine, amines and mercaptans.

Concentrations of ammonia nitrogen (NH_4) and methionine (Meth) in µg per ml

am = amino present Mec = smell of mercaphans

Subject's No.	INITIAL: Ileal pH	Ileal NH_4	Ileal Meth	Ileal Am	Ileal Mec	Duodenal pH	Gastric pH	ILEAL FLUID: +Water pH	+Water NH_4	+Water Meth	+Water Am	+Water Mec	+Methionine pH	+Methionine NH_4	+Methionine Meth	+Methionine Am	+Methionine Mec
Cirrhosis of the Liver																	
2	7·7	20	70	-	-	-	-	-0·8	+207	0	0	+	-0·7	+234	-200	0	+
3	8·1	33	33	0	0	-	7.5	-0·8	+215	+61	0	0	-0·9	+288	-404	0	+
9	7·5	66	98	-	0	-	-	-	+152	-	-	-	-	+160	-	-	-
18	8·1	0	17	0	0	-	2·5	-	-	-	-	-	-	-	-	-	-
19[x]	7·8	28	61	0	0	7·2	1·8	+0·4	-9	+9	0	0	+0·5	+71	-158	0	+
20	8·0	6	108	-	0	-	-	-	-	-	-	-	-	-	-	-	-
21	7·7	8	98	0	0	-	6.7	-0·5	+197	-2	0	0	-0·5	+193	-215	0	+
23	6·3	6	83	0	0	1·8	2·0	-	-	-	-	-	-	-	-	-	-
24	8.1	10	16	-	0	-	-	-1·0	+298	+28	0	0	-	+320	-	-	-
25	8·4	5	6	0	0	1·8	-	-1·0	+301	+24	+	0	-	-	-	-	-
26	6·4	28	21	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Mean	7·6	18	55					-0·7	+197	+20			-0·4	+211	-244		
Gastro-intestinal disorders																	
45	6·8	70	124	0	0	-	-	-	-	-	-	-	-	-	-	-	-
46	7.1	46	75	-	0	-	-	-	-	-	-	-	-	-	-	-	-
47	-	-	-	-	-	5·6	-	-	-	-	-	-	-	-	-	-	-
48	7·9	2	55	-	0	-	2·1	-0·2	+19	+67	0	0	-0·2	+32	-144	0	+
49	5·8	19	156	0	0	-	-	-0·5	+25	-30	0	0	-0·1	+25	-276	0	0
Mean	6·9	34	102					-0·3	+22	+18			-0·1	+28	-210		
Normal subjects																	
63	8·1	4	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-
64	8·0	43	77	-	0	3·6	-	-0·2	+169	-71	0	0	-0·2	+245	-14	0	0
65	8·2	0	7	-	0	-	1.4	-	+201	-	-	-	-	+216	-	-	-
66	8·9	53	-	-	0	-	1·7	-	-	-	-	-	-	-	-	-	-
67	7·2	1	57	0	0	-	-	+0·1	+1	+6	0	0	+0·8	+7	-29	0	0
68	7·6	5	111	0	0	-	5·0	+0·6	+2	-11	0	0	+0·5	+10	-20	0	0
Mean	7·9	18	64					+0·2	+93	-25			+0·4	+122	-21		

Subject's No.	CHANGES AFTER INCUBATION: +Glucose pH	+Glucose NH_4	+Glucose Meth	+Glucose Am	+Glucose Mec	+Glucose +Methionine pH	+Glucose +Methionine NH_4	+Glucose +Methionine Meth	+Glucose +Methionine Am	+Glucose +Methionine Mec	+Methionine +Chlortetracycline pH	+Methionine +Chlortetracycline NH_4	+Methionine +Chlortetracycline Meth	+Methionine +Chlortetracycline Am	+Methionine +Chlortetracycline Mec	+Glucose +Methionine +Chlortetracycline pH	+Glucose +Methionine +Chlortetracycline NH_4	+Glucose +Methionine +Chlortetracycline Meth	+Glucose +Methionine +Chlortetracycline Am	+Glucose +Methionine +Chlortetracycline Mec
Cirrhosis of the Liver																				
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-2·8	+52	+9	+	0	-2·8	+56	-111	+	0	-	-	-	-	-	-0·1	+10	-7	0	0
19[x]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+0·1	-6	-5	0	0
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-1·3	+263	-16	+	+	-3·5	+55	-15	+	0	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	-1·8	-208	+12			-2·5	+55·5	-140								0	+2	-6		
Gastro-intestinal disorders																				
45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-2·2	+4	+49	0	0	-2·7	+8	-46	0	0	-	-	-	-	-	-0·1	+2	+14	0	0
49	-1·5	+19	+2	0	0	-1·4	+10	-102	0	0	-0·5	-6	-8	-	0	-0·2	+3	-6	0	0
Mean	-1·8	+12	+25			-2·0	+9	-74								-0·1	+2	+4		
Normal subjects																				
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
64	-	-	-	-	-	-3·1	+67	+118	+	-	-	-	-	-	-	-	-	-	-	-
65	-1·4	-	+8	+	0	-1·0	+67	-94	+	+	-	-	-	-	-	-	-	-	-	-
66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
67	-1·6	+7	-13	0	0	-1·6	+50	-85	0	0	+0·7	+1	-10	-	0	+0·6	+4	-8	-	0
68	+0·2	+1	-16	0	0	-0·6	+40	-141	0	0	+0·5	+5	-41	-	0	+0·1	+61	-90	-	0
Mean	-0·9	+4	-7			-1·6	+56	-50			+0·6	+3	-25			+0·3	+32	-49		

[x] Ileal juice incubated with Lactose: pH -2·9, NH_4 +39, Meth -9, Incubated Lactose and methionine pH -1·2, NH_4 +13, Meth -40. Incubated +Lactose, methionine and chlortetracycline, pH +0·3, NH_4 -6, Meth -12.

TABLE D

Bacterial growth and ammonia production in Ileal fluid during incubation.

Figures represent ammonia in µg N/ml. Bacterial growth is expressed semi-quantitatively (See P 46).

Subject's No.	Organisms	Initial Content	Content after incubation with: Water	Glucose	Methionine	Methionine + Glucose	Methionine + Chlortetracycline	Methionine + Glucose + Chlortetracycline
Patients with Cirrhosis		20	+207		+234			
2	Coliform orgs.	+++						
	Strep.faecalis	++	?		?			
	Proteus	+++						
3		33	+215		+288			
	Coliform orgs.	+++	+++		+++			
	Strep.faecalis	++	+++		+++			
	Bacteroides	+++	-		-			
	Proteus	+++	-		-			
9		66	+169		+160			
	E.coli	+++						
	Strep.faecalis	++	?		?			
	Bacteroides	+						
	Proteus	+						
18		0		+52		+56		
	Klebsiella	+		+++		+++		
19		28	-9	+39'	+71	+13'		-6'
	E.freundii	-	-	+++	-	-		±
	A.cloacae	-	+++	-	+++	+++		-
	Strep.faecalis	-	-	-	+++	+++		-
	Resp.orgs.	±	-	-	+++	+++		-
	Candida	±	-	+	-	-		-
	Lactobacilli	+	-	-	-	-		±
21		8	+197		+193			
	Coliform orgs.	+++	?		?			
24		10	+298		+320			
	E.freundii	±	+++		+++			
	Strep.faecalis	++	+++		+++			
	Resp.orgs.	++	-		-			
25		5	+301	+263		+55		
	Coliform orgs.	±	+++	+++		+++		
	Ps.pycocyanea	±	+++	+++		+++		
	Resp.orgs.	++	-	-		-		
Patients with gastro-intestinal disorders								
48		2	+19	++	+32	+8		+2
	E.freundii	±	?	+++	+++	+++		++
	Strep.faecalis	±		-	+	-		-
49		19	+25	+19	+25	+10	-6	+3
	E.coli	+	+++	+++	+++	+++	++	++
	E.freundii	±	+++	+++	+++	+++	+	+
Normal subjects								
64		43	+169		+245	+67		
	E.coli	-	+++		+++	+++		
	Resp.orgs.	+++	-		-	-		
	Anaerobic streps.	+	-		-	-		
65		0	+201		+216	+67		
	E.coli	-	+++		+++	+++		
	Strep.faecalis	-	+		+	+		
	Ps.pyocyanea	-	-		+	-		
	Bact.alkalig.	±	-		-	-		
67		1	+1	+7	+7	+50	+1	+4
	E.freundii	-	+++	+++	+++	+++	+	-
	Strep.faecalis	-	-	+	-		+	+
	Respiratory orgs.	±	-	-	-		-	-
	Anaerobic Streps.	±	-	-			-	-
68		5	+2	+1	+10	+40	+5	+61
	E.coli	-	-	-	-	+++	-	±
	Paracolon Bac.	-	-	-	++	+++	-	-
	Strep.faecalis	-	-	-	++	-	-	-
	Bact.alkalig.	-	++	++	-	-	-	-
	Lactobacilli	++	-	-	-	-	-	-
	Resp.orgs.	++	-	-	-	-	++	++
	Anaerobic streps.	-	-	-	-	+++	-	-
	Mean	17	+138	+55	+116	+41	0	+14

' Lactose added instead of glucose.

"Respiratory Organisms" include one or more of the following: Strep.viridans, Micrococci, Staph. albus and Diphtheroids.

TABLE E

Bacterial growth and changes in Methoinine content of ileal fluid during incubation

Figures represent Methoinine in ug N/ml. Bacteria growth is expressed semi-quantitatively (See P 46).

Subject's No	organisms	Initial content	Water	Glucose	Methionine	Methionine + Glucose	Methionine + Chlortetracyecline	Methionine + Glucose + Chlortetracycline
Patients with Cirrhosis								
2		70	0		-200			
	Coliform orgs.							
	Strep.faecalis	++	?		?			
	Protens	+++						
3		33	+61		-404			
	Coliform orgs.	+++	+++		+++			
	Strep.faecalis	++	+++		+++			
	Bacteroids	+++	-		-			
	Protens	+++	-		-			
18		11		+9	-111			
	Klebsiella	+		+++	+++			
19		61	+9	-9'	-158	-40'		-12'
	E.freundii	-	-	+++	-	-		±
	A.Cloacae	-	+++	-	+++	+++		-
	Strep. faecalis	-	-	-	+++	+++		-
	Resp. orgs.	±	-	-	+++	+++		-
	Candida	±	-	+	-	-		-
	Lactobacilli	+	-	-	-	-		±
21		98	-2		-215			
	Coliform Orgs.	+++	?		?			
24		16	+28					
	E.freundii	±	+++					
	Strep.faecalis	++	+++					
	Resp. orgs.	++	-					
25		6	+24	-16		-15		
	Coliform orgs.	±	+++	+++		+++		
	Ps.pyocyanea	±	+++	+++		+++		
	Resp. orgs.		-	-		-		
Patients with gastro-intestinal disorders								
48		55	+67	+49	-144	-46		+14
	E. freundii	±		+++	+++	+++		++
	Strep.faecalis	±	?	-	-	-		-
49		156	-30	+2	-276	-102	-8	-6
	E.coli	+	+++	+++	+++	+++	++	++
	E.freundii	±	+++	+++	+++	+++	+	+
Normal subjects								
64		77	-71		-14	+118		
	E.coli	-	+++		+++	+++		
	Respiratory orgs.	+++	-		-	-		
	Anaerobic Streps.	±	-		-	-		
65		7		+8		-94		
	E.coli	-		+++		+++		
	Strep.faecalis	-		+		+		
	Ps.pycyanea	-		-		-		
	Bact.alkalig.	+		-		-		
67		57	+6	-13	-29	-85	-10	-8
	E.freundii	-	+++	+++	+++	+++	+	-
	Strep.faecalis	-	-	+	-	-	+	+
	Respiratory orgs.	±	-	-	-	-	-	-
	Anaerobic Streps.	±	-	-	-	-	-	-
68		117	-11	-16	-20	-141	-41	-90
	E.coli	-	-	-	-	+++	-	±
	Paracolon Bacillus	-	-	-	++	+++	-	-
	Strep.faecalis	-	-	-	++	-	-	-
	Bact.alkalig.	-	++	++	-	-	-	-
	Lactobacilli	++	-	-	-	-	-	-
	Resp.orgs.	++	-	-	-	-	++	++
	Anaerobic Streps.	-	-	-	-	+++	-	-
	Mean	63	+75	+2	-157	-50	-20	-18

' Lactose added instead of glucose.

"Respiratory organisms" include one or more of the following: Strep.viridans, Micrococci, Staph. Albus and Diphtheroids.

FAECES BEFORE THERAPY

Subject's No. and	Date of spec	S.C.	Coli	St.f.	St.an.	Bac.	Lac.	Cl.w.	Others
Dates of Therapy	24.10[x]	$2{\cdot}5 \times 10^{10}$	$4{\cdot}3 \times 10^{8}$	5×10^{7}	-	-	-	±	-
	28.10	-	$3{\cdot}2 \times 10^{8}$	6×10^{8}	-	-	-	-	-
1 (A)		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
30.10 - 4.11									
	24.9[x]	-	1×10^{6}	$2{\cdot}2 \times 10^{8}$	-	-	-	$3{\cdot}9 \times 10^{8}$	-
	27.7	-	1×10^{8}	$1{\cdot}1 \times 10^{8}$	-	-	-	$6{\cdot}4 \times 10^{7}$	-
2 (B)	29.9	$1{\cdot}8 \times 10^{11}$	9×10^{8}	$1{\cdot}2 \times 10^{8}$	-	-	-	$1{\cdot}9 \times 10^{8}$	-
		-	-	-	-	-	-	-	-
29.9 - 4.10		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
25.10 - 29.10		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
13.4 - 20.4	13.4	$6{\cdot}2 \times 10^{10}$	9×10^{8}	$8{\cdot}5 \times 10^{8}$	-	$4{\cdot}5 \times 10^{9}$	-	-	-
3 (C)	25.11	$5{\cdot}5 \times 10^{10}$	$5{\cdot}5 \times 10^{7}$	6×10^{8}	-	3×10^{8}	-	-	-
	27.11	$3{\cdot}8 \times 10^{10}$	$4{\cdot}6 \times 10^{8}$	6×10^{7}	-	2×10^{9}	-	-	-
27.11 - 2.12									
	22.3[x]	-	$2{\cdot}7 \times 10^{4}$	7×10^{8}	-	$1{\cdot}3 \times 10^{10}$	-	-	-
5.4 - 8.4	5.4	$1{\cdot}2 \times 10^{11}$	$4{\cdot}7 \times 10^{8}$	$1{\cdot}4 \times 10^{9}$	-	8×10^{10}	-	-	$1{\cdot}1 \times 10^{9}$ St.albus
		-	-	-	-	-	-	-	-
19.5 - 27.5	19.5	-	$4{\cdot}5 \times 10^{7}$	-	-	1×10^{10}	2×10^{8}	2×10^{8}	1×10^{9} St.albus
	16.11[x]	$3{\cdot}6 \times 10^{10}$	$2{\cdot}5 \times 10^{9}$	5×10^{7}	-	3×10^{9}	-	-	-
4 (D)	23.11	$2{\cdot}9 \times 10^{10}$	$1{\cdot}1 \times 10^{8}$	3×10^{7}	$4{\cdot}6 \times 10^{8}$	$9{\cdot}9 \times 10^{8}$	-	-	-
	24.11	$5{\cdot}2 \times 10^{10}$	$5{\cdot}9 \times 10^{9}$	9×10^{7}	7.5×10^{8}	$1{\cdot}5 \times 10^{9}$	-	-	-
27·11 - 3.12		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
2.4 - 5.4	2.4	-	$3{\cdot}5 \times 10^{8}$	$1{\cdot}5 \times 10^{9}$	-	8×10^{9}	±	-	-
		-	-	-	-	-	-	-	-
5 (E)	2.3[x]	-	$5{\cdot}3 \times 10^{8}$	$1{\cdot}3 \times 10^{9}$	-	9×10^{8}	-	1×10^{8}	$1{\cdot}5 \times 10^{8}$ (Mic)
	4·3[x]	-	3×10^{8}	$2{\cdot}2 \times 10^{9}$	-	5×10^{8}	-	2×10^{8}	-
13.3 - 21.3	7.3[x]	$4{\cdot}5 \times 10^{10}$	$1{\cdot}1 \times 10^{8}$	1×10^{8}	9×10^{8}	$1{\cdot}3 \times 10^{9}$	-	$1{\cdot}5 \times 10^{8}$	-
	10·3	-	$4{\cdot}4 \times 10^{8}$	$8{\cdot}5 \times 10^{8}$	-	+	+	+	-
	12.3	-	$1{\cdot}3 \times 10^{8}$	$4{\cdot}5 \times 10^{8}$	8×10^{8}	$7{\cdot}5 \times 10^{8}$	-	$7{\cdot}5 \times 10^{8}$	-
7.5 - 13.5		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-

[x] Specimens not shown in diagrams.

Abbreviations as for Table A. In addition (1) St.f.(h) = haemolytic strep faecalis. (2) St.alb. = Staph.albus. (3) St.pyog. = Staph.pyogenes.

TABLE F (I).

The effect of Chlortetracycline on the faecal flora.

FAECES DURING THERAPY

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
1.11	$2{\cdot}3 \times 10^{10}$	$2{\cdot}9 \times 10^{7}$	$1{\cdot}3 \times 10^{8}$	$6{\cdot}6 \times 10^{7}$	1×10^{8}	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
1.10	$6{\cdot}3 \times 10^{9}$	$2{\cdot}3 \times 10^{7}$	-	-	-	-	-	-	4×10^{6}	7×10^{7} (St.albus)
4.10	-	-	1×10^{6}	$5{\cdot}6 \times 10^{7}$	-	$1{\cdot}1 \times 10^{7}$	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
27.10	$3{\cdot}1 \times 10^{10}$	-	$2{\cdot}1 \times 10^{9}$	-	$1{\cdot}5 \times 10^{9}$	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
15.4	$1{\cdot}5 \times 10^{10}$	-	-	-	-	-	-	5×10^{9}	-	$8{\cdot}5 \times 10^{8}$ Mic. $8{\cdot}5 \times 10^{8}$ St.pyogenes
29.11	$1{\cdot}5 \times 10^{10}$	1×10^{7}	$7{\cdot}2 \times 10^{8}$	$9{\cdot}4 \times 10^{8}$	-	-	-	-	-	-
30.11	-	1×10^{7}	$4{\cdot}8 \times 10^{8}$	$1{\cdot}3 \times 10^{9}$	-	$3{\cdot}8 \times 10^{9}$	-	-	-	-
7.4	-	4×10^{8}	-	7×10^{8}	-	-	1×10^{9}	-	-	$4{\cdot}7 \times 10^{8}$ St.albus
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
21.5	-	5×10^{6}	-	-	-	-	5×10^{9}	$1{\cdot}2 \times 10^{9}$	-	1×10^{9} St.albus
27.5	-	-	-	5×10^{8}	1×10^{9}	-	-	$2{\cdot}5 \times 10^{9}$	-	
29.11	$9{\cdot}5 \times 10^{9}$	-	$7{\cdot}5 \times 10^{8}$	-	-	-	$7{\cdot}5 \times 10^{8}$	-	-	-
2.12	$8{\cdot}7 \times 10^{9}$	-	8×10^{8}	$2{\cdot}5 \times 10^{7}$	-	-	-	-	-	-
3.12	$2{\cdot}5 \times 10^{10}$	$2{\cdot}5 \times 10^{8}$	$2{\cdot}5 \times 10^{8}$	-	$1{\cdot}5 \times 10^{8}$	-	-	4×10^{9}	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
5.4	-	2×10^{7}	5×10^{8}	4×10^{7}	-	5×10^{9}	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
15.3	-	1×10^{8}	-	$1{\cdot}5 \times 10^{8}$	-	1×10^{8}	-	-	-	-
19.3	$3{\cdot}5 \times 10^{10}$	1×10^{7}	$2{\cdot}1 \times 10^{8}$	9×10^{8}	-	-	-	-	-	-
21.3	-	-	5×10^{8}	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
9.5	-	2×10^{7}	-	$4{\cdot}4 \times 10^{8}$	$6{\cdot}5 \times 10^{8}$	1×10^{9}	1×10^{10}	-	$6{\cdot}5 \times 10^{8}$	-
13.5	-	-	5×10^{6}	$2{\cdot}5 \times 10^{8}$	6×10^{8}	2×10^{8}	-	-	-	-

3

FAECES AFTER THERAPY

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
5.11	$2{\cdot}4 \times 10^{10}$	-	$2{\cdot}9 \times 10^{8}$	-	-	-	-	-	-	-
12.11	$7{\cdot}6 \times 10^{10}$	$4{\cdot}3 \times 10^{7}$	$6{\cdot}5 \times 10^{8}$	$7{\cdot}5 \times 10^{6}$	-	-	-	-	-	-
3.12[x]	$4{\cdot}9 \times 10^{10}$	4×10^{7}	$1{\cdot}8 \times 10^{9}$	7×10^{9}	-	-	5×10^{9}	+	-	-
10.12[x]	-	$1{\cdot}1 \times 10^{7}$	5×10^{9}	-	-	-	5×10^{10}	-	-	$1{\cdot}1 \times 10^{7}$ (Candida)
6.10	6×10^{8}	-	$1{\cdot}4 \times 10^{8}$	-	$2{\cdot}2 \times 10^{7}$	$4{\cdot}3 \times 10^{7}$	-	-	-	-
6.10	$6{\cdot}7 \times 10^{7}$	-	$7{\cdot}7 \times 10^{7}$	-	$1{\cdot}6 \times 10^{7}$	$5{\cdot}7 \times 10^{7}$	-	-	-	-
19.10[x]	-	-	-	-	$9{\cdot}6 \times 10^{7}$	$2{\cdot}4 \times 10^{7}$	$2{\cdot}4 \times 10^{8}$	-	$6{\cdot}4 \times 10^{7}$	-
19.10[x]	4×10^{8}	-	-	-	$9{\cdot}9 \times 10^{7}$	$2{\cdot}5 \times 10^{8}$	-	-	4×10^{7}	-
21.10[x]	$2{\cdot}9 \times 10^{10}$	$1{\cdot}3 \times 10^{8}$	-	$2{\cdot}5 \times 10^{7}$	-	1×10^{8}	-	-	$3{\cdot}7 \times 10^{7}$	-
2.11	$1{\cdot}8 \times 10^{10}$	8×10^{8}	1×10^{8}	-	$4{\cdot}8 \times 10^{8}$	-	-	-	-	-
4.11	-	$1{\cdot}1 \times 10^{8}$	$3{\cdot}7 \times 10^{8}$	$1{\cdot}7 \times 10^{8}$	5×10^{7}	-	-	-	-	-
10.1[x]	$3{\cdot}1 \times 10^{10}$	$1{\cdot}1 \times 10^{8}$	6×10^{8}	-	-	-	1×10^{10}	-	1×10^{8}	-
28.2[x]	$1{\cdot}3 \times 10^{11}$	$6{\cdot}5 \times 10^{9}$	-	-	-	$1{\cdot}1 \times 10^{10}$	6×10^{9}	-	-	-
10.3[x]	$6{\cdot}5 \times 10^{10}$	$2{\cdot}3 \times 10^{8}$	-	1×10^{9}	-	-	$3{\cdot}2 \times 10^{9}$	-	-	-
15.3[x]	$3{\cdot}7 \times 10^{10}$	8×10^{8}	-	-	-	-	+	+	-	-
	-	-	-	-	-	-	-	-	-	-
28.4	$1{\cdot}2 \times 10^{9}$	-	-	-	-	-	4×10^{8}	-	-	-
13.12	3.9×10^{10}	2×10^{8}	$3{\cdot}7 \times 10^{8}$	$6{\cdot}1 \times 10^{9}$	-	-	-	-	-	2×10^{8} (St.albus)
15.12	$2{\cdot}2 \times 10^{10}$	$2{\cdot}5 \times 10^{7}$	$2{\cdot}8 \times 10^{8}$	8×10^{7}	-	-	-	-	-	-
13.4	$3{\cdot}5 \times 10^{10}$	$2{\cdot}5 \times 10^{8}$	4×10^{7}	6×10^{8}	-	1×10^{9}	$7{\cdot}5 \times 10^{8}$	-	-	-
22.4	-	1×10^{9}	4×10^{8}	-	$1{\cdot}5 \times 10^{8}$	±	$2{\cdot}5 \times 10^{9}$	±	-	-
26.4[x]	-	$7{\cdot}5 \times 10^{8}$	-	$7{\cdot}5 \times 10^{8}$	-	-	1×10^{10}	-	-	-
4.5[x]	-	$3{\cdot}1 \times 10$	-	1×10^{9}	1×10^{8}	-	$2{\cdot}4 \times 10^{10}$	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
5.12	3×10^{10}	$9{\cdot}9 \times 10^{8}$	1×10^{7}	-	-	-	$1{\cdot}5 \times 10^{10}$	4×10^{8}	-	-
9.12	-	$4{\cdot}8 \times 10^{9}$	5×10^{8}	-	$2{\cdot}5 \times 10^{8}$	-	1×10^{10}	-	-	-
28.12[x]	$2{\cdot}2 \times 10^{10}$	$1{\cdot}5 \times 10^{7}$	-	$8{\cdot}5 \times 10^{7}$	-	-	1×10^{9}	-	-	-
15.2[x]	$6{\cdot}9 \times 10^{10}$	$2{\cdot}6 \times 10^{9}$	2×10^{8}	$4{\cdot}4 \times 10^{6}$	-	-	1×10^{11}	-	-	-
4.3[x]	$8{\cdot}5 \times 10^{10}$	$1{\cdot}8 \times 10^{9}$	5×10^{6}	$1{\cdot}8 \times 10^{8}$	-	-	1×10^{10}	-	-	-
14.3[x]	$7{\cdot}5 \times 10^{10}$	$8{\cdot}4 \times 10^{8}$	-	$3{\cdot}4 \times 10^{8}$	-	4×10^{9}	$1{\cdot}5 \times 10^{10}$	-	-	-
24.3[x]	-	$1{\cdot}2 \times 10^{9}$	-	+	-	-	+	+	-	-
27.3[x]	-	2.6×10^{9}	-	±	-	-	$1{\cdot}2 \times 10^{9}$	±	-	-
14.4	-	-	$2{\cdot}5 \times 10^{7}$	9×10^{8}	-	-	-	-	-	-
18.4	$1{\cdot}2 \times 10^{11}$	$1{\cdot}8 \times 10^{8}$	5×10^{6}	$7{\cdot}5 \times 10^{7}$	-	-	5×10^{8}	-	-	-
25.3	2.5×10^{11}	$2{\cdot}2 \times 10^{9}$	$2{\cdot}5 \times 10^{7}$	-	5×10^{7}	±		±	-	-
5.4	$5{\cdot}2 \times 10^{10}$	$1{\cdot}9 \times 10^{9}$	1×10^{7}	5×10^{8}	-	-	$3{\cdot}3 \times 10^{9}$	-	-	-
2.5[x]	-	$3{\cdot}8 \times 10^{9}$	-	$1{\cdot}3 \times 10^{9}$	-	-	3×10^{9}	5×10^{9}	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-

SUBJECTS WITHOUT LIVER DISEASE

BEFORE THERAPY

No.	Date	S.C.	Coli	St.f.	St.an.	Bac.	Lac.	Cl.w.	Others
57 (T)									
9.2 - 12.2	9.2	4×10^{10}	1×10^{7}	-	-	-	6×10^{9}	-	-
		-	-	-	-	-	-	-	-
60 (X)	11.8	-	$3{\cdot}3 \times 10^{8}$	$3{\cdot}5 \times 10^{7}$	-	3×10^{9}	-	5×10^{7}	$3{\cdot}2 \times 10^{8}$ (Mic)
		-	-	-	-	-	-	-	-
11.8 - 17.8		-	-	-	-	-	-	-	-
61 (Y)	12.8	-	$2{\cdot}5 \times 10^{8}$	-	5×10^{7}	$2{\cdot}3 \times 10^{9}$	-	-	-
		-	-	-	-	-	-	-	-
13.8 - 20.8		-	-	-	-	-	-	-	-
70 (V)	9.2	-	$3{\cdot}2 \times 10^{6}$	$1{\cdot}5 \times 10^{5}$	-	4×10^{8}	9×10^{8}	-	-
9.2 - 12.2		-	-	-	-	-	-	-	-
71 (W)	22.2	$7{\cdot}4 \times 10^{10}$	8×10^{8}	8×10^{7}	-	$2{\cdot}4 \times 10^{9}$	-	-	-
		-	-	-	-	-	-	-	-
22.2 - 25.2									
77 (Z)		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
13.8 - 21.8		-	-	-	-	-	-	-	-

TABLE F (II)

DURING THERAPY

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
13.2	$6{\cdot}3 \times 10^{10}$	8×10^{7}	-	2×10^{8}	$5{\cdot}5 \times 10^{7}$	-	-	5×10^{9}	-	-
15.2	$9{\cdot}5 \times 10^{9}$	4×10^{8}	5×10^{7}	4×10^{9}	$5{\cdot}5 \times 10^{9}$	-	-	$1{\cdot}8 \times 10^{9}$	-	-
8.3	$4{\cdot}4 \times 10^{10}$	$7{\cdot}4 \times 10^{8}$	1×10^{7}	-	$7{\cdot}5 \times 10^{9}$	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
18.4	-	-	-	1×10^{9}	$2{\cdot}2 \times 10^{9}$	$2{\cdot}1 \times 10^{9}$	-	-	-	$2{\cdot}7 \times 10^{8}$ (St.pyogenes)
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
14.2	$2{\cdot}6 \times 10^{10}$	$5{\cdot}5 \times 10^{7}$	$1{\cdot}3 \times 10^{8}$	$2{\cdot}2 \times 10^{8}$	-	9×10^{8}	-	$1{\cdot}4 \times 10^{9}$	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
18.7	-	-	-	-	$1{\cdot}5 \times 10^{9}$	-	-	-	-	$2{\cdot}8 \times 10^{9}$ (St.pyogenes)
	-	-	-	-	-	-	-	-	-	$1{\cdot}5 \times 10^{9}$ (Mic)
27.2	-	-	-	3×10^{8}	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
25.6	-	$3{\cdot}3 \times 10^{6}$	$2{\cdot}5 \times 10^{7}$	1×10^{5}	-	-	-	-	-	1×10^{7} (St.albus)
	-	-	-	-	-	-	-	-	-	-
13.6	-	$6{\cdot}5 \times 10^{8}$	5×10^{6}	5×10^{6}	-	1×10^{8}	-	$1{\cdot}5 \times 10^{8}$	-	-
	-									
	-	-	-	-	-	-	-	-	-	-
13.8	-	4×10^{9}	2×10^{7}	-	-	-	$1{\cdot}8 \times 10^{10}$	-	-	-
15.8	-	$2{\cdot}4 \times 10^{10}$	-	-	-	-	-	-	-	-
18.8	-	6×10^{9}	-	7×10^{8}	-	-	-	-	-	-
10.2	-	6×10^{7}	-	-	-	-	-	-	-	-
12.2	$4{\cdot}4 \times 10^{10}$	1×10^{9}	-	$1{\cdot}5 \times 10^{7}$	-	-	-	$3{\cdot}1 \times 10^{9}$	-	-

AFTER THERAPY

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
18.2	$6{\cdot}3 \times 10^{10}$	-	$4{\cdot}8 \times 10^{8}$	$1{\cdot}8 \times 10^{10}$	-	$3{\cdot}6 \times 10^{9}$	-	-	-	-
25.2	$5{\cdot}1 \times 10^{10}$	$3{\cdot}6 \times 10^{8}$	$1{\cdot}1 \times 10^{9}$	-	$3{\cdot}5 \times 10^{9}$	-	1×10^{9}	-	-	1×10^{9} (Mic)
11.3	$7{\cdot}5 \times 10^{9}$	-	$3{\cdot}8 \times 10^{8}$	3×10^{8}	$1{\cdot}1 \times 10^{9}$	$2{\cdot}3 \times 10^{8}$	-	-	-	-
15.3	-	$5{\cdot}3 \times 10^{8}$	1×10^{9}	3×10^{9}	-	-	-	-	-	-
19.3	-	$1{\cdot}2 \times 10^{9}$	3×10^{8}	$3{\cdot}5 \times 10^{8}$	1×10^{9}	2×10^{9}	7×10^{9}	±	-	-
22.3	-	$2{\cdot}4 \times 10^{8}$	2×10^{7}	2×10^{7}	1×10^{8}	-	$2{\cdot}5 \times 10^{8}$	3×10^{9}	-	-
28.5[x]	$6{\cdot}4 \times 10^{10}$	5×10^{9}	5×10^{7}	1×10^{8}	-	9×10^{8}	5×10^{9}	-	-	-
14.4[x]	4×10^{10}	$2{\cdot}8 \times 10^{8}$	-	$8{\cdot}5 \times 10^{7}$	5×10^{7}	$1{\cdot}3 \times 10^{9}$	$6{\cdot}7 \times 10^{8}$	-	-	-
25.4	-	-	$2{\cdot}9 \times 10^{8}$	-	$1{\cdot}9 \times 10^{9}$	-	-	-	-	-
3.5	-	6×10^{8}	$1{\cdot}5 \times 10^{7}$	1×10^{9}	2×10^{9}	$7{\cdot}5 \times 10^{8}$	-	-	-	-
11.8	-	-	$2{\cdot}5 \times 10^{8}$	-	-	-	-	-	-	-
16.8	-	5×10^{8}	-	-	-	-	-	-	-	-
18.2	-	$1{\cdot}3 \times 10^{10}$	8×10^{9}	$2{\cdot}8 \times 10^{8}$	-	-	8×10^{8}	-	$2{\cdot}7 \times 10^{9}$	-
25.2	-	$7{\cdot}4 \times 10^{10}$	5×10^{9}	-	$1{\cdot}9 \times 10^{9}$	$1{\cdot}2 \times 10^{9}$	5×10^{9}	8×10^{9}	-	-
4.3[x]	-	-	$2{\cdot}6 \times 10^{9}$	-	-	-	$1{\cdot}3 \times 10^{9}$	$7{\cdot}5 \times 10^{9}$	-	-
8.3[x]	-	$4{\cdot}7 \times 10^{10}$	6×10^{8}	-	6×10^{8}	-	6×10^{8}	-	-	-
17.3[x]	-	$1{\cdot}2 \times 10^{11}$	$1{\cdot}5 \times 10^{8}$	-	6×10^{9}	-	-	$1{\cdot}2 \times 10^{10}$	-	-
22.3[x]	-	-	8×10^{8}	-	9×10^{7}	-	-	±	$1{\cdot}3 \times 10^{9}$	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
29.6	-	1×10^{8}	1×10^{7}	1×10^{8}	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
25.6	-	-	7.5×10^{9}	$1{\cdot}5 \times 10^{9}$	-	1×10^{8}	-	3×10^{9}	-	-
1.7	-	-	$2{\cdot}5 \times 10^{8}$	$1{\cdot}2 \times 10^{9}$	-	-	-	-	-	-
7.7	-	2.8×10^{8}	9×10^{7}	$1{\cdot}5 \times 10^{8}$	-	-	1×10^{10}	-	-	-
23.8	-	$8{\cdot}5 \times 10^{7}$	$1{\cdot}5 \times 10^{7}$	$1{\cdot}5 \times 10^{8}$	-	-	-	-	-	-
25.8	-	$2{\cdot}8 \times 10^{9}$	$7{\cdot}5 \times 10^{7}$	$1{\cdot}1 \times 10^{9}$	-	-	-	-	-	-
14.2	$2{\cdot}7 \times 10^{10}$	$2{\cdot}5 \times 10^{8}$	$4{\cdot}5 \times 10^{7}$	-	-	-	-	$2{\cdot}3 \times 10^{9}$	-	-
18.2	-	$2{\cdot}4 \times 10^{9}$	±				$3{\cdot}2 \times 10^{9}$	-		

BEFORE THERAPY

No.	Date	S.C.	Coli	St.f.	St.an.	Bac.	Lac.	Cl.w.	Others
7 (F)	9.2	$4{\cdot}8 \times 10^{10}$	$9{\cdot}4 \times 10^{8}$	1×10^{8}	-	$1{\cdot}7 \times 10^{9}$	-	-	-
10.2 - 16.2		-	-	-	-	-	-	-	-
3.3 - 8.3		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
16.4 - 23.4	16.4	$4{\cdot}5 \times 10^{10}$	$7{\cdot}5 \times 10^{7}$	-	-	$4{\cdot}4 \times 10^{9}$	-	-	-
		-	-	-	-	-	-	-	-
8 (R)		-	-	-	-	-	-	-	-
4.8 - 8.8		-	-	-	-	-	-	-	-
	10.2	$3{\cdot}6 \times 10^{10}$	$1{\cdot}6 \times 10^{9}$	-	$2{\cdot}8 \times 10^{9}$	$1{\cdot}9 \times 10^{9}$	$2{\cdot}7 \times 10^{9}$	-	-
9 (G)		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
10.2 - 16.2		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
18 (H)	10.7	-	$4{\cdot}5 \times 10^{7}$	-	$2{\cdot}5 \times 10^{9}$	-	6×10^{7}	-	-
10.7 - 18.7		-	-	-	-	-	-	-	-
29 (K)	20.7	-	1×10^{6}	4×10^{7}	-	-	-	-	3×10^{6} (Alk)
20.6 - 27.6									
30 (M)		-	-	-	-	-	-	-	-
18.6 - 24.6									
31 (N)	22.6	-	3×10^{8}	6×10^{7}	-	-	-	-	-
22.6 - 25.6									
36 (O)		-	-	-	-	-	-	-	-
11.6 - 14.6									
25.6 - 28.6		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
37 (P)		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
11.8 - 21.8		-	-	-	-	-	-	-	-
20 (I)	9.2	4×10^{10}	1×10^{7}	-	-	-	6×10^{9}	-	-
9.2 - 12.2									

DURING THERAPY

TABLE F (III).

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
10.2	-	6×10^7	-	-	-	-	$3{\cdot}5 \times 10^8$		-	-
11.2	$4{\cdot}4 \times 10^{10}$	1×10^9	-	$1{\cdot}5 \times 10^7$	-	$2{\cdot}1 \times 10^9$	$7{\cdot}5 \times 10^8$	$2{\cdot}8 \times 10^9$	-	-
13.8	-	$2{\cdot}5 \times 10^7$	-	$1{\cdot}1 \times 10^8$	-	-	5×10^8	-	-	-
15.8	-	$4{\cdot}7 \times 10^8$	8×10^7	$1{\cdot}6 \times 10^8$	-	-	-	-	-	-
17.8	-	$1{\cdot}2 \times 10^9$	-	5×10^9	-	-	-	-	-	-
15.8	-	$1{\cdot}3 \times 10^9$	-	-	-	-	-	$1{\cdot}3 \times 10^9$	-	-
18.8	-	$1{\cdot}8 \times 10^9$	$2{\cdot}5 \times 10^7$	$2{\cdot}3 \times 10^9$	-	-	-	$8{\cdot}5 \times 10^8$	-	-
20.8	-	$1{\cdot}2 \times 10^9$	3×10^8	3×10^9	-	-	-	$\pm$	-	-
10.2	-	$1{\cdot}5 \times 10^9$	-	-	-	-	5×10^9	-	-	-
16.2	-	-	-	$1{\cdot}2 \times 10^9$	-	-	-	$1{\cdot}2 \times 10^9$	-	-
24.2	6×10^{10}	8×10^8	-	-	$2{\cdot}6 \times 10^9$	-	-	-	-	-
25.2	$2{\cdot}7 \times 10^{10}$	-	-	$2{\cdot}5 \times 10^9$	$2{\cdot}8 \times 10^{10}$	-	-	-	-	-
14.8	-	1×10^8	-	$2{\cdot}5 \times 10^8$	-	-	$9{\cdot}4 \times 10^9$	-	-	-
18.8	-	2×10^8	7×10^8	-	$3{\cdot}5 \times 10^8$	$1{\cdot}3 \times 10^9$	-	-	-	-
20.8	-	$1{\cdot}8 \times 10^9$	-	-	$2{\cdot}5 \times 10^8$	$8{\cdot}5 \times 10^6$	-	-	-	-

9

AFTER THERAPY

Date	S.C.	Coli	P.	St.f.	St.f.(H)	St.an.	Bac.	Lac.	Cl.w.	Others
14.2	$1{\cdot}9 \times 10^{10}$	$1{\cdot}3 \times 10^{9}$	$6{\cdot}5 \times 10^{8}$	$2{\cdot}5 \times 10^{9}$	-	-	-	1×10^{9}	-	-
18.2	-	$5{\cdot}5 \times 10^{7}$	-	$1{\cdot}5 \times 10^{7}$	-	-	4×10^{9}	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
14.2	9×10^{9}	1×10^{7}	1×10^{8}	$1{\cdot}8 \times 10^{9}$	-	-	-	2×10^{8}	-	-
	-	-	-	-	-	-	-	-	-	-
28.2	3.7×10^{10}	$1{\cdot}1 \times 10^{9}$	-	1×10^{9}	$6{\cdot}2 \times 10^{9}$	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
23.8		$7{\cdot}9 \times 10^{9}$	$4{\cdot}5 \times 10^{7}$	$5{\cdot}5 \times 10^{9}$	-	-	-	-	-	-
29.8		$1{\cdot}3 \times 10^{8}$	-	-	$3{\cdot}5 \times 10^{7}$	-	$4{\cdot}8 \times 10^{9}$	-	-	-
		-	-	-	-	-	-	-	-	-

TABLE G

Ammonia production by gram positive bacteria.

Cultures grown in Amino Acid medium containing 0·5% glucose.

NH_4 production and growth in µg N/ml.

pH	6		7.2		8	
Species	Growth	NH_4 production	Growth	NH_4 production	Growth	NH_4 production
Clostridium Welchii			257 281 105 91 251 105 87	32 37 37 38 42 42 52		
Mean ± S.D.			182(±91)	40(±6)		
Lactobacillus Plantarum	15 4 63 17 66	2 2 5 6 8	10 16 24 43 10 46 39	0 2 4 4 5 5 7	30 37 15 10	3 3 12 19
Mean ± S.D.	33(±29)	5·0(±3)	27(±15)	4(±1)	23(±13)	9(±6)
Lactobacillus Bifidus			17 149 163 123 45	0 0 0 11 31		
Mean ± S.D.			99(±65)	8(±15)		
Cl. sporogenes			186 178 173 202 164 166 223 253	67 73 99 119 140 142 195 214		
Mean ± S.D.			193(±27)	131(±53)		
Staphylococcus Pyogenes			11 13 59 106 106 151	7 20 46 39 50 53		
Mean ± S.D.			74(±56)	36(±18)		
Strep. faecalis (Haemolytic)			73 22 113 119 69	5 6 32 36 46		
Mean ± S.D.			79(±39)	25(±18)		
Strep. faecalis (non-haemolytic)			113 119 69 106 99 82 79	29 32 37 38 40 47 53		
Mean ± S.D.			95(±19)	39(±7)		
Anaerobic Streptococcus			9 2 7 9 9 32 15 15 85 29	2 3 4 5 6 13 13 24 27 40		
Mean ± S.D.			21(±27)	14(±13)		

TABLE II

Ammonia production by gram negative organisms in the amnio acid medium.

Glucose when present at concentration of 0·5%.

NH_4 production and bacterial growth (Turbidity) in µg N/ml.

	6		7.2				8	
Glucose	-		-		+		-	
Species	Growth	NH_4	Growth	NH_4	Growth	NH_4	Growth	NH_4
E. freundii	125	173	131	154	75	88	42	145
	166	190	130	155	51	101	60	191
			102	166	62	104	85	202
			75	178	77	120		
					75	132		
Mean ± S.D.	145(±29)	181(±15)	109(±27)	163(±11)	68(±11)	109(±16)	62(±22)	179(±30)
E. coli					97	85		
					107	86		
					178	101		
					106	110		
Mean ± S.D.					122(±38)	95(±12)		
Klebsiella					187	53		
					185	55		
					183	81		
					130	97		
Mean ± S.D.					146(±39)	71(±21)		
Bact. alkaligenes			27	72				
			31	70				
Mean ± S.D.			29(±2)	71(±1)				
P. mirabilis	25	148	36	173	130	96	37	126
	42	150	36	195	123	100	47	137
					157	100		
					173	102		
					123	102		
					85	107		
					79	122		
					60	183		
					87	199		
					239	216		
Mean ± S.D.	33(±12)	149(±1)	36(±0)	184(±16)	127(±53)	133(±47)	43(±7)	131(±8)
P. vulgaris					136	74		
					140	79		
					129	85		
					139	85		
					220	90		
Mean ± S.D.					153(±38)	82(±6)		
P. morgani			80	149	161	83		
			77	150	165	83		
					138	133		
					108	138		
					140	146		
					140	150		
					189	166		
Mean ± S.D.			78(±2)	150(±1)	149(±26)	128(±28)		
Ps. pyocyanea					165	4		
					143	73		
					142	78		
					164	87		
					149	173		
Mean ± S.D.					155(±23)	83(±62)		
Bacteroides	6	4			70	9	1	7
	4	5			2	12	2	11
	2	9			8	15	2	19
	6	10			3	16	3	20
	3	13			2	17	4	21
	3	19			2	17		
	4	20			7	20		
	2	24			15	28		
Mean ± S.D.	4(±2)	13(±7)			13(±23)	17(±6)	2(±2)	16(±6)

TABLE I

The effect of chlortetracycline at concentrations just limiting growth on NH_4 production growth and NH_4 in µg N/ml.

Chlortetracycline	-		+		Concentration
Species	Growth	NH_4 Produced	Growth	NH_4 Produced	of Chlortetracycline
E. freundii	118	119	94	110	2·5
	114	132	86	98	
	104	122	86	92	
			79	86	
	80	120			
Mean	104	123	86	96	
S.D.	15	9	8	9	
E. coli	148	118	75	124	2·5
	129	151	72	122	
	127	105	60	94	
	106	158	60	93	
Mean	122	133	67	108	
S.D.	40	22	4	17	
			100	28	
Klebsiella	132	45	89	8	2·5
	132	41	89	7	
	92	95			
	81	81			
			52	18	
Mean	109	65	82	15	
S.D.	24	24	20	9	
Strep. facalis (haemolytic)	69	43	58	7	50
	69	54	56	4	
	69	43	56	14	
	65	40	52	17	
Mean	68	45	55	10	
S.D.	2	5	8	6	
Proteus mirabilis	162	116	112	103	50
	158	100	111	106	
			111	106	
	124	109	103	98	
	421	106			
Mean	141	107	109	103	
S.D.	21	19	8	8	
Proteus morgani	130	150	109	170	50
	130	146	91	178	
	109	167	82	173	
	101	158			
Mean	117	155	94	173	
S.D.	17	12	11	15	
Proteus vulgaris	134	121	78	146	50
	117	124	76	148	
Mean	125	122	77	147	
S.D.	14	3	1	1	
Lactobacillus plantarum	30	7	21	11	10
	24	4	18	1	
	24	0	13	10	
	23	2	11	5	
	21	9	11	5	
			9	0	
Mean	24	4	14	5	
S.D.	5	4	4	4	
Anaerobic Streptococcus	72	31	15	30	10
	31	19	8	30	
	12	20	8	16	
Mean	38	23	10	25	
S.D.	25	7	4	8	

TABLE X

The relationship of gastric and duodenal acidity to the bacterial population of the small intestine

BIOCHEMISTRY			BACTERIOLOGY			
Subject's No	Gastric PH	Duodenal PH	Gastric Flora	Duodenal Flora	Jejeunal Flora	Ileal Flora
Patients with Cirrhosis						
3	7·5			Bact.coli 6×10^6 Strep.faecalis 3×10^5		Bact.coli 9×10^7 Strep.faecalis 1×10^7
18	2·5			Bact.coli 6×10^2 Anitratum 4×10^2	Bact.coli 3×10^4 Strep.viridans 2×10^5 Anitratum 2×10^5	Bact.coli 2×10^5 Anitratum 1×10^5
19	1·8		Candida $6{\cdot}7 \times 10^5$ M.citreus $1{\cdot}7 \times 10^5$	Lactobacilli 7×10^4 Staph.pyogenes 2×10^3 Candida 2×10^3	Staph.pyogenes 1×10^3 Candida 1×10^3 Lactobacilli 3×10^5	Strep.viridans 4×10^3 Lactobacilli + Candida + Staph.pyogenes +
21	6·1		Bact.coli 1×10^6 Micrococcus + Strep.viridans +		Bact.coli 3×10^6 Strep.faecalis 2×10^8	Bact.coli 5×10^7
23	2·0	1·8	Micrococcus $8{\cdot}4 \times 10^3$ Diphtheroids $4{\cdot}2 \times 10^3$ Strep.viridans $1{\cdot}7 \times 10^3$	..... sterile	Bact.coli 2×10^4 Strep.faecalis 2×10^4	Bact.coli 5×10^3 Strep.faecalis 2×10^4 Ps.pyocyanea 4×10^2
25		1·8		..... sterile	Bact.coli 3×10^2 Strep.viridans + Ps.pyocyanea 1×10^2	Bact.coli 4×10^2 Strep.viridans 1×10^5 Ps.pyocyanea 1×10^2
Patients with gastro-intestinal disorders						
43	±			P.mirabilis 6×10^2 Strep.viridans 1×10^5 Diphtheroids 2×10^4		Bact.coli 7×10^2 Diptheroids) 4×10^3 Micrococci)
44	±				Bact.coli 4×10^6 Strep.faecalis 4×10^7	Bact.coli 1×10^7 Strep.faecalis 8×10^7
46	±				Bact.coli 4×10^7	Bact.coli 4×10^7 P.vulgaris 3×10^6
47	±	5·6		Bact.coli 6×10^5 Ps.pyocyanea 1×10^6		Ps.pyocyanea 2×10^7
48	2·1		..... sterile	..... sterile	Bact.coli 1×10^5 Strep.faecalis 3×10^4	Bact.coli 8×10^4 Strep.faecalis 4×10^4
Normal subjects						
64		3·6		Anaerobic strep. ± Lactobacilli 4×10^4	..... sterile	Strep.viridans 2×10^8 Anaerobic strep. 1×10^3
65	1·4		Strep.viridans $5{\cdot}5 \times 10^4$	Strep.viridans 1×10^3	Strep.viridans 5×10^2 Anaerobic strep. 5×10^2	Bact.alkalig. 3×10^2
66	1·7		..... sterile	..... sterile	..... sterile	Bact.coli 1×10^3 Bact.alkalig. 1×10^2
68	5·0		Staph.pyogenes 1×10^2 Micrococcus) 1×10^5 Diphtheroids)	Strep.viridans + Micrococcus + Haemophilus +	Strep.viridans 1×10^6 Lactobacilli 5×10^5	Strep.viridans 5×10^6 Lactobacilli 1×10^6

± = free acid present in test meal in normal or increased amounts.

± = a few colonies isolated.

\+ = moderate numbers isolated but not counted

) = this aspirate was repeated and the results were Bact.coli $1{\cdot}2 \times 10^6$, Micrococcus +, Strep.viridans +

"Bact.coli" includes all gram negative lactose fermenting organisms.

TABLE L

A Comparison of the types of coliform organisms isolated from faeces and ileum

1) Patients with cirrhosis

Patient No.	Faeces: Typical E.coli number	antigen	L	G	M	D	Suc	Sal	Faeces: Atypical coliform organisms number	antigen	L	G	M	D	Suc	Sal	Ileum: Typical E.coli number	antigen	L	G	M	D	Suc	Sal	Ileum: Atypical coliform organisms number	antigen	L	G	M	D	Suc	Sal
2	8×10^8	09	+	+	+	+	-	+	3×10^9	F	+	+	+	+	+	-	+	07	+	+	+	+	-	-	+	F	+	+	+	+	+	-
																	+	0?	+	+	+	-	+	-								
3	+	091	+	+	+	-	-	-	+	F	+	+	+	+	+	-	+	091	+	+	+	-	-	-	+	F^x	+	+	+	+	+	-
4	+	?	+	+	+	-	+	-	+	F	+	+	+	-	+	-									+	F	+	+	+	-	+	+
									+	F	+	+	+	-	-	-																
9	+	?	+	+	+	-	-	-									+	?	+	+	+	-	-	-								
18	+	?	+	+	+	+	+	-																	+	K	+	+	+	-	+	+
19									6×10^7	F	+	+	+	-	+	-																
									1.5×10^8	K	+	+	+	-	+	+																
22									+	F	+	+	+	-	-	-	+	06	+	+	+	-	-	-	+	K_{53}	+	+	+	-	+	+
									+	K_{17}	+	+	+	+	+	+																
									+	K_{53}	+	+	+	-	+	+																
23	+	081	+	+	+	-	±	-																	+	F	+	+	+	-	±	-
25	+	?	⊥	⊥	⊥	-	-	-									+	?	⊥	⊥	⊥	-	-	-	+	F	+	+	+	-	-	-
	+	?	+	+	+	+	-	-									+	?	+	+	+	-	-	-								
26	+	?	+	+	+	+	+	-																								
No. of patients from whom isolated								8								5								5								7

11) Subjects without liver disease

48	+	?	+	+	+	±	-	-																	+	F	+	+	+	-	±	-
49	10^8	?	+	+	+	+	±	-	10^7	F^x	+	+	+	-	+	+	6×10^5	?	+	+	+	+	±	-	2.2×10^4	F^x	+	+	+	-	+	+
64	+	432	+	+	+	-	-	+																								
	+	088	+	+	+	+	-	+																								
	+	025	+	+	+	+	+	+																								
65	+	02	+	+	+	+	-	±																								
66	5×10^8	017	+	+	+	-	+	-									1×10^2	0?	+	+	+	+	+	-	1.5×10^3	F	+	+	+	+	+	-
	3×10^8	0?	+	+	+	+	+	-																								
	2×10^8	0?	+	+	+	+	-	-																								
67	+	?	+	+	+	+	±	-																								
		?	+	+	+	+	-	+																								
68	+	?	+	+	+	+	+	-	+	F	+	+	+	-	+	-																
69	+	?	+	+	+	-	-	-									+	?	+	+	+	+	+	-								
	+	?	-	+	+	±	-	-																								
No. of subjects from whom isolated								8								2								3								3

Numbers of orgs expressed per g. in the case of faeces, per ml. in the case of ileal fluid.

+ = number of orgs not counted. - = orgs present in numbers below the lower limit of the counting method.

The number following an O or H indicates the antigenic composition of E.coli strains. O? = unknown O groups. ? = O group not determined.

F = E.freundii, K = Klebsiella. The number following K indicates the capsular type.

L, G, M, D, Suc, Sal indicates fermentation of lactose, glucose, Mancitol, Dulcitol, Sucrose and Selicin. + = Fermentation with acid and gas
± = Fermentation " " " " (late)
⊥ = production of acid only.

Faecal and ileal strains, put in red, were identical biochemically and in some cases antigenically.

F^x = Strain of E.freundii identical also by colicine typing.

FROM
THE POSTGRADUATE MEDICAL SCHOOL OF LONDON
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METHIONINE TOXICITY IN LIVER DISEASE AND ITS PREVENTION BY CHLORTETRACYCLINE.

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METHIONINE TOXICITY IN LIVER DISEASE AND ITS PREVENTION BY CHLORTETRACYCLINE.

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NITROGENOUS substances given to certain patients with hepatic cirrhosis can precipitate neurological changes, indistinguishable from spontaneous hepatic coma (17, 23, 38, 47, 49). These include a high protein diet, ammonium salts and urea. There have also been reports that methionine, an amino-acid often recommended for the treatment of liver disease, might have a similar action. Watson (53) reports a patient with cirrhosis developing confusion after 8 g. methionine and Kinsell and co-workers (22) describe a patient with cirrhosis who became mentally disorientated when given 9 g. dl-methionine daily for three days. Singh, Barclay and Cooke (48) noted neurological deterioration in a patient with hepatic cirrhosis after 10 g. dl-methionine by mouth, and similar changes directly attributable to methionine have been reported in four other patients with hepatic cirrhosis (37, 47). The present paper describes investigations into the mechanism of methionine toxicity and the effect of chlortetracycline in delaying the development of symptoms.

PATIENTS STUDIED.

Twenty-eight patients with liver disease were investigated. Patients one to nine who were studied in detail suffered from cirrhosis of the liver and had previously experienced episodes of impending hepatic coma (*Chronic portal systemic encephalopathy*). Electroencephalograms were compatible with this diagnosis. In eight, the extent of the portal venous collateral circulation was assessed by transplenic portal venography (1) and a very extensive collateral circulation was demonstrated. In the ninth patient (Case seven) disturbance of the blood clotting mechanism prohibited this investigation.

In addition fifteen patients were observed with portal cirrhosis, one patient with biliary cirrhosis, one patient with extra-hepatic portal vein obstruction without cirrhosis and two patients with acute virus hepatitis. Six of these patients had previously experienced the neurological complications of liver disease (Table I).

Twelve patients were included, suffering from non-hepatic diseases.

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We wish to thank Dr. C. Dalgleish for biochemical advice, Dr. W. Hayes for guidance in the bacteriological studies, Dr. Harriet J. Warrack of the Wellcome Foundation for typing Clostridium Welchii strains, and Sisters B. D. Griffiths, S.R.N. and P. R. Richards, S.R.N. for enthusiastic nursing co-operation.

TABLE I.

Patients, diagnosis, extent of portal venous collateral circulation and serum bilirubin and albumin levels.

Patient	Sex	Age	Diagnosis	Portal-systemic collateral circulation	Serum bilirubin (mg. per cent.)	Serum albumin (g. per cent.)
1*	F	56	Portal cirrhosis	Thrombosed portal vein	1·4	2·8
2*	F	52	,, ,,	,, ,,	2·0	2·3
3*	M	42	,, ,,	Patent porta-caval anastomosis	1·0	3·2
4*	F	42	,, ,,	Extensive; patent umbilical vein	2·1	2·7
5*	F	69	,, ,,	Thrombosed portal vein	2·2	2·8
6*	M	59	,, ,,	,, ,,	1·7	3·1
7*	F	58	,, ,,	†	1·8	2·4
8*	M	39	,, ,,	Extensive; patent umbilical vein	1·2	3·2
9*	M	36	,, ,,	Extensive	1·4	2·8
10	F	34	,, ,,	Extensive	1·5	3·0
11	M	35	,, ,,	Slight	1·1	3·2
12	M	34	,, ,,	†	1·3	4·3
13*	M	59	,, ,,	†	0·3	3·9
14	F	54	,, ,,	None	1·1	4·0
15	F	28	,, ,,	Extensive	1·2	3·2
16	M	23	,, ,,	,,	1·2	4·1
17	M	48	,, ,,	†	0·6	4·2
18	M	38	,, ,,	None	0·4	2·5
19	F	45	Virus hepatitis	†	11·0	2·9
20	M	18	Portal pylephlebitis	Thrombosed portal vein	0·7	4·9
21	M	52	Portal cirrhosis	,, ,,	0·5	3·5
22	F	66	,, ,,	†	0·3	3·0
23*	F	75	,, ,,	Extensive	2·2	2·2
24*	F	26	,, ,,	Slight	7·1	1·4
25*	M	65	,, ,,	Slight	1·9	2·5
26*	F	58	,, ,,	None	2·0	2·3
27*	M	60	Biliary cirrhosis	None	22·0	2·7
28*	F	23	Portal cirrhosis	Porta-caval anastomosis	2·2	2·8

* Patients with neurological complications. † Not investigated.

METHODS.

Neuro-psychiatric assessment.

The degree of neurological disorder was assessed by two independent observers. The following grades were recognised:

0 Normal.

1 Trivial lack of awareness, apathy or euphoria with or without objective neurological signs.

2 Obvious personality change, with neurological abnormality; gross facade of personality preserved.

3 Advanced confusion and disorientation.

4 Stuporose but responsive.

5 Comatose.

Biochemical methods.

Blood ammonium levels were estimated by a slight modification of Conway's method (12, 37).

Plasma dl-methionine, — plasma 1 ml. was de-proteinised with 5 ml. acetone. After evaporation of acetone, it was desalted by the Dent modification of the Consden, Gordon and Martin method (11). Volumes of each specimen containing 1–30 μg. methionine, together with a range of standards, were run on buffered one-way chromatograms (28). After lightly spraying with ninhydrin, the concentration of amino-acid in each methionine spot and hence in the original specimen was estimated either by the method of Yemm and Cocking (56) or that of Naftalin (34).

Blood bicarbonate, serum bilirubin and albumin were estimated by routine procedures and arterial blood pH by the method of Rosenthal (43).

Bacteriological methods.

Fresh fæces (200 mg.) were weighed on a clean piece of X-ray film and placed in a bottle containing 100 ml. of 0·85% sodium chloride solution. The bottle was shaken at 37°C. for one hour at 270 oscillations a minute, for homogenization.

A standard loopful (0·01 ml.) of a 1: 100 dilution in saline of this suspension was spread evenly over plates containing the following media:

(A) Blood agar.

(B) Blood agar containing 6% agar (20).

(C) MacConkey's medium.

In some cases Sabouraud's medium was used in addition. These plates were incubated ærobically for 48 hours.

For anærobic cultivation 0·01 ml. of a 1 : 1,000 dilution of the original suspension was spread on—

(A) Blood agar

(B) Blood agar containing 6% agar and incubated for 48 hours anærobically in a Fildes-McIntosh jar containing 5% CO_2.

A tomato agar medium for lactobacilli (5) and Fildes peptic blood digest medium for Bacteroides (45) were also used in several cases.

TABLE

Effect of daily oral methionine with and without chlortetracycline on

		Methionine alone									
		Days of control period				Days after start of methionine					
		4	3	2	1	0*	1	2	3	4	5
Patient	Dose					10	10	6			
	CNS			3	3	4	5	5	3	3	
	NH_4N			1·7		2·4	3·0	2·9	2·2	1·5	
1†	Dose					10	10				
	CNS	2		2	2	2	3	4	2	1	
	NH_4N	2·7				1·7	2·4	2·0	2·0		
	Methionine	13				18		123			
	Dose					8	8	10	10	10	
	CNS			3	2	2	2	2	3	4	
	NH_4N			2·7		3·0			1·6		
2†	Dose					10	10	10	10		
	CNS		0	0	0	0	1	2	3	0	
	NH_4N			1·0		1·2			0·7		
	Methionine					9			75		
	Dose					8	8	8	8		
3†	CNS		1	1	1	2	1	1	1	5	2
	NH_4N		1·0		1·2			1·1		1·3	
	Dose					10	4				
4†	CNS			3	2	2	5	4	3	3	
	NH_4N			1·9		3·4	3·0			2·5	
	Methionine					26	84				
	Dose					10	1				
5†	CNS			1	1	3	5	4	3	2	
	NH_4N			0·8		4·3	2·5	1·6	1·2		
	Methionine					39	96	47	30		
	Dose					20					
6†	CNS		2	2	2	2	5	3	2		
	NH_4N		1·5		1·7	1·5	2·6	1·7	1·3		
	Dose					10	10	10	2		
7†	CNS	0		0		0	1	2	3	1	
	NH_4N	1·1		2·2		2·2		2·4	2·9	2·0	
	Dose					8	8	10	10	14	16
8†	CNS		1	1	1	1	1	1	1	2	1
	NH_4N		2·3			0·9				2·4	
	Dose					8	12	12	12	12	12
9†	CNS			3		3	3	3	3	3	3
	NH_4N			1·8		1·1		2·8			
	Methionine					13		142			
	Dose					10	10	10	10	10	10
10	CNS			0		0	0	0	0	0	0
	NH_4N			1·2		1·1		1·0		0·8	
	Methionine					12				70	

II.

the neurological condition, blood methionine and ammonium (NH_4N) levels.

		Methionine and chlortetracycline									
feeding		Days of control period			Days after start of methionine feeding						
6	8	3	2	1	0*	1	2	3	4	5	6
		1	1	1 1·4	10 1 40	10 1 2·1	10 1 1·5 193				
			0 1·6	0	10 0 1·3 15	10 0	10 0	10 0 1·1 150			
2 1·9	1		1 1·4		10 1 1·3	10 1	10 1 1·5	10 1	10 1 1·7	12 1 1·2	
		3 2·3 23	3	2	8 2 2·6 0	8 3	8 3	8 5 1·5 205	3	3	2
		1	1	1	10 2 1·4 39	10 2	10 2	10 2 1·8 190	10 2	10 1	10 1 1·6 183
1 1·3											
12 3 0·9 133											
10 0 0·5 83											

TABLE II

		Methionine alone									
		Days of control period				Days after start of methionine					
		4	3	2	1	0*	1	2	3	4	5
Patient 11	Dose					12	12	12	12	12	12
	CNS					0	0	0	0	0	0
	NH_4N					1·3					
	Methionine					20					
12	Dose					8	12	12	12	12	12
	CNS				0	0	0	0	0	0	0
	NH_4N				1·1						0·7
	Methionine				24						87
13	Dose					10	12	12	12	14	14
	CNS					0	0	0	0	0	0
	NH_4N					0·5	0·7		0·5		
	Methionine					15	116		135		
14	Dose					10	10	10	10	10	10
	CNS				0	0	0	0	0	0	0
	NH_4N				1·7				1·1		
	Methionine				15				159		
15	Dose					10	10	10	10	10	
	CNS					0	0	0	0	0	0·6
	NH_4N					1·5					
	Methionine					27				109	50
16	Dose					20	20	20			
	CNS			0	0	0	0	0	0		
	NH_4N			1·0		0·8			1·2		
20	Dose					10	10	12	12	12	12
	CNS			0	0	0	0	0	0	0	0
	NH_4N			0·9		0·8			0·9		

Bacterial metabolism of methionine in vitro.

Strains of Bact. coli and Proteus vulgaris were grown in 0·5% glucose broth for 24 hours, washed in 0·85% saline and resuspended in 0·02M phosphate buffer at pH 6 and pH 8. Since the pH in the intestine is variable (30) 1,000 μgm methionine/ml. was added to the suspensions which contained approx. 70 μgm/ml. total nitrogen. A Bacteroides strain was grown in 0·5% glucose broth for 48 hours in a Fildes-McIntosh jar and then a similar suspension was prepared. The effect of the addition of 2·5 μg./ml. glucose on methionine breakdown was studied in all species and that of 5 μg./ml. chlortetracycline in *Bact. coli* and *Pr. vulgaris*. Chlortetracycline was not added to Bacteroides suspensions, since these organisms disappear from the gut when the antibiotic is administered. Anærobic conditions for Bacteroides were obtained by the addition of 0·1 mg./ml. thioacetic acid.

Production of sulphates (25) and amines (4) were measured.

Methionine was given to patients with liver disease with their consent and co-operation as part of a necessary assessment of their mental and neurological condition or as a preliminary to consideration of porta-caval anastomosis. Patients

—*continued.*

feeding		
6	8	
12 0 1·0 74		
		Doses of methionine in grams. Chlortetracycline 2 g. per day started 2 days before methionine.
14 0 0·7 123	14 0 115	CNS—neurological grade. Blood levels of NH_4N (normal < 1) and methionine (normal < 20) in μg./ml..
10 0 0·7 150		† Patients who had experienced neurological complications. * Values before commencing methionine.
12 0	 0 0·7	

with severe impairment of liver function were excluded. Dietary protein intake was maintained constant during the period of observation. Patients with neurological complications had been under observation in hosptial for at least one week and the neurological condition was steady.

Enteric coated tablets containing 250 mg. dl-methionine were given in divided doses between 6 a.m. and 9 p.m.. The total daily dose, usually 10 g., varied between 8 and 20 g.. Two patients received two courses. The drug was withdrawn when unequivocal neurological deterioration occurred or after five to seven days. Blood levels of methionine, ammonium, bicarbonate and serum bilirubin were estimated at intervals during the control period, during the administration of methionine and following its withdrawal.

In ten patients, 6 g. methionine in 300 ml. 5% dextrose was given intravenously at a constant rate during a 30 minute period. Peripheral blood samples were taken before the infusion and 15, 60, 120 and 240 minutes after its completion and analysed for methionine and ammonium.

Five patients sensitive to methionine by mouth later received chlortetracycline (0·5 g. 4 times per day) for two days before the addition of a second course of oral

TABLE III.

The effect in sensitive subjects of oral methionine compared with that of methionine together with chlortetracycline.

Patient	Methionine					Methionine with chlortetracycline				
	Dose		Neurological deterioration (grades)	Blood methionine (μg./ml.)	Blood ammonium (μg. NH_4N/ml.)	Dose		Neurological deterioration (grades)	Blood methionine (μg./ml.)	Blood ammonium (μg. NH_4N/ml.)
	Total (g.)	Days				Total (g.)	Days			
1	20	2	2	123	2·0	30	3	0	193	1·5
(bis)	26	2½	2	—	2·9	—	—	—	—	—
2	40	4	3	75	0·7	40	4	0	150	1·1
(bis)	46	5	2	—	1·6	—	—	—	—	—
3	32	4	4	—	1·3	62	6	0	—	1·2
4	14	1½	3	84	3·0	32	4	2	205	1·5
5	11	1	4	96	2·5	70	7	0	183	1·6
6	20	1	3	—	2·6	—	—	—	—	—
7	32	3	2	—	2·9	—	—	—	—	—
Mean	27	2½	3	94·5	2·17	47	5	0	182·7	1·38

methionine. Both drugs were stopped simultaneously. In these patients fæces were collected before starting chlortetracycline, when the patient was receiving chlortetracycline alone, and later during combined treatment with chlortetracycline and methionine.

RESULTS.

Clinical investigations.

The effect of oral methionine (Tables II and III). The drug imparted a characteristic odour to the breath and rarely caused slight nausea.

Neurological status. Nine patients had portal cirrhosis and pre-existing neurological complications associated with an extensive portal systemic collateral circulation. During the administration of methionine, neurological deterioration of at least two grades occurred in seven (patients 1 to 7) and in two, this effect was reproduced when the drug was administered a second time. Changes followed a similar pattern to those seen during spontaneous deterioration. They occurred from one to four days after commencing and after total doses of 11 to 46 g. methionine. Two of the patients (No. 8 and 9) tolerated 80 and 99 g. methionine without neurological change.

Seven patients with portal cirrhosis (patients 10 to 16) who had never exhibited neurological complications, tolerated 50–102 g. methionine without neurological change. Liver function was considered to be less severely impaired than in the patients who were sensitive to the drug and an extensive collateral circulation was demonstrated in only three (patients 10, 15, 16). The patient with extra-hepatic portal vein obstruction (patient 20) also showed no change.

Blood ammonium levels. Control fasting blood ammonium levels were raised in five of the seven patients who later deteriorated with methionine. There was an inconstant change following methionine, the level rising five times, remaining unchanged once and falling on three occasions. The mean level at the height of deterioration showed no significant difference from the control values.

Two patients who had previously experienced neurological complications, but were unaffected by oral methionine, had raised control blood ammonium levels. In one instance, methionine was followed by a rise and in the other by a fall in the blood ammonium level.

Eight patients without neurological changes showed an inconstant change in the blood ammonium level after methionine.

Blood methionine levels. In four patients who later deteriorated after methionine, the control blood methionine levels were normal in two and raised in two. The level was increased in one and normal in five of the patients who were unaffected by methionine feeding.

In both groups, the rise in blood methionine level after methionine administration was comparable.

Serum bilirubin levels and evidence of acidosis. Serum bilirubin level did not change after methionine feeding (Table IV).

There was no clinical evidence of increasing acidosis. The serum bicarbonate showed a slight but significant decrease ($P = 0{\cdot}01$). In two patients in which it was measured, the arterial pH did not change. In one patient the renal compensatory mechanisms were studied before and after seven days of methionine administration (total dose: 70 g.) and was found to be adequate. Titratable urinary acid rose from 38 to 87 mEq. per day (normal value 20–50), while urinary NH_4 rose from 25 to 126 mEq. daily (normal average value 50).

TABLE IV.

Serum bilirubin and blood bicarbonate levels before and after the administration of oral methionine.

Patient	Control levels		Levels after methionine	
	Plasma biliburin (mg./100 ml.)	Blood bicarbonate (mEq./l.)	Plasma bilirubin (mg./100 ml.)	Blood bicarbonate (mEq./l.)
1†	0·9	—	0·9	—
2†	3·8	24·0	1·7	22·7
4†	2·2	27·3	2·1	—
5†	0·5	27·9	2·7	26·8
5*	2·3	27·1	1·6	25·6
6†	2·2	—	2·4	23·8
8†	0·5	—	1·6	—
9†	0·8	26·2	1·2	21·8
10	1·5	28·5	1·2	25·1
28†	1·3	23·0	1·3	21·0
Mean	1·6	26·3	1·7	23·8

† Neurological deterioration after methionine.

* Methionine given with chlortetracycline.

Effect of intravenous methionine. Intravenous methionine was given to four patients who had previously deteriorated with oral methionine. In three there was no change in neurological status and in the fourth there was "two-grade" deterioration, occurring two hours after stopping the infusion (Table V).

Six patients without previous neurological changes were unaffected by the infusion.

There was no conspicuous change in blood ammonium level, except in the patient who showed neurological deterioration when the methionine was stopped. In this patient the level rose from 2·7 to 4·1 μg./ml..

Methionine tolerance was impaired in all seven patients with liver disease (patients 1, 2, 3, 4, 17, 18, 19) and blood methionine levels remained elevated four hours after stopping the infusion while in three normal subjects (patients 29, 30, 31) control values were regained within four hours of the infusion.

Effect of chlortetracycline on methionine toxicity. Five patients who had previously deteriorated when given oral methionine were completely or partially protected by the simultaneous administration of chlortetracycline (Tables II, III). Blood methionine levels were higher than those obtained with methionine alone. Blood ammonium levels did not show any conspicuous change during administration of chlortetracycline with methionine, but levels recorded when the drugs were withdrawn were usually lower than those recorded at the height of deterioration following methionine alone.

Conclusions. Oral methionine is toxic to some patients with cirrhosis of the liver and an extensive portal systemic collateral circulation, who have previously shown neurological complications. This toxicity could not be correlated with the changes in blood ammonium levels. Intravenous methionine is apparently non-

TABLE V.

Effect of intravenous methionine on the blood levels of methionine and ammonium.

Patient	Methionine						Ammonium N.					
	Time in hr. after infusion						Time in hr. after infusion					
	0	¼	1	2	3	4	0	¼	1	2	3	4
1*	39	89	94	101	—	94	0·9	2·5	2·2	1·2	—	1·5
2*	12	290	275	205	155	—	0·6	1·1	0·8	0·9	—	1·2
3*	46	286	189	176	—	138	2·1	1·7	2·6	2·6	—	2·0
4*	16	157	141	124	—	109	2·7	3·0	3·2	3·1	—	4·1
17	24	199	123	108	—	87	0·4	0·6	0·9	1·3	—	1·6
18	20	170	120	106	—	88	0·9	1·5	1·4	0·8	—	0·6
19	40	311	—	214	—	228	0·9	1·5	—	1·2	—	1·2
29	20	118	74	50	—	18	0·5	0·5	0·5	0·8	—	0·3
30	18	156	110	76	—	25	0·5	0·5	0·7	0·9	—	0·3
31	11	96	75	—	—	—	0·7	1·0	0·7	1·2	—	—

(Blood levels in μg./ml.).

* Patients who had previously experienced neurological complications.

toxic despite high blood methionine levels. Oral chlortetracycline given with methionine prevented or retarded toxic effects, although blood methionine levels were higher than with oral methionine alone. It therefore seemed likely that the toxicity was due to some substance other than methionine. The protection afforded by chlortetracycline suggested that this toxic factor might be derived from methionine by the action of intestinal bacteria which were affected by the " wide spectrum " antibiotic, and bacteriological observations were therefore made.

Bacteriological investigations.

No difference in the fæcal viable count was found between nine patients without liver disease and twenty patients with liver disease, thirteen of whom had at some

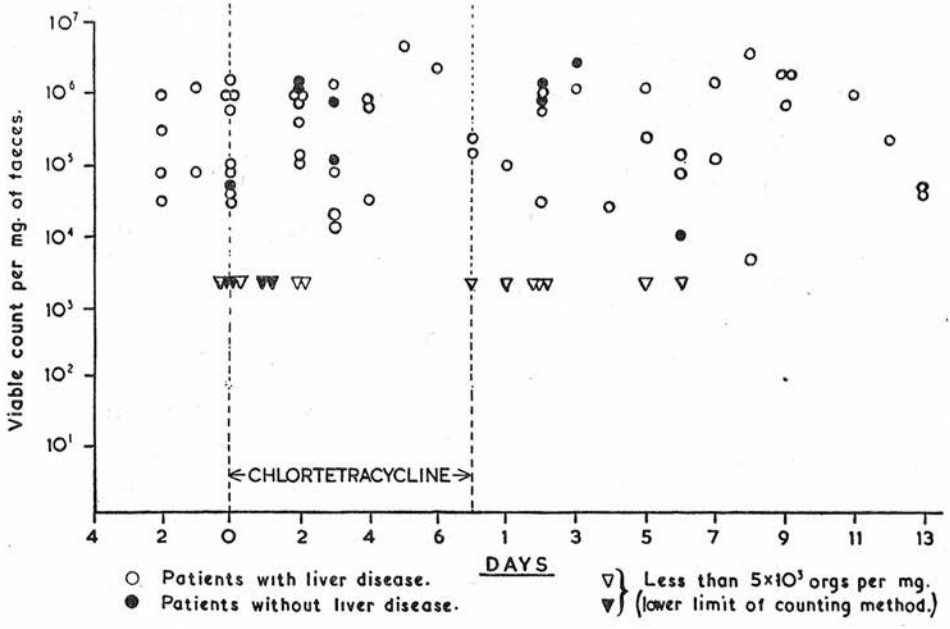

Fig. 1. Effect of chlortetracycline on the fæcal viable count of *Streptococci.*

time had neurological symptoms. *Cl. welchii Type A*, however, was isolated in six of twenty patients with liver disease and in only one of the nine other patients. It was not more common in those with neurological complications.

Oral methionine did not have a significant effect on the fæcal flora of seven patients with liver disease, three of whom had neurological complications.

Effect of chlortetracycline (Figures 1–4).

Chlortetracycline was given for three to eight days to sixteen patients, thirteen of whom had cirrhosis of the liver, all but one with neurological complications. Two patients had three courses of the antibiotic. Seventy two specimens of fæces were examined during the three days preceding and the thirteen days following chlortetracycline. The total viable count showed no sustained fall during therapy. The fæcal flora changed however, the picture being the same in all patients.

Lactose fermenting gram negative rods (*Bact. coli*) were eliminated at some stage in ten of the sixteen patients, whereas before treatment they were absent in

only one. The viable count during therapy fluctuated widely and some patients showed no diminution. No increase in resistance to chlortetracycline during therapy was detected (Fig. 4).

The total count of streptococci during treatment showed no change, but sometimes there was a complete change of strain. In seven patients after treatment a hæmolytic *Str. fæcalis* and in three patients a micro-ærophilic streptococcus, both resistant to chlortetracycline, replaced a sensitive *Str. fæcalis*. A hæmolytic *Str. fæcalis* was never isolated in significant numbers before treatment (Fig. 1).

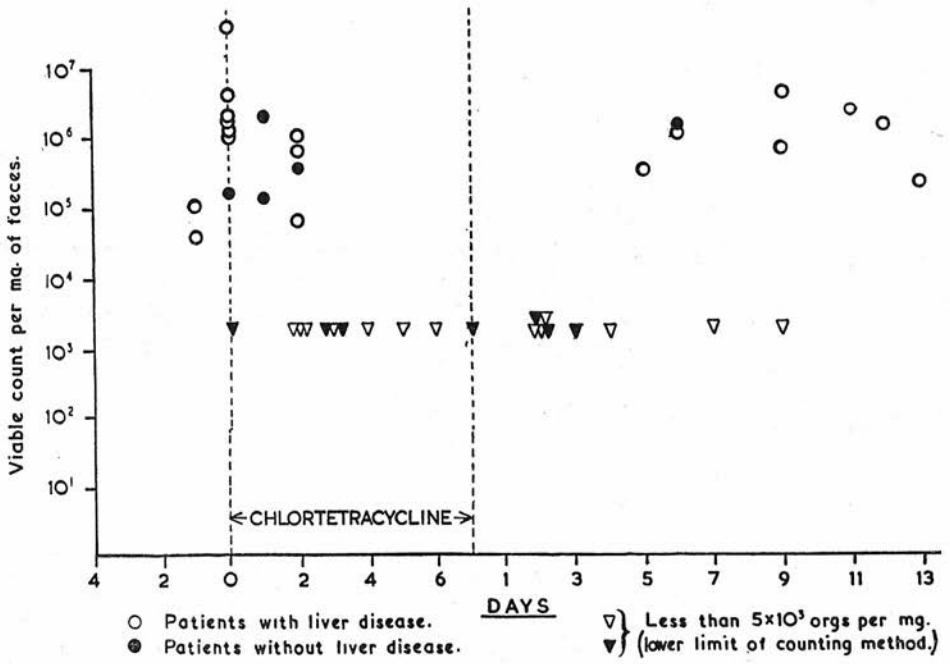

Fig. 2. Effect of chlortetracycline on the fæcal viable count of *Bacteroides*.

Proteus was not isolated from any patient before therapy. During and after chlortetracycline, proteus appeared in thirteen of sixteen patients. Of those organisms investigated, five were *Pr. mirabilis*, three *Pr. vulgaris* and two *Pr. morganii*. Proteus was usually eliminated from the fæces in two to three weeks, but in some cases (especially after a second course of chlortetracycline), proteus persisted for two to three months (Fig. 2).

Gram negative anærobic bacilli (*Bacteroides*) were investigated in 8 patients, and were present before treatment but disappeared before the fourth day. They reappeared four days after the cessation of the antibiotic (Fig. 2).

In one patient a lactobacillus was present in large numbers before treatment. In five patients chlortetracycline was followed by the appearance of resistant lactobacilli.

Clostridium welchii Type A was isolated initially in three patients and in every instance was eliminated by chlortetracycline.

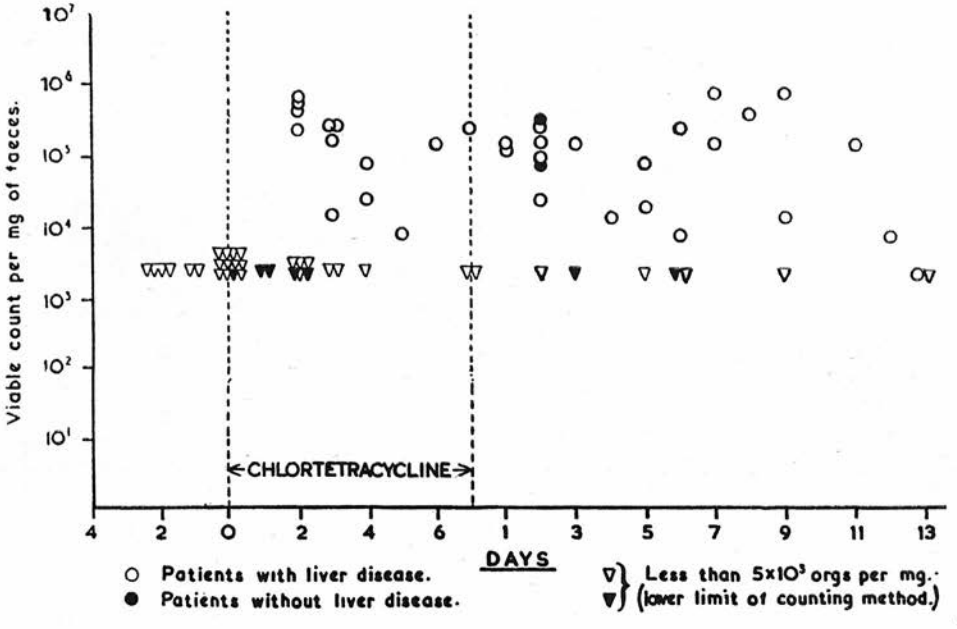

Fig. 3. Effect of chlortetracycline on the fæcal viable count of *Proteus*.

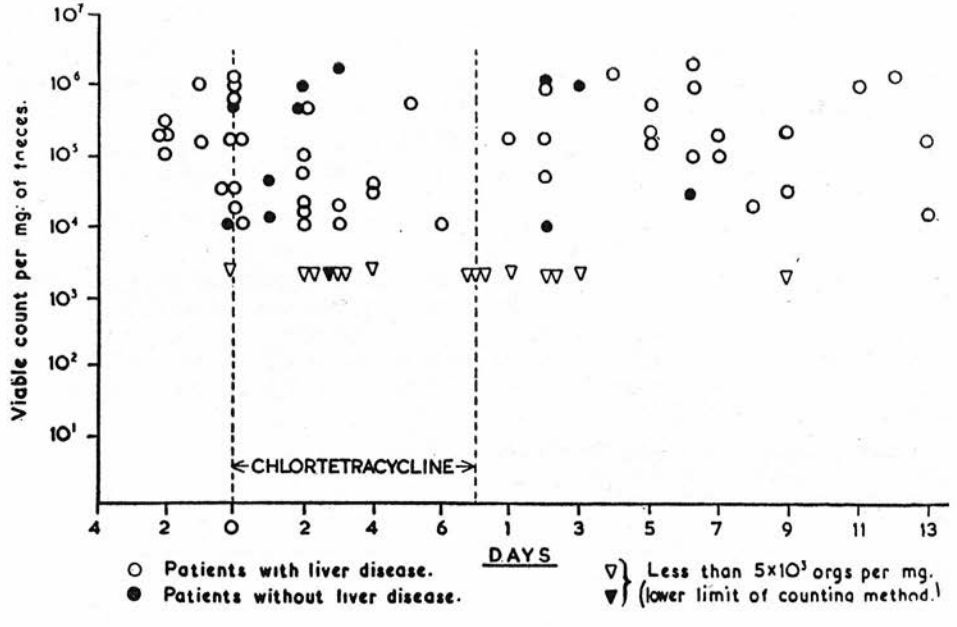

Fig. 4. Effect of chlortetracycline on the fæcal viable count of *Bact. coli*.

Staph. pyogenes resistant to chlortetracycline appeared in large numbers in three patients during treatment.

Candida was found in large numbers in only one patient during therapy.

Detailed bacteriological results are shown in the Appendix.

Conclusion. The fæcal flora of patients with liver disease with and without neurological complications does not differ from that of subjects without liver disease. Chlortetracycline effects an inconstant fall in *Bact. coli*, the strain of streptococci often changed and proteus usually appeared while bacteroides fell and lactobacilli frequently increased.

The action *in vitro* of *Bact. coli*, proteus and bacteroides on methionine with and without chlortetracycline was therefore investigated.

Action of Bact. coli, Pr. vulgaris and Bacteroides on methionine.

Bact. coli, *Pr. vulgaris* and *Bacteroides* did not grow in cultures in which methionine was the only source of nitrogen. Resting suspensions were therefore set up in a solution of methionine. After incubation NH_4 production in these cultures was slight. In four experiments at pH 8 *Bact. coli*, *Pr. vulgaris* and *Bacteroides* produced respectively 2·0 ($\pm$ 1·0), 10·0 ($\pm$ 5·4) and 0·8 ($\pm$ 0·7) μg. NH_4N per ml.. Further experiments at pH 6 and with and without added glucose gave similar results.

Since 14 μg. NH_4N are equivalent to 149 μg. methionine and since 1,000 μg./ml. of methionine were present initially, it is not surprising that no decrease in the methionine concentration was detected. There was also no detectable increase in SO_4 production.

No evidence was obtained for methionineamine production either from methionine alone (4 occasions) or in a mixed amino-acid medium by growing bacteria (13 occasions), and in the latter medium, no evidence for methionine breakdown was found, by resting suspensions (4 occasions).

Discussion.

A methionine deficient diet is followed by severe liver injury in rats (21). These observations have been enthusiastically applied to human liver disease and methionine is freely prescribed (8, 26). It is of importance, therefore, to realise that this amino-acid in therapeutic amounts has been found to reproduce "impending hepatic coma" in some patients with hepatic cirrhosis. As there is no conclusive evidence that methionine affects the course of liver damage in man (36, 55), this therapy should now be abandoned.

Patients exhibiting neurological deterioration after methionine all had large collateral channels between the portal systemic venous system, with chronic portal systemic encephalopathy (47). These patients can show a perplexing variety of neurological and mental changes, and exacerbation by methionine may provide a convenient method of inducing a diagnostic neurological effect. Chronic portal systemic encephalopathy may follow a surgical porta-caval anastomosis in about 10% of patients (41), and a course of oral methionine given pre-operatively may provide a suitable screening test to determine the likelihood of this occurrence.

The toxic effects of methionine were not related to alterations in the liver function tests. They are not due to unaltered methionine, for there was no correlation of symptoms with the blood methionine level, which rose equally in those who deteriorated and those who did not. The breakdown products of methionine which might be responsible are numerous, and only a few possibilities can be considered.

Our *in vitro* studies cannot be taken as evidence that bacteria do not attack methionine for three reasons. Firstly, metabolism of resting suspensions is often very different from that of growing bacteria. Secondly, no attempt was made to induce enzyme formation by growing the bacteria in the presence of high concentrations of methionine. Thirdly, no precautions were taken while washing the bacteria, to keep them under reduced conditions and the enzyme systems which attack methionine may well possess sulphhydryl groups which are oxygen labile.

Ammonium can be derived from methionine and ammonium toxicity has been related to the neurological changes which precede hepatic coma (3, 38, 42). Ammonium production in the intestine seems unlikely to cause the deterioration which follows methionine, for changes in blood ammonium levels were inconstant and alterations of a similar order occurred when chlortetracyline was given with the methionine, yet without neurological deterioration.

A further possibility is that ammonium intoxication may result from disturbed glumatic acid metabolism (54). Glumatic acid is important for cerebral respiration possibly by combining with ammonium to form glutamine. It has been shown that methionine sulfoxide, a substance that can readily be derived from methionine, inhibits this synthesis in *Lactobacillus arabinosis* (51). Accumulation of methionine sulfoxide might explain the cerebral features of methionine toxicity. Moreover, methionine sulfoxide has possibly been detected in the cerebro-spinal fluid of two patients with hepatic coma (52). Current methods of identification, however, are unreliable and this observation needs confirmation. Methionine sulfoxide has proved non-toxic to dogs and rats (2, 50).

Methyl mercaptan can be produced from methionine directly (9) or *via* cysteine, by proteus and sometimes by *Bact. coli* in the presence of glucose (24). Recently a patient with massive hepatic necrosis and fœtor hepaticus was found to have methyl mercaptan in the urine (10) and we have identified this substance on two of four occasions in the urine of four patients in hepatic coma. These are observations of great interest, although the effect of mercaptans on the central nervous system are unknown. Some mercaptans, however, have been found to have anti-hypertensive properties (44) and this might contribute to the hypotension of patients with cirrhosis of the liver.

Butt and Mason (6) considered that fœtor hepaticus was due to a tertiary amine. Methionineamine has never been isolated naturally, and we have failed to find it on ten occasions in the urine of patients with liver disease. It could theoretically be derived from methionine, however, and some amines are known to affect oxidative metabolism in the brain (39, 40).

Methionine may produce acidosis from the metabolism of sulphur and inorganic sulphate, but in the doses we used, adequate renal and pulmonary function should compensate. In our patients, the decrease in arterial pH and blood alkali reserve was slight. Moreover, increasing acidosis has not been reported in spontaneous hepatic coma (7, 38, 46).

At present, the toxic substance derived from methionine and the mechanism of its action remain unknown. Further study of the mercaptans and amines derived from methionine seem worth while.

The observed effects of chlortetracycline on the fæcal flora are in general agreement with those of other investigators, who found some elimination of *Bact. coli* and a rise in proteus, streptococci and staphlococci (14, 33). Pappenfort and Schnall (35) noted the appearance after chlortetracycline therapy of *Candida*, which we found in considerable numbers in only one patient. Loh and Baker (27) found an individual variation. The changes they report are less marked than those

previously described, but very similar to our own. The rise in proteus, however, was more conspicuous in our series, but the increase in *Staphlococcus pyogenes* occurred less often. We confirmed the observation of Dearing and Heilman (14) that Bacteroides were eliminated by chlortetracycline in every patient. In agreement with Loh and Baker, the total anærobic count was found to be less reduced than the count of Bacteroides ; this was due to multiplication of lactobacilli and anærobic streptococci.

Although the changes in fæcal flora show a similar pattern, exact results of therapy in any individual cannot be predicted. They presumably depend on the initial presence in small numbers of strains, resistant to the antibiotic, which multiply when sensitive organisms are depressed during therapy. McVay (29) showed that organisms at higher levels of the intestine are suppressed earlier and to a greater extent than those present in the fæces. The changes effected in the ileum and proximal colon, where the antibiotic is present in higher concentration, may be more clear-cut than those seen in the fæces.

The protection afforded by chlortetracycline might be due to a change in intestinal flora, or it could be a direct metabolic effect. Chlortetracycline does not reduce the amount of ammonium produced from methionine by bacterial suspensions and it seems unlikely that the protection is due to less amino-acid being metabolised.

Chlortetracycline prevents massive hepatic necrosis in rats fed on a necrogenic diet (16, 19), an effect attributed to elimination of intestinal bacteria, which may utilise protective constituents of food (18). This long term effect is hardly relevant to our observations and sparing of an essential food factor seems unlikely. Chlortetracycline might affect liver function directly, but this could not be shown by routine liver function tests. Mann and co-workers (31) found that antibiotics failed to reduce blood ammonium levels in patients with liver disease.

Chlortetracycline may decrease amine production in the gut (32). De la Huerga and Popper (15) showed that chlortetracycline inhibited the formation of total tri-methylamine from choline through suppression of choline-utilising bacteria. The possible toxic effects of amine have already been discussed.

" Hepatic coma" shows such natural fluctuations that it is difficult to assess therapy in any individual patient. The present observations, in which patients served as their own control, shows that chlortetracycline is at least of benefit in methionine-induced neurological complications of liver disease. The neurological picture in this condition is identical with that caused by other nitrogenous substances and that seen in many instances of spontaneous " hepatic coma ", although the metabolic relationship between these conditions remains unknown. It does seem worth while, however, to apply our observations to the whole group of neurological complications of liver disease, and it is our clinical impression that chlortetracycline therapy is of benefit in spontaneous hepatic coma.

SUMMARY.

1. Oral methionine caused neurological deterioration in seven of nine patients with portal cirrhosis and chronic portal systemic encephalopathy. In eight of the nine patients, large portal systemic venous collateral channels were demonstrated. It was without effect in seven patients with hepatic cirrhosis, three of whom had an extensive portal systemic circulation and one with extra-hepatic portal vein obstruction. These patients had never experienced neurological complications.

2. Intravenous methionine was without effect in three of those who reacted to the oral amino-acid and in one there was a delayed exacerbation.

3. Neurological deterioration occurred without significant change in blood ammonium, blood pH, or serum bilirubin level.

4. Blood methionine levels rose equally in those who deteriorated and those who did not.

5. Oral chlortetracycline prevented or delayed the neurological deterioration in five sensitive patients who received methionine although the blood levels of that substance were even higher.

6. Fæcal flora of patients with liver disease and neurological complications, did not differ from normal subjects and patients with uncomplicated cirrhosis.

7. Methionine did not change the fæcal flora, but chlortetracycline in all groups resulted in a rise in proteus with elimination of Bacteroides and an inconstant fall in *Bact. coli.* The streptococcal types changed and lactobacilli increased.

8. The toxicity of methionine in patients with chronic portal systemic encephalopathy is due to some breakdown product of methionine other than ammonium.

9. It is suggested that chlortetracycline may be of benefit in " spontaneous hepatic coma ".

APPENDIX.

Detailed bacteriological data.

Patient	Organism	Control	After methionine	During and after chlortetracycline			Dates of chlortetracycline
				Date			
Without liver disease				14/2	18/2		
32	Bact. coli	$1{\cdot}0 \times 10^{4}$		$1{\cdot}3 \times 10^{8}$	$5{\cdot}5 \times 10^{4}$		9/2–12/2
	Str. fæcalis	—		$2{\cdot}5 \times 10^{6}$	$1{\cdot}5 \times 10^{4}$		
	Proteus	—		$6{\cdot}5 \times 10^{5}$	—		
	Bacteroides	—		—	$4{\cdot}0 \times 10^{6}$		
	Lactobacillus acidophilus	$6{\cdot}0 \times 10^{5}$		$1{\cdot}0 \times 10^{6}$	—		
				12/2	14/2		
33	Bact. coli	$3{\cdot}2 \times 10^{3}$		—	$1{\cdot}0 \times 10^{4}$		9/2–12/2
	Str. fæcalis	$1{\cdot}5 \times 10^{2}$		$1{\cdot}2 \times 10^{6}$	$1{\cdot}8 \times 10^{6}$		
	Proteus	—		—	$1{\cdot}0 \times 10^{5}$		
	Bacteroides	$4{\cdot}0 \times 10^{5}$		—	—		
	Lactobacillus acidophilus	$4{\cdot}0 \times 10^{5}$		$1{\cdot}2 \times 10^{6}$	$2{\cdot}0 \times 10^{5}$		
				25/2	28/2		
34	Bact. coli	$8{\cdot}0 \times 10^{5}$		—	$1{\cdot}0 \times 10^{6}$		25/2–28/2
	Str. fæcalis (hæmolytic)	—		$2{\cdot}8 \times 10^{7}$	$6{\cdot}2 \times 10^{6}$		
	Str. fæcalis (non-hæmolytic)	$8{\cdot}0 \times 10^{4}$		$2{\cdot}5 \times 10^{6}$	$1{\cdot}0 \times 10^{6}$		
	Bacteroides	$2{\cdot}4 \times 10^{6}$		—	—		
35	Bact. coli	$5{\cdot}5 \times 10^{4}$					
	Str. fæcalis	$2{\cdot}5 \times 10^{4}$					
	Anærobic strep.	$1{\cdot}0 \times 10^{6}$					
	Bacteroides	$5{\cdot}0 \times 10^{5}$					
36	Bact. coli	$2{\cdot}3 \times 10^{5}$					
	Str. fæcalis	$5{\cdot}0 \times 10^{3}$					
	Bacteroides	$2{\cdot}0 \times 10^{5}$					

APPENDIX—*continued.*

Patient	Organism	Control	After methionine	During and after chlortetracycline			Dates of chlortetracycline
				Date			
Without liver disease—continued.							
37	Bact. coli	$2{\cdot}4 \times 10^9$					
	Bacteroides	$2{\cdot}5 \times 10^9$					
38	Bact. coli	$1{\cdot}3 \times 10^5$					
	Str. fæcalis	$1{\cdot}5 \times 10^4$					
	Bacteroides	$1{\cdot}3 \times 10^9$					
39	Bact. coli	$1{\cdot}5 \times 10^4$					
	Str. fæcalis	$1{\cdot}3 \times 10^5$					
	Bacteroides	$1{\cdot}2 \times 10^9$					
40	Bact. coli	$1{\cdot}4 \times 10^4$					
	Str. fæcalis	$4{\cdot}1 \times 10^4$					
	Bacteroides	Many					
	Alkaligenes	$8{\cdot}4 \times 10^4$					
With liver disease				1/11	5/11	10/12	
1	Bact. coli	$4{\cdot}6 \times 10^5$	$3{\cdot}2 \times 10^5$	$2{\cdot}9 \times 10^4$	—	$1{\cdot}1 \times 10^4$	30/10–4/11
	Str. fæcalis (hæmolytic)	—	—	$1{\cdot}0 \times 10^5$	—	—	
	Str. fæcalis (non-hæmolytic)	$5{\cdot}0 \times 10^4$	$6{\cdot}0 \times 10^5$	$6{\cdot}6 \times 10^4$	—	—	
	Proteus vulgaris	—	—	$9{\cdot}3 \times 10^5$	$2{\cdot}9 \times 10^5$	$5{\cdot}0 \times 10^6$	
	Bacteroides	—	—	—	—	$5{\cdot}0 \times 10^7$	
	Cl. welchii	Few	—	—	—	—	
	Candida	—	—	—	—	$1{\cdot}1 \times 10^4$	
				4/10	21/10		
2	Bact. coli	$1{\cdot}2 \times 10^3$		—	$1{\cdot}3 \times 10^5$		29/9–4/10
	Str. fæcalis (hæmolytic)	—		$5{\cdot}6 \times 10^4$	—		
	Str. fæcalis (non-hæmolytic)	$2{\cdot}2 \times 10^5$		$1{\cdot}1 \times 10^4$	$1{\cdot}3 \times 10^5$		
	Proteus	—		$1{\cdot}4 \times 10^3$	—		
	Cl. welchii	$3{\cdot}9 \times 10^5$		—	$3{\cdot}7 \times 10^4$		
				27/10	10/1	28/2	
	Bact. coli			—	$1{\cdot}1 \times 10^5$	$6{\cdot}0 \times 10^4$	25/10–29/10
	Paracolon			—	—	$5{\cdot}6 \times 10^5$	
	Str. fæcalis (hæmolytic)			$1{\cdot}5 \times 10^6$	—	—	
	Str. fæcalis (non-hæmolytic)			—	—	$1{\cdot}1 \times 10^7$	
	Proteus			$2{\cdot}1 \times 10^6$	$6{\cdot}0 \times 10^5$	—	
	Bacteroides			—	$1{\cdot}0 \times 10^7$	$6{\cdot}0 \times 10^6$	
	Lactobacillus			—	—	—	
	Cl. welchii			—	—	—	
	Staph.			—	$1{\cdot}0 \times 10^5$	—	
	Micrococci			—	—	—	
				15/4			
	Lactobacillus			$5{\cdot}0 \times 10^6$			13/4–20/4
	Staphs.			$8{\cdot}5 \times 10^5$			
	Micrococci			$8{\cdot}5 \times 10^5$			

APPENDIX—*continued.*

Patient	Organism	Control	After methionine	During and after chlortetracycline			Dates of chlortetracycline
				Date			
With liver	*disease—continued.*						
				29/11			
3	Bact. coli	$4{\cdot}0 \times 10^5$		$1{\cdot}0 \times 10^4$			27/11–2/12[m]
	Str. fæcalis (non-hæmolytic)	$1{\cdot}5 \times 10^5$		$9{\cdot}5 \times 10^5$			
	Proteus mirabilis	—		$7{\cdot}2 \times 10^5$			
				13/4			
	Bact. coli			$2{\cdot}5 \times 10^5$			5/4–8/4
	Str. fæcalis (non-hæmolytic)			$6{\cdot}0 \times 10^5$			
	Anærobic strep.			$1{\cdot}0 \times 10^6$			
	Proteus vulgaris			$4{\cdot}0 \times 10^4$			
	Bacteroides			$7{\cdot}5 \times 10^5$			
				29/11	8/12	18/12	
4	Bact. coli	$2{\cdot}5 \times 10^6$	$1{\cdot}1 \times 10^5$	—	$4{\cdot}8 \times 10^6$	$1{\cdot}5 \times 10^4$	27/11–2/12
	Str. fæcalis (hæmolytic)	—	—	—	$2{\cdot}5 \times 10^5$	—	
	Str. fæcalis (non-hæmolytic)	$5{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^4$	—	—	$8{\cdot}5 \times 10^4$	
	Anærobic strep.	—	$4{\cdot}6 \times 10^5$	—	—	—	
	Proteus mirabilis	—	—	$7{\cdot}4 \times 10^5$	$5{\cdot}0 \times 10^5$	—	
	Bacteroides	$3{\cdot}0 \times 10^6$	$9{\cdot}9 \times 10^5$	—	$1{\cdot}0 \times 10^7$	$1{\cdot}0 \times 10^6$	
				5/4	14/4		
	Bact. coli			$2{\cdot}0 \times 10^4$	—		2/4–5/11
	Str. fæcalis (non-hæmolytic)			$4{\cdot}0 \times 10^4$	$9{\cdot}0 \times 10^5$		
	Proteus mirabilis			$5{\cdot}0 \times 10^6$	—		
	Anærobic Strep.			$5{\cdot}0 \times 10^5$	$2{\cdot}5 \times 10^4$		
				18/4			
	Bact. coli			$1{\cdot}8 \times 10^4$			14/4–18/4
	Str. fæcalis (non-hæmolytic)			$7{\cdot}5 \times 10^4$			
	Proteus mirabilis			$5{\cdot}0 \times 10^3$			
	Bacteroides			$5{\cdot}0 \times 10^5$			
				15/3	21/3	25/3	
5[o]	Bact. coli	$5{\cdot}3 \times 10^5$	$3{\cdot}0 \times 10^5$	$1{\cdot}0 \times 10^5$	—	$2{\cdot}2 \times 10^6$	13/3–21/3
	Str. fæcalis (hæmolytic)	—	—	—	$9{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^4$	
	Str. fæcalis (non-hæmolytic)	$1{\cdot}3 \times 10^6$	$2{\cdot}2 \times 10^6$	$1{\cdot}5 \times 10^6$	—	—	
	Proteus mirabilis	—	—	—	$5{\cdot}0 \times 10^5$	$2{\cdot}5 \times 10^4$	
	Bacteroides	$9{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^5$	$1{\cdot}5 \times 10^5$	—	—	
	Cl. welchii	$1{\cdot}0 \times 10^5$	$2{\cdot}0 \times 10^6$	—	—	—	
	Micrococci	$1{\cdot}5 \times 10^5$	—	—	—	—	
	Aerobic spore bearer	—	$1{\cdot}5 \times 10^6$	—	—	—	
				25/3			
6	Bact. coli	$7{\cdot}5 \times 10^4$		—			18/3–22/3
	Str. fæcalis (non-hæmolytic)	$1{\cdot}1 \times 10^6$		Present			
	Proteus morganii	—		Present			
	Bacteroides	$8{\cdot}0 \times 10^6$		—			

APPENDIX—*continued.*

Patient	Organism	Control	After methionine	During and after chlortetracycline			Dates of chlortetracycline
				Date			
With liver	*disease—continued.*			14/2	18/2	25/2	
7	Bact. coli	$9{\cdot}4 \times 10^5$		$4{\cdot}0 \times 10^5$	—	$4{\cdot}6 \times 10^5$	10/2–16/2
	Str. fæcalis (hæmolytic)	—		$5{\cdot}5 \times 10^6$	—	—	
	Str. fæcalis (non-hæmolytic)	$1{\cdot}0 \times 10^5$		$4{\cdot}0 \times 10^6$	$1{\cdot}8 \times 10^7$	$3{\cdot}5 \times 10^6$	
	Anærobic strep.	—		—	$3{\cdot}6 \times 10^6$	—	
	Proteus mirabilis	—		$5{\cdot}0 \times 10^4$	$4{\cdot}8 \times 10^5$	$1{\cdot}0 \times 10^6$	
	Bacteroides	$1{\cdot}7 \times 10^6$		—	—	$1{\cdot}0 \times 10^6$	
	Lactobacillus	—		$1{\cdot}8 \times 10^6$	—	—	
	Micrococci	—		—	—	$1{\cdot}5 \times 10^6$	
				8/3	11/3		
	Bact. coli			$7{\cdot}4 \times 10^5$	—		3/3–8/3
	Str. fæcalis (hæmolytic)			$7{\cdot}5 \times 10^6$	$1{\cdot}1 \times 10^6$		
	Str. fæcalis (non-hæmolytic)			—	$1{\cdot}1 \times 10^6$		
	Proteus mirabilis			$1{\cdot}0 \times 10^4$	$3{\cdot}8 \times 10^5$		
				18/4	25/4		
	Str. fæcalis (hæmolytic)			$2{\cdot}2 \times 10^6$	$1{\cdot}9 \times 10^6$		16/4–23/4
	Str. fæcalis (non-hæmolytic)			$3{\cdot}1 \times 10^6$	—		
	Proteus (morganii and mirabilis)			—	$2{\cdot}9 \times 10^5$		
	Staph. pyogenes			$2{\cdot}7 \times 10^5$	—		
				11/3	16/8		
8	Bact. coli			—	$3{\cdot}4 \times 10^5$		4/8–11/8
	Paracolon			—	$2{\cdot}5 \times 10^5$		
	Proteus morganii			$2{\cdot}5 \times 10^5$	—		
				14/2	25/2		
9	Bact. coli	$5{\cdot}1 \times 10^6$		$5{\cdot}5 \times 10^4$	$5{\cdot}0 \times 10^4$		10/2–16/2
	Str. fæcalis (hæmolytic)	—		$2{\cdot}2 \times 10^5$	$1{\cdot}2 \times 10^6$		
	Str. fæcalis (non-hæmolytic)	—		—	$1{\cdot}9 \times 10^6$		
	Anærobic strep.	$2{\cdot}8 \times 10^6$		$9{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^6$		
	Proteus mirabilis	—		$1{\cdot}3 \times 10^5$	—		
	Bacteroides	$1{\cdot}9 \times 10^6$		—	$8{\cdot}0 \times 10^6$		
	Lactobacillus	$2{\cdot}7 \times 10^6$		$1{\cdot}4 \times 10^6$	—		
11	Bact. coli	$1{\cdot}0 \times 10^6$	$2{\cdot}3 \times 10^6$				
	Str. fæcalis (non-hæmolytic)	$3{\cdot}1 \times 10^6$	—				
	Lactobacillus bifidus	—	$2{\cdot}0 \times 10^5$				
12	Bact. coli	$1{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^5$				
	Str. fæcalis (non-hæmolytic)	$8{\cdot}0 \times 10^5$	—				
	Bact. alkaligenes	$2{\cdot}4 \times 10^6$	—				
	Lactobacillus	—	$5{\cdot}0 \times 10^5$				
15	Bact. coli	$8{\cdot}5 \times 10^4$	$4{\cdot}5 \times 10^4$				
	Str. fæcalis (non-hæmolytic)	$7{\cdot}0 \times 10^4$	—				
	Bacteroides	$1{\cdot}0 \times 10^6$	—				
	Cl. welchii	—	Few				
	Cl. Sporogenes	—	Few				

APPENDIX—*continued.*

Patient	Organism	Control	After methionine	During and after chlortetracycline			Dates of chlortetracycline
				Date			
With liver	*disease—continued.*						
19	Bact. coli Str. fæcalis (non-hæmolytic) Bacteroides Cl. welchii	$4{\cdot}0 \times 10^5$ $9{\cdot}4 \times 10^4$ $3{\cdot}8 \times 10^4$ $5{\cdot}3 \times 10^3$	$2{\cdot}2 \times 10^4$ $3{\cdot}5 \times 10^4$ — $1{\cdot}3 \times 10^3$				
21	Bact. coli Str. fæcalis (non-hæmolytic) Bacteroides Lactobacillus	$7{\cdot}0 \times 10^5$ $1{\cdot}0 \times 10^4$ $7{\cdot}0 \times 10^7$ $1{\cdot}0 \times 10^6$					
				10/2	14/2		
22	Bact. coli Str. fæcalis (non-hæmolytic) Proteus Bacteroides Lactobacillus	$1{\cdot}2 \times 10^6$ $1{\cdot}5 \times 10^6$ — $2{\cdot}0 \times 10^6$ —		$2{\cdot}8 \times 10^6$ $4{\cdot}0 \times 10^5$ — Few —	$2{\cdot}5 \times 10^5$ — $4{\cdot}5 \times 10^5$ — $2{\cdot}3 \times 10^6$		9/2–12/2
				27/7			
23	Bact. coli Str. fæcalis (non-hæmolytic) Bact. alkaligenes	$1{\cdot}0 \times 10^3$ $3{\cdot}0 \times 10^4$ $4{\cdot}0 \times 10^3$		— $3{\cdot}0 \times 10^5$ —			20/7–27/7
				29/6			
24	Bact. coli Str. fæcalis (non-hæmolytic) Proteus	$2{\cdot}0 \times 10^4$ $5{\cdot}0 \times 10^3$ $3{\cdot}0 \times 10^3$		$1{\cdot}0 \times 10^5$ $1{\cdot}0 \times 10^5$ $1{\cdot}0 \times 10^4$			18/6–24/6
				25/6			
25	Bact. coli Str. fæcalis (non-hæmolytic) Proteus vulgaris Staph. albus	$3{\cdot}0 \times 10^5$ $6{\cdot}0 \times 10^4$ — —		$2{\cdot}3 \times 10^3$ $1{\cdot}0 \times 10^5$ $2{\cdot}5 \times 10^4$ $6{\cdot}0 \times 10^4$			22/6–25/6
26	Bact. coli Bacteroides	$4{\cdot}4 \times 10^6$ $3{\cdot}5 \times 10^6$					
27	Bact. coli Str. fæcalis (non-hæmolytic)	$1{\cdot}2 \times 10^6$ $9{\cdot}6 \times 10^5$					

* *Case* 5:—On date 5/4 bacterial counts were Bact. coli $1{\cdot}9 \times 10^6$, Str. fæcalis (non-hæmolytic) $5{\cdot}0 \times 10^5$, Proteus mirabilis $1{\cdot}0 \times 10^4$ and Bacteroides $3{\cdot}3 \times 10^6$.

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THE BIOLOGIC ASSAY OF L-METHIONINE USING A MUTANT OF BACT. COLI STRAIN K-12

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THE BIOLOGIC ASSAY OF L-METHIONINE USING A MUTANT OF BACT. COLI STRAIN K-12

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THE liver plays an important part in protein and amino acid metabolism. The function of methionine in experimental hepatic necrosis (Himsworth[1]) and its metabolism in patients with liver disease (Kinsell and associates[2]) have been investigated particularly carefully. Many patients with liver disease have a high fasting plasma methionine level and are unable to metabolize methionine. In this paper, a new method for the estimation of L-methionine in plasma is described employing a methionine-requiring mutant of *Bacterium coli* Strain K-12.

Certain mutant strains of *Bact. coli* K-12 (mutants 58/161 and J^{5-3}) employed in work on genetic recombination, unlike typical *Bact. coli* strains, cannot grow in a basic synthetic medium containing only glucose and ammonium salts as source of carbon and nitrogen, respectively. Mutant 58/161 requires L-methionine and mutant J^{5-3} requires both L-methionine and L-proline for growth, and these amino acids must be added to the medium. Since as little as 5 μg per milliliter methionine promotes good growth of the mutants in synthetic fluid medium, whereas 0.5 μg per milliliter still allows the development of a visible turbidity, these mutants appeared to be suitable for the estimation of methionine levels in the blood. With limiting concentrations of methionine, the amount of growth (judged nephelometrically) is proportional to the methionine concentration.

As the methionine-requiring mutant will grow in the absence of methionine if plasma proteins are present, a protein-free extract of the plasma must be used for assay. Dilutions of this extract are made in fluid synthetic medium, a small inoculum of washed bacteria is added to each, and the tubes are incubated at 37° C. At the same time, a set of standard tubes containing known concentrations of methionine are similarly set up and incubated. After incubation, the turbidity of each culture is compared with the standard series in a photoelectric nephelometer.

METHOD

1. *Strains of Bacterium coli Employed.*—The L-methionine-requiring strain of *Bact. coli* K-12, mutant 58/161, was principally used in this work. It was found, however, that its requirement for methionine could be fully satisfied by either homocysteine or cystathionine,

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which constitute prior stages in methionine synthesis so that the biochemical block in this strain arises before the cystathionine stage. The following amino acids and growth factors were investigated to see whether they could support growth of this strain in the absence of methionine: cysteine, threonine, alanine, proline, aspartic acid, glycine, tryptophane, norleucine, glutamic acid, isoleucine, vitamin B_{12}, choline, calcium pantothenate, para-aminobenzoic acid, and adenine.

None of these substances was found to be capable of replacing methionine in supporting growth. Recovery and dilution experiments strongly suggested that the methionine precursors cystathionine and homocysteine were not present in biologic fluids in significant amounts. This was confirmed by repeating some of the assays with another mutant strain of *Bact. coli* K-12 (J^{5-3}) which requires both methionine and proline. Work on genetic recombination (Clowes and Rowley[3]) had suggested that in this strain the mutation to methionine-dependence had occurred at a different genetic locus from that of mutant 58/161. This suggestion was supported by the finding that homocysteine and cystathionine could not substitute for methionine in promoting growth of this mutant, nor could cysteine, vitamin B_{12}, aspartic acid, or adenine. Results of assays with the two strains were very similar, thus confirming that homocysteine and cystathionine are not normally present in the plasma in amounts which could interfere with the assay of methionine by mutant 58/161 of *Bact. coli* K-12.

2. *Preparation of Inoculum.*—An overnight broth culture of the assay strain was washed three times in 0.85 per cent sodium chloride and resuspended to the original volume in saline. One drop of this suspension (0.02 ml.) was used to inoculate the 10 ml. of medium in each assay tube.

3. *Medium.*—The medium employed had the following constitution:

Na_2 H PO_4	7 Gm.
K H_2 PO_4	3 Gm.
Mg. SO_4 (10 per cent soln.)	0.1 ml.
N H_4 Cl	1 Gm.
Dist. water to	1,000 ml.

The pH was adjusted to 7.2 and the medium sterilized by autoclaving. Before use, 1.25 ml. of sterile 20 per cent glucose solution was added to each 100 ml., giving a final glucose concentration of 0.25 per cent in the medium.

For assays employing strain J^{5-3}, 20 mg. of L-proline was added to each 1,000 ml. of the medium.

4. *Preparation of a Standard Curve.*—Tubes were prepared containing 10, 5, 4, 3, 2, 1 and 0.5 μg per milliliter of L-methionine in 10 ml. medium. After inoculation with the test organism, the tubes were incubated at 37° C. for forty-eight hours and the turbidity of each tube measured with a photoelectric nephelometer.* The turbidity in arbitrary units was then plotted against the concentration of L-methionine in μg per milliliter. Between 0.5 μg per milliliter and 5 μg per milliliter there is virtually a linear relationship (see Fig. 1).

5. *Deproteinization of Plasma.*[4]—Eleven and two-tenths milliliters of distilled water was added to 8 ml. of plasma in a bottle. This was then stoppered, shaken, and 2.8 ml. of 0.6 N H_2 SO_4 added slowly with mixing, followed by 2 ml. of 10 per cent sodium tungstate. The plasma was therefore diluted 1 in 3 in the preparation of a protein-free supernatant.

The bottle was then shaken for two to three minutes and centrifuged. The protein-free supernatant was pipetted off and used for the methionine assay after adjustment of the pH to 7.2 and sterilization by boiling for two minutes.

6. *Preparation of Assay Tubes.*—As the turbidity is proportional to the methionine concentration only over a limited range, several different dilutions of the supernatant were

*An "EEL" photoelectric nephelometer (made by Evans Electroselenium Ltd., Harlow, Essex, England) was used which measures, on an arbitrary scale, the amount of light reflected by the bacteria in the suspension. In order to obtain comparable readings, the instrument was adjusted each day so that a turbidity standard (known to be equivalent to 3.6 mg. of protein nitrogen of *Bact. coli* K-12) read 100 arbitrary units.

usually made in the basal medium. With low concentrations of methionine, such as are found in the fasting state, 5 ml. of plasma supernatant was added to 1 ml. of ten times concentrated synthetic medium and the total volume made up to 10 ml. with distilled water. Thus, the final dilution of the supernatant in the medium was 1 in 2. With higher methionine concentrations, 1 ml. or 0.5 ml. of plasma supernatant was added to 9 or 9.5 ml. of the synthetic medium, giving a dilution of the supernatant in the medium of 1 in 10 or 1 in 20.

7. *Calculation of Plasma Methionine Concentration.*—From the standard curve, the methionine concentration in micrograms per milliliters in one of the dilutions of the supernatant set up was determined. This figure was then multiplied by the dilution factor of the supernatant in the medium of the particular tube used, and also by three, which is the dilution of the original plasma in the protein-free supernatant.

Thus: Methionine concentration in μg per milliliter of the original plasma = Methionine concentration in the test tube read off the standard curve × Dilution of supernatant in the medium × 3

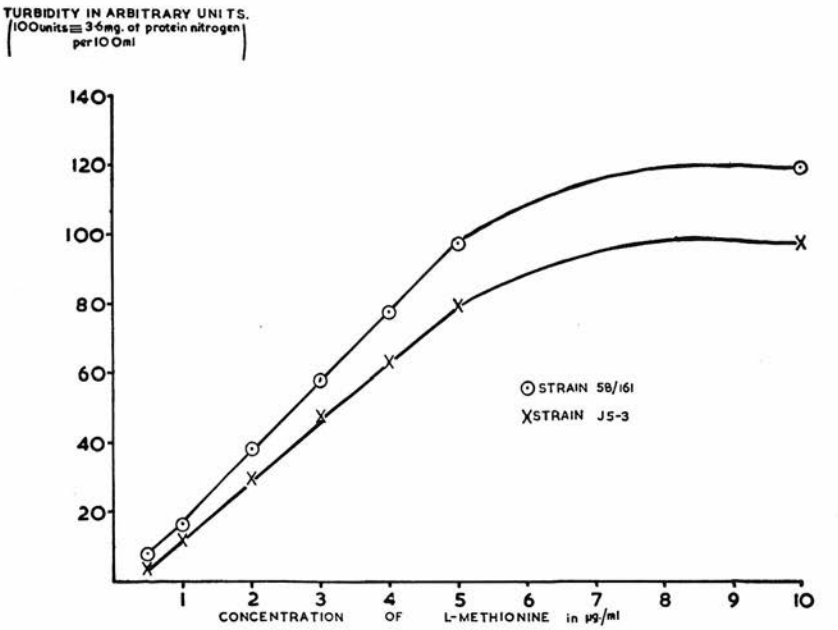

Fig. 1.—Standard curve of growth in arbitrary turbidimetric units as related to concentration of L-methionine in medium.

RESULTS

1. *Recovery Experiment.*—The L-methionine in normal plasma and in the same sample of plasma to which 100 μg per milliliter of L-methionine had previously been added was estimated.

	NEPHELOMETER READINGS IN ARBITRARY UNITS		ESTIMATED CONCENTRATION OF METHIONINE IN MICROGRAMS PER MILLILITER
	DILUTIONS OF SUPERNATANT IN MEDIUM		
	1:2	1:20	
Plasma	20	–	6
Plasma methionine	–	34	108

2. *L-methionine Levels in Patients With Cirrhosis of the Liver.*—Ten grams of DL-methionine was given daily for two to six days to 9 patients with cirrhosis of the liver. The L-methionine morning plasma levels varied from 9 to 69 μg per milliliter before the administration of methionine. They rose to 20 μg per milliliter, or above, in every patient receiving this amino acid. In one case, after six days a level of 375 μg per milliliter was reached.

In a series of 10 parallel estimations of a single specimen, the average reading was 43.1 arbitrary units and the standard deviation ± 2.

DISCUSSION

Although quantitative chromatographic methods for the estimation of amino acids in biologic fluids have recently been developed (for example, column chromatography by Moore and Stein,[5] and paper chromatography by Smith and Tompsett[6]), microbiologic methods probably remain more specific and sensitive than chemical estimations (Wheeler and Gyorgy[7]). The usual microbiologic methods employ *Streptococcus faecalis* (Stokes and co-workers[8]) or *Leuconostoc mesenteroides* P_{60} for L-methionine and *Lactobacillus fermentans* 36 for DL-methionine (Kinsell and associates[9]). All these organisms require complex synthetic media for assay.

The *Bact. coli* mutants used in this method require L-methionine for growth. Except for cystathionine and homocysteine, which can replace this amino acid as an essential growth factor for strain 58/161, none of the amino acids, B vitamins, and growth factors tested could replace methionine in promoting growth. Both strains gave very similar results, and it is concluded that cystathionine and homocysteine are not present in the plasma in amounts sufficient to invalidate this assay method.

The medium used in this method is much simpler than that required by the other test organisms. There appears to be no reason why other amino acids and B vitamins should not be assayed by a similar method using *Bact. coli* mutants.

The results obtained by this method are very similar to those of Kinsell and associates[2] who administered 9 Gm. of DL-methionine daily, as compared to 10 Gm. in the present series. Using *Leuconostoc mesenteroides* P_{60}, they found that elevation of the fasting level to above 20 μg per milliliter occurred in all patients with liver disease, but not in normal subjects. The present investigation thus confirms that methionine utilization is impaired in patients with cirrhosis of the liver.

SUMMARY

A microbiologic method for the assay of L-methionine in plasma, using a methionine-requiring *Bact. coli* mutant, is described. The medium used is much simpler than that required by other test organisms. The method has been applied to the investigation of the abnormal methionine metabolism in patients with cirrhosis of the liver.

It is suggested that other amino acids and B vitamins might be assayed using *Bact. coli* mutants.

I wish to thank Dr. Sheila Sherlock for stimulating my interest in methionine metabolism, Lord Stamp for his encouragement, Dr. W. Hayes for much help and advice and also for strain 58/161, and Dr. C. E. Dalgliesh for valuable suggestions.

I am also grateful to Dr. R. C. Clowes of the Wright Fleming Institute, St. Mary's Hospital, London, for strain J^{5-3} and Dr. D. D. Woods, F.R.S., of the Biochemistry Department, Oxford University, for a gift of cystathionine.

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THE *IN VITRO* PRODUCTION OF AMMONIUM AND AMINES BY INTESTINAL BACTERIA IN RELATION TO NITROGEN TOXICITY AS A FACTOR IN HEPATIC COMA

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LARGE amounts of ammonium salts are normally present in portal blood and are then metabolised to urea in the liver (Bollman and Mann, 1930). In patients with poor liver function and a portosystemic collateral circulation, and also in dogs with a portocaval shunt (Eck fistula), ammonium may by-pass the liver and reach high levels in the general circulation (Burchi, 1927 ; White, Phear, Summerskill and Sherlock, 1955). There is evidence that the presence of ammonium salts in the peripheral circulation may have toxic effects. For example, Bollman and Mann (1930) found that ammonium salts were toxic to dogs with an Eck fistula. A correlation between a high peripheral blood ammonium level and nervous symptoms has been noticed particularly after administration of a high protein diet in Eck fistula dogs (Monguio and Krause, 1934) or urea in man (Phillips, Schwartz, Gabuzda and Davidson, 1952) or after haemorrhage from intestinal varices (Riddell, 1955).

In addition to the possible toxicity of ammonium salts there is some evidence that methionine breakdown products, other than ammonium, may also be toxic. Thus, Phear, Ruebner, Sherlock and Summerskill (1956) have shown that after oral methionine certain cirrhotic patients exhibit nervous disturbances clinically indistinguishable from hepatic coma. In view of the fact that ammonium in particular is found in very high concentrations in the portal vein it seems probable that its source is the intestine (Cholopoff, 1927). Since bacteria are known to produce ammonium from both the breakdown of amino acids and the splitting of urea, it seems likely that the ammonium in the portal vein is the result of bacterial metabolism in the intestine. Attempts have therefore been made to treat cases of hepatic coma by the administration of antibacterial agents. Farquhar, Stokes, Whitlock, Bluemle and Gambescia (1950) found that chlortetracycline benefited such patients while Phear *et al.* (1956) found that the nervous disturbances following oral methionine, mentioned above, were partly or completely prevented by the administration of the same antibiotic. Dintzis and Hastings (1953) and Chao and Tarver (1953) found that the feeding of sulphonamides to animals inhibits the formation of ammonium from urea in the intestine. Sulphonamides have also been shown to lower the blood ammonium level in a patient with a porto-caval anastomosis (McDermott and Adams, 1954). Again, Martini, Phear, Ruebner and Sherlock (1956) during an investigation of the intestinal flora of cirrhotics found that when ileal fluid was incubated there was an increase of coliforms and *Streptococcus faecalis* with production of ammonium and break-

* In receipt of a grant from the Medical Research Council

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down of methionine. However, the addition, before incubation, of chlortetracycline to the ileal fluid prevented almost completely the multiplication of the bacteria and the biochemical changes.

The object of the present study was to investigate the bacterial production of ammonium under *in vitro* conditions approximating to those probably existing in the intestine. The production of amines which may also be toxic (Melnykowycz and Johannson, 1955) was also examined. Work on bacterial nitrogen metabolism has mainly dealt with the action of bacterial suspensions on individual nitrogenous substances. We studied the changes produced by bacteria growing in media containing a mixture of amino acids. The strains used were intestinal bacteria isolated during the investigations of Phear *et al.* (1956) and Martini *et al.* (1956). An attempt was made to identify the principal ammonium producers and to study the effect of chlortetracycline on their growth and ammonium production with a view to applying rational chemotherapy to this condition or to changing the intestinal flora by dietary means.

MATERIAL AND METHODS

Strains of bacteria

The organisms used in this investigation were isolated mainly from the faeces of patients with cirrhosis of the liver before and after chlortetracycline therapy (Phear *et al.*, 1956). A few were obtained from the ileal fluid of cirrhotic patients (Martini *et al.*, 1956) and a few others from normal subjects. Strains representing each of the commoner intestinal organisms were chosen.

Sensitivity to chlortetracycline

This was measured by a tube dilution method in 0·5 per cent glucose broth (pH 7·2). The concentrations of chlortetracycline used varied from 250 to 0·12 μg./ml. Results were read after 18 hr. incubation for aerobes and after 2 days' incubation in a Fildes McIntosh jar for anaerobes. The end-point was taken as the lowest concentration of the antibiotic which inhibited growth. The Oxford Staphylococcus was used as a standard organism throughout. The results of sensitivity tests were in general agreement with those of other investigators (Ungar, 1951).

Culture media

A synthetic amino acid medium of known composition was used for most of the work. The amino acid mixture chosen was that of Stokes, Gunness, Dwyer and Caswell (1945). ' Yeastrel ' was added to 0·5 per cent to supply the various members of the B group of vitamins. To each litre of medium were added 5 g. NaCl, 10 mg. $MgSO_4$, 7 g. Na_2HPO_4, 3 g. KH_2PO_4. The pH was adjusted to 7·2. The final nitrogen concentration was 130 mg. per cent. The amino acids remaining after incubation were identified by paper chromatography.

A casein hydrolysate medium was used for some of the work, and had the same composition except that the 130 mg. per cent nitrogen was derived from casein by acid hydrolysis.

A peptic digest of blood was prepared similar to that of Fildes (Mackie and McCartney, 1953) except that horse blood was substituted for sheep blood. Its nitrogen content was adjusted to 130 mg. per cent. Yeastrel and salts were added in the same concentrations as in the amino acid medium.

The buffer concentration (0·02 M) was insufficient to maintain the pH in the presence of added carbohydrate, and after incubation the pH fell. The final pH values of cultures inoculated with an atypical coliform (*Bacterium freundii*), *Proteus morgani* and a non-haemolytic streptococcus in the presence of glucose were respectively 5·2, 4·9 and 4·4.

Anaerobic conditions, when necessary, were obtained by boiling the medium and then adding 0·01 per cent thiolacetic acid. Other substances when added were present in the following concentrations : glucose 500 mg., lactose 500 mg. and urea 100 mg. per cent.

Medium in 10 ml. quantities was placed in 20 ml. screw-capped bottles, inoculated with a drop (0·02 ml.) of a broth culture of each strain and incubated at 37°.

Assessment of bacterial growth

After incubation, growth was usually assessed by the turbidity of the medium. An EEL photoelectric nephelometer (Evans Electroselenium Ltd.) was used. In order to obtain comparable readings the instrument was adjusted each day so that an opacity standard read 100 arbitrary units. The amount of bacterial growth of each species was assumed to be proportional to the nephelometer reading in arbitrary units. In order to compare the growth of different species it was decided to assess bacterial growth in μg. N/ml. One strain of each genus was grown in 0·5 per cent glucose broth, washed three times in 0·85 per cent sodium chloride and re-suspended. The N content of a suspension of standard turbidity (100 arbitrary units) was then determined.

Growth in the blood digest medium was not assessed turbidimetrically as turbidity estimations gave irregular results believed to be due to a breakdown product derived from the medium. When this medium was used in an experiment, growth was assessed by viable counts using the method of Miles and Misra (1938). It was realised that this was less reliable for the assessment of growth than a nephelometric method.

Time of incubation

To find the most suitable time for incubating the cultures before measuring growth and ammonium production a series of cultures of *Bact. freundii* was set up and their turbidity, viable count and ammonium content were measured daily. Growth and ammonium production were almost maximal after 1 day. Cultures of *Bact. freundii* and other aerobes were therefore examined after 18 hr. incubation. Anaerobes including microaerophilic streptococci and lactobacilli grew more slowly and were examined after 48 hr.

Biochemical methods

Ammonium production was measured by the microdiffusion method of Conway (1950). Total nitrogen was measured by Nesslerisation.

DL-methionine was measured by running the specimens, together with a range of standards, on buffered filter paper (McFarren, 1951) and then extracting and measuring the concentration of methionine in the spots by the method of Naftalin (1948) or of Yemm and Cocking (1955).

After making the medium alkaline, amines were extracted into ether. They were then re-extracted into a small volume of hydrochloric acid and run on unidimensional chromatograms using butanol-acetic acid as solvent. Chromatograms were run on Whatman No. 1 filter paper at room temperature. They were sprayed with ninhydrin. It was not possible to identify the amines fully, but known amines were run on each chromatogram, and their Rf values compared with those of the amines produced by the bacteria. No exact quantitative measurements of amine production were made, but the amount of substance in each spot was graded 0 to 6.

RESULTS

Growth and ammonium production by different species

The ammonium content of the medium was increased by the growth of all the species investigated except the lactobacilli which in some experiments did not produce any ammonium (Table I). In the absence of glucose the various species did not differ in the quantities of ammonium produced in relation to growth expressed in terms of

$$\frac{\text{production of } NH_4 \text{ nitrogen}}{\text{production of bacterial protein nitrogen.}}$$

The addition of glucose to the medium, while increasing the growth of all strains except *Bact. freundii*, decreased NH_4 production This agrees with the findings of Stephenson and Gale (1937). In the presence of glucose the most potent NH_4 producers were the coliform, *Proteus* and *Bacteroides* strains and the NH_4N produced in relation to growth was greater from Gram-negative bacteria

TABLE I.—*NH_4N Production by Different Species of Bacteria.*

Species.	Glucose. − Growth.	− NH_4N production.	− NH_4N/ growth.	Glucose. + Growth.	+ NH_4N production.	+ NH_4N/ growth.
Gram-negative bacteria						
Bact. freundii	109 (27)	163 (11)	1·5	68 (11)	109 (16)	1·6
Bact. coli	—	—	—	122 (38)	95 (12)	0·8
Bact. aerogenes	—	—	—	146 (39)	71 (21)	0·5
Bact. alkaligenes	29 (2)	71 (1)	2·4	—	—	—
Proteus mirabilis	36 (0)	184 (16)	5·2	127 (53)	133 (47)	1·1
P. vulgaris		—		153 (38)	82 (6)	0·5
P. morgani	78 (2)	150 (1)	1·9	149 (26)	128 (28)	0·9
Bacteroides	—	—	—	13 (23)	17 (6)	1·3
Ps. pyocyanea	—	—	—	155 (23)	83 (62)	0·5
		Mean	2·8		Mean	0·9
Gram-positive bacteria						
Cl. welchii	—	—	—	182 (91)	40 (6)	0·2
Cl. sporogenes	—	—	—	144 (77)	108 (74)	0·8
L. plantarum	—	—	—	27 (15)	4 (1)	0·2
L. bifidus	—	—	—	99 (65)	8 (15)	0·08
Staph. pyogenes	14 (2)	42 (11)	3·0	74 (56)	36 (18)	0·5
Str. faecalis (haemolytic)	—	—	—	79 (39)	25 (18)	0·3
Str. faecalis (non-haemolytic)	10 (3)	48 (1)	4·8	95 (19)	39 (1)	0·4
Microaerophilic streptococcus	—	—		21 (24)	14 (13)	0·6
		Mean	3·9		Mean	0·4

All cultures were grown in the amino acid medium of initial pH 7·2. Average growth and NH_4 production in μg.N/ml. Standard deviations in brackets.

(mean 0·9, range 0·5–1·6) than from the Gram-positive organisms (mean 0·4, range 0·08–0·8).

Altering the pH of the glucose-containing synthetic amino acid medium to pH 6 and pH 8 had little effect on the growth and ammonium production of *Bact. freundii*, *P. mirabilis*, *L. acidophilus* and *Str. faecalis*.

The substitution of lactose for glucose did not lower the NH_4 production of *Bact. freundii* to the same extent as glucose although this organism ferments both sugars. On the other hand, while *P. morgani* (a non-lactose fermenter) was unaffected by lactose, the growth and NH_4 production of *Str. faecalis* in this sugar did not differ from those in glucose.

Comparison of ammonium production in an amino acid, a casein hydrolysate and a blood digest medium

Ammonium production in relation to growth was similar in the amino acid and casein media but much less in the blood digest medium. The initial ammonium content of the casein medium was 400 μg. N per ml., while those of the amino acid and blood media were 43 and 62 μg. N per ml. Thus neither growth nor ammonium production was related to the initial ammonium content of the medium. In the blood medium there was little difference in the ammonium production in relation to growth by the three strains but in the casein and amino acid media *Bact. freundii* was the most potent ammonium producer followed by *P. mirabilis* and *Str. faecalis* (Table II).

TABLE II.—*Comparison of Growth and Ammonium Production in Amino Acid, Casein and Blood Media*

Species.	Amino acid. Viable count × 10^9/ml.	Amino acid. NH_4N production.	Amino acid. NH_4N/viable count.	Casein. Viable count × 10^9/ml.	Casein. NH_4N production.	Casein. NH_4N/viable count.	Blood. Viable count × 10^9/ml.	Blood. NH_4N production.	Blood. NH_4N/viable count.
Bact. freundii	1·6	142		2·5	126		2·9	29	
	2·5	139		3·0	135		2·5	30	
	2·0	102		2·9	155		4·7	41	
	1·9	119		3·0	151		—	—	
Average	2·0 (±0·3)	125 (±16)	63	2·8 (±0·2)	142 (±12)	50	3·4 (±1·0)	33 (±5)	9·7
Str. faecalis (non-haemolytic)	2·0	43		2·4	93		1·5	9	
	2·3	43		3·2	93		1·6	8	
	1·5	39		2·0	77		1·4	24	
	1·7	39		2·1	77		1·5	26	
Average	1·9 (±0·3)	41 (±2)	21	2·4 (±0·5)	85 (±8)	35	1·5 (±0·1)	17 (±8)	11
P. mirabilis	2·3	111		2·7	186		3·6	53	
	2·6	118		4·2	201		3·7	59	
	3·8	96		5·0	134		3·3	59	
	4·8	87		5·5	199		3·5	59	
Average	3·4 (±0·9)	103 (±13)	38	4·4 (±1·1)	180 (±27)	41	3·5 (±0·1)	57 (±8)	16

Glucose was added to all cultures.
Ammonium production expressed in μg. NH_4N/ml.

Urease activity

Table III shows the difference in ammonium production with and without added urea, among those strains found to possess urease activity. *Bact. aerogenes* and 3 species of *Proteus* were most active, producing more than 200 μg. per ml. more ammonium, in the presence of urea. *Staphylococcus pyogenes* and one strain of *Bact. coli* had less activity, producing about 20 μg. more ammonium, in the presence of urea. The breakdown of urea by *Bact. coli* confirmed the suggestion of Christensen (1946) that occasional strains might possess urease activity. The microaerophilic streptococcus and *Ps. pyocyanea* showed little activity, producing only about 5 μg. per ml. additional ammonium. No urease activity was observed in *Bact. alkaligenes*, a second *Bact. coli* strain, strains of *Str. faecalis*, lactobacilli, *Bacteroides* or *Clostridium welchii*.

The effect of chlortetracycline on growth and ammonium production

When carrying out chlortetracycline sensitivity tests it was found that tubes containing a concentration of drug which would just inhibit growth over 18 hr. showed good growth after 40 hr. This is probably accounted for by the instability of chlortetracycline (Ungar, 1951). Table IV shows the ammonium production in relation to growth in the presence of quantities of chlortetracycline which partially inhibited growth over a 40-hour period. It will be seen that chlortetracycline decreased the ammonium production in relation to growth of *Bact. aerogenes* and of a haemolytic *Str. faecalis* while having no effect on that of *Bact. coli*, *Bact. freundii*, *P. morgani*, *P. vulgaris*, *P. mirabilis* and *L. plantarum*. Ammonium production in relation to growth was increased in a micro-aerophilic streptococcus.

Amine production

A comparison was made of the amine production of *Bact. freundii*, *P. mirabilis* and a haemolytic *Str. faecalis* in the amino acid, blood and casein media, and in the last with and without added glucose. Under all conditions *P. mirabilis*

produced most amines, and casein was found to be the best medium for amine production by all species. The addition of thiolacetic acid slightly increased amine production. Glucose, while increasing the growth of all three species, decreased amine production by *Bact. freundii* and *Proteus*, while increasing that of the haemolytic *Str. faecalis*.

TABLE III.—*Urease Activity of Bacteria Possessing this Enzyme in an Amino Acid Medium Containing Glucose*

Growth and NH_4 production in μg.N/ml.

	Urea.				
	−		+		
Species.	Growth.	NH_4 produced.	Growth.	NH_4 produced.	Increase in the NH_4N production in the presence of urea.
Bact. aerogenes	183	81	183	468	387
			173	425	344
P. vulgaris	220	90	297	443	353
			258	417	327
P. morgani	189	166	239	500	334
			239	506	340
P. mirabilis	239	216	254	470	254
			254	423	207
Staph. pyogenes	151	53	138	98	45
			152	80	27
Bact. coli	178	101	156	123	22
			156	123	22
Anaerobic streptococcus	85	27	74	32	5
			83	31	4
Ps. pyocyanea	164	87	150	94	7
			155	89	2

TABLE IV.—*Effect of Chlortetracycline at Concentrations Just Limiting Growth on Ammonium Production in Amino Acid Medium Containing Glucose*

	Chlortetracycline										
	−					+					
	Growth.		NH_4N production.			Growth.		NH_4N production.			Concentration of chlortetracycline (in μg./ml.).
Species.	Average.	S.D.	Average.	S.D.	NH_4N growth.	Average.	S.D.	Average.	S.D.	NH_4N growth.	
Bact. freundii	104	(15)	123	(9)	1·2	86	(8)	96	(9)	1·1	2·5
Bact. coli	122	(40)	133	(22)	1·1	67	(4)	108	(17)	1·6	2·5
Bact. aerogenes	109	(24)	65	(24)	0·6	82	(20)	15	(9)	0·2	2·5
Str. faecalis (haemolytic)	68	(2)	45	(5)	0·6	55	(8)	10	(6)	0·2	50
P. mirabilis	141	(21)	107	(14)	0·8	109	(8)	103	(8)	0·9	50
P. morgani	117	(17)	155	(12)	1·3	94	(11)	173	(15)	1·8	50
P. vulgaris	125	(14)	122	(3)	1·0	77	(1)	147	(1)	2·0	50
L. plantarum	24	(5)	4	(4)	0·2	14	(4)	5	(4)	0·3	10
Microaerophilic streptococcus	38	(25)	23	(7)	0·6	10	(4)	25	(8)	2·5	10

It appeared that amines of Rf 0·04—0·06 are agmatine, histamine and cadaverine, while that of Rf 0·45–0·47 is probably tyramine with a front retarded by other substances present in the extract. It is not known to which amines the other substances correspond, but that of Rf 0·69–0·72 seems to be similar to the ephedrine-like spot of Melnykowycz and Johansson (1955). Spots with Rf of 0·21–0·23, 0·45–0·47 and 0·69–0·72 gave a positive Pauly's reaction.

Having determined that the casein medium was most favourable to amine production a comparison was made of amine production by these and some other species (Table V). Glucose was added to increase growth. *P. mirabilis* was most active, and lactobacillus, *Ps. pyocyanea*, *Bact. aerogenes* and *Bacteroides* less so. No amines were detectable in the medium after culture of *Cl. welchii* and *Bact. alkaligenes*. It was not possible to identify all the amines produced in our cultures but results are in general agreement with those of previous workers (Gale, 1946).

Chlortetracycline in the same subinhibitory concentrations as in Table IV prevented amine formation by *Bact. freundii* and *Bact. aerogenes* while having little effect on that of the haemolytic *Str. faecalis* and none on that of *P. mirabilis* (Table V).

TABLE V.—*Comparison of Amine Production by Different Species in a Casein Hydrolysate Medium with and without Subinhibitory Concentrations of Chlortetracycline*

Species.	Chlortetracycline.	Time of incubation in days.	Rf. 0·04–0·06	0·12–0·15	0·21–0·23	0·35–0·36	0·45–0·47	0·57–0·59	0·61–0·63	0·69–0·72	0·78–0·83
Bact. freundii	—	1	..	1	..	..	..	..	..	2	..
	+	1	—	..	..	..	..	..	..	..	..
P. mirabilis	—	1	1	1	..	2	2	4	3	5	1
	+	1	1	1	..	2	2	4	3	5	1
Str. faecalis (haemolytic)	—	1	..	..	..	..	3	..	2	3	..
	+	1	..	..	..	..	2	..	1	2	..
Cl. sporogenes	—	2	1	..	1	..	3	..	..	1	1
L. plantarum	—	2	..	..	..	..	..	..	..	1	..
Ps. pyocyanea	—	2	..	..	..	..	..	..	1	..	..
Bact. aerogenes	—	2	1	..	..	..	..	..	..	..	1
	+	2	—	..	..	..	..	..	..	..	..
Cl. welchii	—	2	—	..	..	..	..	..	..	..	..
Bacteroides	—	2	..	..	..	..	..	..	1	..	..
Bact. alkaligenes	—	2	—	..	..	..	..	..	..	..	..

All cultures were initially at pH 7·2 and were incubated anaerobically in the presence of glucose. Numbers represent an estimation of the amount of ninhydrin-positive substance in each spot on an arbitary scale (0–6).

DISCUSSION

The results of our *in vitro* studies on ammonium production cannot unreservedly be applied to the more complex conditions existing in the intestine, although comparable quantities of ammonium were produced in incubation experiments using ileal fluid as a medium (Martini *et al.*, 1956). It would be of interest to know how much ammonium is produced in the gut daily. However, the volume of the gut fluid and its bacterial content are not known and are probably variable. The fact that in our cultures the bacterial cell nitrogen was of

the same order as the NH_4 nitrogen produced and that the nitrogen content of the faeces is 1–3 g. per day, suggests that this is in the order of g. rather than mg. White *et al.* (1955) showed that 3 g. of NH_4Cl may sometimes give rise to nervous symptoms in patients with cirrhosis of the liver. The quantity of ammonium produced by intestinal bacteria may therefore be of clinical significance. However, only work on isolated intestinal loops of animals can solve this problem satisfactorily. Ammonium production also occurred in a medium containing no other source of nitrogen than a peptic digest of blood. The rise in blood ammonium observed in cirrhotic patients after intestinal haemorrhage may therefore in part be due to ammonium produced by bacteria from blood in the intestine. When blood urea levels rise in cirrhosis of the liver the urease activity of *Proteus* and *Bact. aerogenes* strains may be of clinical significance.

In our experiments cultures were made in small screw-capped bottles in order to prevent escape of ammonium, and anaerobic conditions almost certainly prevailed during most of the incubation period. This assumption is supported by two observations. Firstly, *Bact. freundii* broke down aspartic acid, cysteine, threonine and serine, which it attacks by dehydrogenation, rather than glycine, alanine and glutamic acid which it would have attacked by oxidation (Fry, 1955). Secondly, the rate of ammonium production by *Bact. coli* is greatest under aerobic conditions (Stumpf and Green, 1944). Since we found no difference between the ammonium production of *Bact. coli* with and without thiolacetic acid, anaerobic conditions seem to have prevailed even without this reducing substance. As conditions in the large intestine are favourable to the growth of anaerobic bacteria we did not consider that our results were invalidated by anaerobiosis in our cultures. It must be admitted that in the intestine, where mixed bacterial populations compete for available nutrients, bacteria may not show the same degree of ammonium production as did our pure strains in relatively simple media. Although we found that in several species ammonium production at pH 6 and pH 8 did not differ greatly from that at pH 7, further work on ammonium production under more varied conditions is clearly required. In particular the metabolism of mixed cultures of intestinal bacteria and the production of ammonium during the early phases of growth seem to merit further investigation.

The intestinal flora of patients with cirrhosis of the liver differs from that of normal subjects in a greatly increased number of coliforms and *Str. faecalis* in the small intestine. These organisms also occur at a higher level in cirrhotics (Martini *et al.*, 1956). It does not seem probable, however, that the increased blood ammonium levels in these patients are due to this increase in small intestinal flora. Patients suffering from other diseases but with normal liver function may have a similar intestinal flora without showing any clinical or biochemical abnormality (Martini *et al.*, 1956). It seems probable, moreover, that even in patients with cirrhosis of the liver the majority of intestinal bacteria are situated in the large intestine, which therefore remains the main site of ammonium production. The increased blood ammonium levels in cirrhosis are therefore almost certainly not due to increased ammonium production in the intestine but to a decreased ability of the liver to metabolise ammonium or to the presence of a collateral circulation which by-passes the liver.

Reduction in ammonium production by the intestinal flora in patients with hepatic coma might be of benefit. As glucose was found to reduce ammonium production by intestinal bacteria the low protein–high carbohydrate diet

usually prescribed for cirrhotic patients may lower ammonium production partly by lowering the amount of nitrogenous substrate available to bacteria and partly because of the effect of glucose in lowering ammonium production. Lactobacilli which produce little or no ammonium are generally believed to become prominent in the intestinal flora when a milk diet is given (Dudgeon, 1926). Such a diet might therefore be of benefit to patients suffering from the nervous complications of liver disease.

Chlortetracycline was considered to be beneficial in hepatic coma by Farquhar *et al.* (1950) and more recently antibiotics have been advised for the treatment of hepatic coma on the supposition that production of harmful toxic metabolites might be diminished (Riddell, 1955). Investigations of the effect of chlortetracycline on the faecal flora (Phear *et al.*, 1956) showed that the total viable counts were hardly diminished. Although there was a constant depression of *Bacteroides* with a partial lowering of the coliform count, the number of *Proteus*, anaerobic streptococci and lactobacilli increased in many cases and *Str. faecalis* was unaffected. Replacement of *Bacteroides* and some coliform strains by *Proteus*, another strong ammonium producer, may have occurred in the Eck fistula dogs of Mann, Bollman, Huizenga, Farrar and Grindlay (1954) who found that oxytetracycline did not lower the blood ammonium levels in these animals. Our finding that chlortetracycline at subinhibitory levels may lower ammonium production *in vitro* is of interest but of limited importance as the proportion of strains showing this phenomenon was small.

Antibiotics affecting the principal ammonium producers, *Proteus*, coliforms and *Bacteroides*, should cut down the production of ammonium by intestinal bacteria and therefore might benefit patients in impending hepatic coma. Chlortetracycline only fulfils these conditions partially and other wide spectrum antibiotics such as neomycin (Poth, 1954) or a combination of two drugs would probably be more effective.

SUMMARY

A comparison has been made of the *in vitro* ammonium and amine production by different species of growing intestinal bacteria.

When cultivated in a synthetic amino acid medium containing glucose of pH 7·2 all species, except lactobacilli, produced ammonium. Gram-negative bacteria produced more ammonium than Gram-positive organisms. Glucose increased growth in almost all instances and decreased ammonium production. Ammonium production was greater in a casein hydrolysate and less in a blood digest medium than in the synthetic amino acid medium. Urease activity was high in *Proteus* and *Bact. aerogenes* and less in *Staph. pyogenes*, *Bact. coli*, *Ps. pyocyanea* and an anaerobic streptococcus.

Amine production was greater in the casein medium than in that containing synthetic amino acids or a peptic digest of blood. *Proteus* was the most active amine producer.

Chlortetracycline at subinhibitory levels in two strains reduced ammonium production out of proportion to its effect on growth.

As ammonium salts are toxic to patients with cirrhosis of the liver a reduction of intestinal ammonium production might be beneficial. The use of dietary measures and of antibiotics for this purpose is discussed.

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