Clinical and other Notes.

A REPORT ON AN INVESTIGATION INTO THE CHARACTERISTICS OF NEW TYPES OF NON-MANNITOL-FERMENTING BACILLI ISOLATED FROM CASES OF BACILLARY DYSENTERY IN INDIA AND EGYPT.

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The object of this paper is to place on record the findings of an investigation extending over a period of five years into new types of non-mannitol-fermenting organisms isolated from cases of bacillary dysentery in India and Egypt. (The Egyptian strains were received from Lieutenant-Colonel D. W. Beamish, R.A.M.C., in June, 1939.) Type strains and antisera of these new types are available at the District Laboratory, Quetta, India, and the Emergency Vaccine Laboratory, Military Hospital, Tidworth.

It is hoped that the findings of this investigation in conjunction with Boyd's work on the mannitol-fermenting organisms may assist in clarifying the relationship of the dysentery bacilli.

(i) Introduction.

This investigation has been closely associated with the general routine work on dysentery at the District Labatory, Quetta.

In 1932 and 1933 there was a large increase in the number of cases of dysentery in Quetta. It was during this period, when over fifteen hundred cases of dysentery were being investigated, that a number of so-called "inagglutinable" strains of *B. dysenteriæ* Shiga and *B. dysenteriæ* Schmitz was reported by Large. His work is fully described in the *Journal of the Royal Army Medical Corps* for August-November, 1934. The investigation was interrupted for a short period by the disastrous earthquake at Quetta in May, 1935. In October, 1936, the conduct of the investigation came into my hands. Any conclusions reached are based on personal observations, some of which differ from those previously described by Large.

Only two types of non-mannitol-fermenting organisms are at present recognized as being capable of causing bacillary dysentery. These are *B. dysenteriæ* Shiga and *B. dysenteriæ* Schmitz. It would appear that Dudgeon's *B. para Shiga* (Indol +) and Andrews *B. ambiguus* were probably *B. dys.* Schmitz but serological evidence that they are identical is lacking.

Throughout the literature references are made to both indol-negative

and indol-positive non-mannitol-fermenting bacilli which are inagglutinable with *B. dys.* Shiga and *B. dys.* Schmitz antisera. The best known of these are Dudgeon's *B. para* Shiga, and Ornstein's *B. fallax* and *B. inconstans.* But, as serological data of their relationship to other dysentery organisms are not readily available, it is difficult to assess the relative importance of these organisms. Boyd (1935) refers to three non-indol producing strains that he occasionally found. Archer (1933) described a strain, isolated at Wellington, which differed from *B. dys.* Shiga in producing acid in dulcitol.

From October, 1936, to the end of April, 1941, 154 non-mannitol-fermenting strains have been fully investigated. With the exception of two strains received from England and three from Egypt all strains were isolated at different military laboratories in India. All were inagglutinable with *B. dys.* Shiga and *B. dys.* Schmitz antisera.

It was soon found that as the strains first investigated possessed so great a variety of morphological, cultural and biochemical reactions it would be necessary to define the characteristics of members of the group to be studied. These were based on those exhibited by *B. dys.* Shiga and *B. dys.* Schmitz.

Definition of the Non-Mannitol-Fermenting Group.

The organisms are gram negative, non-capsulate, non-sporing and non-motile coliform bacilli. They ferment glucose but neither is lactose nor a 2 per cent solution of mannitol fermented in fourteen days. Gelatin is not liquefied and the growth of the colonies is non-spreading on phenol-agar or 1½ per cent nutrient agar. The different types must have no serological relationship with the Flexner-Boyd-Newcastle group of dysentery bacilli and are divided into two main subgroups according to their production or non-production of indol.

Of the 154 strains investigated 107 were found to have these characteristics and could be grouped serologically as one or other of new non-mannitol-fermenting types. Eight strains were found to be serologically identical with some members of the Flexnor-Boyd-Newcastle group. The remaining thirty-nine strains form a heterogeneous subgroup of organisms, some of which are undoubtedly related to the proteus group. There is no evidence available at present to suggest that any of these organisms are capable of causing dysentery.

(ii) Biochemical Characteristics.

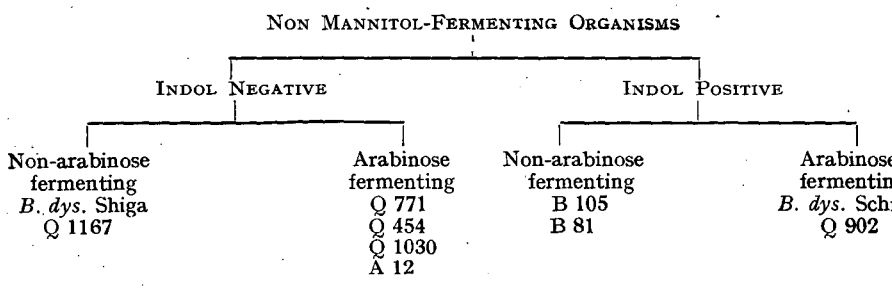
The fermentation reactions of a number of carbohydrates and alcohols were studied—viz. lactose, glucose, mannitol, dulcitol, saccharose, adonite, arabinose, inulin, maltose, raffinose, rhamnose, salicin, xylose and sorbite. With the exception of those given in Table I below, no carbohydrate or alcohol gave reactions which were considered to be of importance in differentiating types.

TABLE I.

	B. dys. Shiga	Q 1167	Q 771	Q 1030	Q 454	A 12	*B. dys.* Schmitz	Q 902	B 105	B 81
Lactose	—	—	—	—	—	—	—	—	—	—
Glucose	A (1)	A (1)	A (1)	A (1)	A (1)	Ag (1)	A (1)	A (1)	A (1) or Ag (1)	A (1)
Mannitol	—	—	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	A (2)	—	—	ALK(1)	ALK(1)	ALK(1)	ALK(1)
Arabinose	—	—	A (1)	A (1)	A (1)	Ag (1)* to A (7)	A (1)	A (1)	—	—
Saccharose	—	—	—	—	—	—	—	—	A (5)	A (4)
Phenol Red Milk	Sl. a (1)	Sl. a (1)	A (1) or Sl. a (1)	A (1)	A (1)	A (2)	A (1–4)	ALK 5	ALK 3	ALK 4
Gelatin†	—	—	—	—	—	—	—	—	—	—
Indol.	—	—	—	—	—	—	+	+	+	+

* Gas is generally absorbed by the seventh day.
† Gelatin not liquefied.

(*a*) The biochemical reactions given in Table I have been so constant throughout the investigation that it is possible to make a preliminary biochemical classification of the members of this group. This is given below:



(*b*) From the findings detailed in Table I it is evident that only Q1167 is biochemically similar to *B. dys.* Shiga.

The types Q771, Q454, Q1030 and A12 differ in being arabinose-fermenters, while Q1030, also differs in being a dulcitol-fermenter.

Of the three new indol-producers Q902 is very similar and only differs from *B. dys.* Schmitz in producing very marked alkalinity in phenol red milk while all strains of the latter examined here were found to produce a permanent acidity.

The types B105 and B81 differ in being saccharose but not arabinose fermenters and by producing a marked alkalinity in milk.

(3) ANTIGENIC STRUCTURE.

(*a*) *Serological Relationship to Mannitol-Fermenting Types.*

Cross agglutination tests failed to show any relationship between the types described and the classical Flexner and Boyd types of mannitol fermenters.

(*b*) *Serological Relationship between the Non-mannitol Type described.*

With the exception of slight cross-agglutination between *B. dysenteriæ* Schmitz and B 105 the types remained serologically distinct.

(*c*) *Bacterial Variation.*

The only variation found up to the present is the S—R type. This characteristic is exhibited by all the new types, but is especially well marked with Q1030, Q771 and Q454. One Egyptian strain of Q771 produced three types of colonies.

(i) Small smooth clear cut colonies which only gave rise to the S type of colony.

(ii) A large rough colony with central papillæ and an indented margin. This gave rise to both S and R type colonies.

(iii) A colony intermediate in size which appeared more R type than S, but with no central papillæ. This gave rise to all three types of colonies.

(4) Distribution and Frequency of Occurrence of the New Types.

The distribution of these new types is given in Table II, and an analysis of the Quetta and all India figures in Tables III and IV.

Table II.

	Quetta	*Karachi*	*Bannu*	*Murree*	*Razmak*	*Kohat*	*Peshawar*	*Lahore*	*Abbotabad*	*Dalhousie*	*Kasauli*	*Bangalore*	*Trimulgherry*	*Allahabad*	*Jhansi*	*Jubbulpore*	*Cairo—Egypt*	*Total*
Q1167	5	–	2	1	3	1	–	–	–	–	–	–	–	–	–	–	–	12
Q1030	10	–	1	–	2	–	–	–	–	–	1	1	–	–	–	–	1	16
Q771	7	–	1	–	1	3	6	3	–	–	–	–	–	–	–	4	2	27
Q454	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	3
A12	9	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	10
Q902	12	1	1	–	3	3	2	–	–	1	1	–	1	1	–	2	–	28
B105	1	–	–	–	–	–	–	–	–	–	–	3	–	–	1	–	–	5
B81	3	–	–	–	–	–	–	–	–	–	–	2	–	1	–	–	–	6
Totals	49	1	5	1	9	7	8	3	1	1	2	6	1	2	1	7	3	107

(*a*) From Table II it would appear that the new types, particularly Q1030, Q771 and Q902 have a wide distribution in India and possibly Egypt.

Table III.—Analysis Quetta and All India Figures for the years 1938–40.

	Mannitol-Fermenting				Non-Mannitol-Fermenting										
					Indol Negative							Indol Positive			
	Andrews V-Z Spectrum	Boyd's types 170, P119 & 103	Boyd's types 88, P288, 274 D1, D19	*B. dys.* Sonne	Non-arabinose fermenting *B. dys.* Shiga	Arabinose fermenting Q1167	Q771	Q1030	Q454	A12	Arabinose fermenting *B. dys.* Schmitz	Non-arabinose fermenting Q902	B105	B81	Totals
Quetta Totals	188	63	88	107	38	1	6	9	2	6	25	12	—	3	548
All India Totals	4945	617	799	1603	1047	9	12	13	3	6	695	24	1	3	9777
Quetta %	34·31	11·5	16·06	19.53	6·93			4·38			4·56		2·74		
All India %	50·58	6·31	8·17	16·4	10·71			·44			7·10		·29		

Table IV.—Analysis Quetta and All India Figures for 1938–40.

	Mannitol-Fermenting	*Non-Mannitol-Fermenting*
Quetta ..	81·39%	18·61%
All India ..	81·46%	18·54%

(*b*) From the above figures it is interesting to note that the total percentage of mannitol and non-mannitol-fermenting organisms (Table IV) for all India and Quetta is almost identical. But on analysing the figures for the different members of each group, it is found that the proportion of new types in both groups is higher for Quetta than for all India.

As these new types are so widely distributed in India, it appears that a number must be missed and, until every strain isolated is fully investigated serologically, this is bound to continue.

(5) Criteria of Pathogenicity.

As stated by Boyd (1940) it is very difficult to satisfy Koch's postulates in the case of dysentery bacilli. He has, however, given certain characteristics which lend strong presumptive evidence of pathogenicity.

First, is the period of the disease during which the suspected organisms are found in the stools. In the case of organisms which are undisputed pathogens, such as Shiga's bacillus, they are present in large numbers, sometimes in pure culture, early in the disease; they become less common and more difficult to isolate as the case advances and, when recovery ensues, they disappear completely.

The day of the disease on which these new types were isolated is given in Table V (*a*). A similar analysis for mannitol-fermenting types isolated during 1940 is given in Table V (*b*).

Table V (*a*).

		Day of Disease							
	Types	1	2	3	4	5	6	7	*Total*
Indol negative	Q1167	4	5	3	–	–	–	–	12
	Q1030	4	7	4	1	–	–	–	16
	Q771	3	13	11	–	–	–	–	27
	Q454	1	2	–	–	–	–	–	3
	A12	1	6	3	–	–	–	–	10
Indol positive	Q902	10	6	3	3	3	1	2	28
	B105	1	3	1	–	–	–	–	5
	B81	1	2	1	1	1	–	–	6
Totals		25	44	26	5	4	1	2	107

Table V (*b*).

	Day of Disease							
Types	1	2	3	4	5	6	7	*Total*
V	–	2	2	1	1	–	–	6
W	4	7	7	6	2	–	–	26
X	3	7	8	4	2	–	3	27
Z	–	4	7	3	1	1	–	16
103	–	1	3	2	2	–	–	8
P119	–	6	5	–	2	1	1	15
170	–	1	3	1	1	1	–	7
88	7	12	5	3	2	1	–	30
288	–	–	–	–	–	1	–	1
Sonne	2	12	6	3	1	–	–	24
Totals	16	52	46	23	14	5	4	160

Second, the bacillus should not be present in the bowel, and so in the stool, of persons who are not suffering from acute or chronic dysentery. The data for this are available from the laboratory records of examinations carried out on cooks, table boys, water carriers and others of this class to ensure that they are not carriers of enteric or dysentery bacilli. During the seven years 1934 to 1940, 5,693 men of this type were examined and 15,903 platings were made from their stools. The following organisms of dysentery or query dysentery bacilli were isolated:—

TABLE VI.

Type	*Mannitol-Fermenting*		*Non-Mannitol-Fermenting*			
	Flexner-Boyd	*Sonne*	*Shiga*	Q1167	*Schmitz*	Q902
Numbers ..	20	3	3	1	2	2
%	0·14		0·05			

In addition Morgan No. 1 bacillus was isolated ten times from menials.

Analysis of the type of exudate present in the cases from which the 107 strains were isolated is given in Table VII (*a*).

TABLE VII (*a*)

	Q1167	Q1030	Q771	Q454	A12	Q902	B105	B81	*Total*
Bacillary exudate	9	11	13	3	7	16	1	2	62
Indefinite exudate	3	5	11	—	2	10	3	4	38
No exudate	—	—	3	—	1	2	1	—	7
Totals	12	16	27	3	10	28	5	6	107

In no case was any other dysentery organism isolated.

For comparison a similar analysis for mannitol-fermenting organisms isolated during 1940 is given in Table VII (*b*).

TABLE VII (*b*)

	V	W	X	Z	103A	P119	170	88	288	*Sonne*	*Total*
Bacillary exudate	3	15	17	12	5	9	3	21	1	18	104
Indefinite exudate	2	10	8	4	2	6	3	6	—	4	45
No exudate	1	1	3	—	1	2	1	3	—	2	14
Totals	6	26	28	16	8	17	7	30	1	24	163

Third, the development, and especially the progressive development, of agglutinins for the suspected organisms in the serum of the patient during the course of the disease is generally to be accepted as an indication that the defences of the body are being called into action to repel the attacks of an invading organism. This evidence is of value when the agglutinins are for the specific antigen of the bacillus.

TABLE VIII.

Suspensions	*Patients' Sera*						
	0	25	50	125	250	500	*Totals*
Q1167	2	1	1	1	1	—	6
Q1030	1	2	4	3	1	—	11
Q7	1	1	8	3	1	—	14
Q454	—	1	—	1	1	—	3
A12	2	1	4	—	1	1	9
Q902	1	2	5	4	3	—	15
B105	—	2	—	—	—	—	2
B81	—	1	1	—	—	—	2
Totals	7	11	23	12	8	1	62

An analysis of the serological findings of sixty-two cases carried out on different dates during the disease showing the maximum titre of the specific agglutinins in the sera are given in Table VIII.

Sera from 200 cases and blood donors sent for the Wassermann tests were tested. No agglutinins for the new types were found among these.

From the tables given in this section it would appear that the following conclusions may be drawn:

(*a*) The greater number of the organisms, i.e. 90 per cent, were isolated during the first three days of the disease.

(*b*) These organisms are not present in the stools of persons except when they are suffering from acute or chronic dysentery. Of the new types 93 per cent were isolated from cases of acute dysentery compared with 91 per cent from the control mannitol-fermenter.

No other pathogenic organism was isolated from any of the cases caused by a mannitol or non-mannitol-fermenter.

(*c*) The great majority of cases showed the presence of a progressive development of homologous agglutinins, rising in some instances to a maximum titre of 250 to 500.

It would therefore appear that there is strong presumptive evidence of pathogenicity for all the new types.

Owing to the relatively few organisms of each type examined, final judgment must be suspended until more organisms are isolated and investigated.

It is, however, interesting to note that in one family both husband and wife were infected by Q1030.

(6) Relationship Between the Infecting Organisms and the Severity of the Disease.

Table IX shows the relationship between the infecting organisms and the severity of the disease.

Table IX.

	Q1167	Q771	Q1030	Q454	A12	Q902	B105	B81
Mild..	6	16	9	2	7	15	3	3
Moderately Severe..	4	8	1	—	1	8	1	3
Severe	2	3	6	1	2	5	1	—
Totals	12	27	16	3	10	28	5	6

(*a*) These new types appear to be capable of producing a mild or severe type of dysentery.

(*b*) Q771, Q902 and A12 appear to be capable of producing a more chronic type of dysentery.

This is especially the case with Q902. Five cases continued to show symptoms from ten to twenty-three days after the onset of the illness; two of these chronic cases responded well to treatment by autogenous vaccine.

Summary and Conclusions.

(1) The biochemical reaction and antigenic structure of eight new types of non-mannitol-fermenting organisms isolated from cases of dysentery occurring in India and Egypt have been described.

(2) A preliminary biochemical classification of non-mannitol-fermenting organisms is given. These new types are widely distributed in India and are not described in "A System of Bacteriology" (1929). With few exceptions they have been found only in the stools of clinical dysentery cases.

(3) The presumptive evidence is in favour of pathogenicity, but until greater numbers of organisms have been isolated and investigated, the matter must rest *sub judice*.

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It is hoped that these findings will stimulate further investigation and enable more data to be accumulated on which to arrive at definite conclusions with regard to the pathogenicity of these new types.

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