TABLE No. 13

ABSORPTION TESTS SHOWING ANTIGENIC RELATIONSHIP BETWEEN SOME OF THE NATIVE FLEXNER STRAINS

(THE REST OF THE DATA ON ABSORPTION TESTS HAS BEEN OMITTED FOR THE SAKE OF BREVITY)

Serum	Absorbing Culture	Testing Culture	Agglutinating Titre	Serum	Absorbing Culture	Testing Culture	Agglutinating Titre	Serum	Absorbing Culture	Testing Culture	Agglutinating Titre
88	90	88	100	17	305	17	1,000	M	305	305	—
		90	—			305	—			M	5,000
90	88	88	—	305	17	17	500	305	M	305	5,000
		90	5,000			305	5,000			M	2,000
88	305	88	1,000	17	P_3	17	5,000	M	P_3	M	5,000
		305	—			P_3	—			P_3	—
305	88	88	200	P_3	17	17	—	P_3	M	M	—
		305	—			P^3	5,000			P_3	5,000
88	17	88	2,000	17	M	M	—	M	17	M	2,000
		17	—			17	1,000			17	—
17	88	88	—	M	17	M	2,000	17	M	M	1,000
		17	5,000			17	—			17	—
88	M	88	200	P_3	90	P_3	5,000	P_4	M	M	—
		M	—			90	—			90	2,000
M	88	88	—	90	P_3	P_3	—	M	P_4	M	500
		M	2,000			90	—			90	100
88	P_3	P^3	—	17	90	17	5,000	305	P_3	305	5,000
		88	200			90	—			P_3	—
P_3	88	P_3	5,000	90	17	17	—	P_3	305	305	100
		88	—			90	5,000			P_3	2,000
88	13	13	—	90	305	90	5,000	98	H D	98	5,000
		88	—			305	—			H D	—
13	88	13	—	305	90	90	5,000	6 Y	M	6 Y	5,000
		88	—			305	2,000			M	—

acidity in maltose after fourteen days. In none of the other sugars tried was acidity produced (Table No. 10). Antiserum of organism No. 61 agglutinated cultures B–1, B–2, B–6 and C–1 in dilutions of one to five hundred, but antiserum of organisms of Group B (Flexner) or C (Sonne) did not agglutinate cultures 61–47 and 127. There is no antigenic relationship to the Shiga or Dispar stock cultures in our possession, nor to cultures of group D. Cultures 55 and 195, are different serologically and culturally from the three already described under this group, however, being non mannite and non lactose fermenters, we have placed them under Group A. These two cultures appeared to be inagglutinable with all the sera with which we have tried them. They produced acidity in sucrose and produced no change in sorbitol, rhamnose, trehalose and galactose, differing in this respect from cultures 61, 127 and 47; they produced indol and no change in milk.

Group B consisted of organisms which did not ferment lactose, produced acidity in mannite and comprised what is recognized as the Flexner group of bacilli. The production of acidity in carbohydrates was rather variable, except in the case of certain of the sugars (Table No. 10). None of the native strains studied acidified lactose or xylose. All of them produced acidity in glucose, mannite, levulose and galactose, and the majority in arabinose, maltose and trehalose. Sorbitol as a rule was not attacked although some of the cultures produced definited acidity in this carbohydrate. Rhamnose as a rule was not attacked. Salicin, inosite, inulin and dulcitol were not attacked. Melezitose, dextrin and glycerine as a rule were not attacked, but six of the cultures produced an initial acidity with reversion to neutrality in melezitose, two produced acidity in dextrin and one, in glycerine. None of the cultures liquified gelatin or produced hydrogen sulphide in lead acetate agar. In milk, the production of acidity was the rule, but in no instance was there coagulation. All the cultures produced indol, both in tryptophane broth and in peptone water. In the group we found six distinct serological varieties and one which was found inagglutinable with our stock and native antisera.

The native cultures were very closely related to each other as is demonstrated by the absorption tests (Table 13) and the agglutination reactions (Table 12) of the different cul-

tures. Representative strains from the various serologic subtypes were compared antigenically with the five classical English strains of mannite-fermenting dysentery bacilli and with other dysentery organisms obtained from different sources in the United States. Three of the native strains were found to be so closely related to the English strains that we considered them as the same subtype (See Table No. 14). None of our strains was of the same serologic type as the American strains, although there was close similarity among some of them in their antigenic structure. The serologic subtypes of our native strains as classified under Group B. (Flexner) were as follows:

I. This group was found to be so closely related to the English "V" strain that we were led to consider it as the same serologic variety. The group is represented by cultures Nos. 88, 98, 283, 202, 77, 286, 160, and 175, all cultures from cases studied during the 1932 epidemic; also culture No. 13 which was isolated by us in 1930 from the epidemic outbreak of bacillary dysentery which occurred at the Insular Penitentiary in Río Piedras.

II. Represented by cultures P–4, 99, 215, P–2, 233, and 206.

III. Represented by cultures 305, 309, 303, 288, 40 and 271.

IV. Represented by cultures P–3, 238, 16 and 250.

V. Represented by culture No. 17. This culture and culture Rob were isolated from two of three members of the same family, continental Americans, that were suffering from acute dysentery. Culture 17 is very similar in antigenic structure to the "Z" culture of the English.

VI. Represented by culture "M". This organism was isolated from a non epidemic case of dysentery. The patient, a soldier, had a severe case of dysentery and died from it. The culture is quite closely related antigenically to the "Y" culture of the English.

VII. Represented by two cultures, both of which were inagglutinable with all the sera with which we tried them, native and stock English and American strains. They were also poor agglutinin producers, the titre of the serum produced being so low that we had to discard

them. These two cultures were similar in their cultural reactions, except that culture No. 33 does not produce acidity in maltose and trehalose, otherwise they are similar in every other respect.

All the six serologic subtypes of the native strains belonging to Group B (Flexner) had some antigenic similarity among themselves, there being a certain antigenic factor common to all. Groups I, II, III and IV, were rather closely related among themselves.

Group C.—Sonne group, first described by Duval in 1904 [31]. Lactose fermenters, no indol production and no action on xylose. This group of organisms has acquired great importance lately as an inciter of dysenteric conditions. Numerous instances of recorded cases and epidemics can be found in the literature, also detailed studies of the organism itself. Among these may be mentioned those of Koser et al [24], Johnston et al [32-33-34-35-36], Brown et al [37], Welch and Mickle [38], Leahy [39], Cann and Navasques [40] and Gilbert and Coleman [41]. In the group of organisms isolated and studied by us we have encountered two (Nos. 151 and 221) which can be classified as Sonne organisms. By absorption tests these two organisms were found to be serologically identical and belonging to the same group as a Sonne Brown strain supplied to us by Koser. Culturally the organisms resemble in every respect the classical Sonne group, no indol is produced, milk is acidified without coagulation and there is a delayed fermentation of lactose and no action on xylose or dulcite.

Group D.—This group resembles the Sonne group in that it ferments lactose but is a different serologic specie. The organisms show characteristics of the classical Dispar group such as the fermentation of lactose and xylose and the production of indol, yet some of the strains studied by us show a characteristic which heretofore has not been found in this group or in the dysentery group, such as the liquefaction of gelatin and the production of acid in salicin. The cultural characteristics and biochemical reactions of each of these organisms studied can be seen from Table No. 8. By agglutination reactions (Table 12) and by absorption tests, the group was subdivided into three subtypes and an inagglutinable strain.

I. In this subgroup we have included two cultures, which,

although serologically different species, are so closely related antigenically and their cultural reactions have so many points in common that we have grouped them as different species in the same subgroup. These cultures showed many of the characteristics of the Dispar group, differing in that they repeatedly liquified gelatin.

(*a*) Culture No. 129. This organism is slightly related in its antigenic composition to the Sonne organism (151). It is not however related to the Flexner group but, in antigenic structure, to the Dispar stock organism.

(*b*) Culture 170. This organism differs culturally from 129 in that it fermented sorbitol and dulcitol. The organism, although very closely related antigenically to No. 129, is of a less complex structure, being able to absorb all its agglutinins from serum 129, but the absorbed serum continues to agglutinate culture 129 to high titre. It is very distantly, if at all, related in antigenic composition to the Sonne organism, but is markedly related to the stock Dispar culture, more closely than culture 129.

II. Culture No. 83.—Is not antigenically related to the Sonne organism, but bears some genetic relationship to our stock Dispar culture and to culture No. 129. It ferments dulcite and salicin with the production of acid, but does not attack sucrose and produces indol.

III. Culture No. 146.—This organism, culturally, could not be placed in either Group C or Group D, although it is a late lactose fermenter and produced indol; it does not produce acidity in xylose but produces acidity in salicin, in this respect resembling culture 83. Antigenically the two cultures are only very slightly related, being two different organisms. We have finally placed this organism in the Dispar group on account of its marked antigenic relationship with culture 170, although they are different organisms, culture 170 being able to absorb all its agglutinins from serum 146, yet the serum continues to agglutinate to full titre culture 146.

IV. Culture Rob and 189 are inagglutinable strains, and we have been unable to classify them serologically into any group. Culturally, Rob produces acidity in glucose, lactose, mannite, maltose, xylose, sucrose, sorbitol, dulcitol, rhamnose, arabinose, trehalose, glycerine, glactose and levulose. Culture 189 resembles the Sonne and Dispar group, in that it pro-

duced acidity in lactose; however, in its other characteristics it does not appear to fall into either of these groups. It produces acidity with coagulation in milk, no indol production, acidity in glucose, lactose, xylose, arabinose and galactose, and no change in the other sugars tried.

IV. Summary and Conclusions

I. A study of bacillary dysentery in Puerto Rico is presented, which includes a historical study and a review of the regional literature, an epidemiological and bacteriological study of three epidemic outbreaks of the disease and a cultural and serologic study and classification of native strains of dysentery bacilli.

II. Dysentery, an epidemic disease characterized by diarrhea with blood and mucus in the stools, accompanied by fever, was of frequent occurrence in Puerto Rico before the Island was discovered by Columbus in 1493.

III. Acute attacks of diarrhea following the ingestion of human flesh in Puerto Rico brought about the cessation of cannibalistic habits among the Indians of the Windward Islands a few years prior to 1569.

IV. A severe epidemic of dysentery occurred during the summer of 1598 among the invaders and natives during the British attack and occupation of the city of San Juan.

V. Diarrhea accompanied by blood and mucus in the stools occurred in epidemic form in 1865, in 1872, and again following the earthquakes of 1873.

VI. Severe and widespread epidemics of dysentery followed the hurricanes of October 29, 1867; August 8, 1899, September 13, 1928 and September 26, 1932.

VII. Flexner in 1902, was the first to demonstrate the actual etiological agent of some of the epidemic dysentery prevalent in the Island, when he isolated a Flexner bacillus from a case of chronic dysentery contracted in Puerto Rico during the Spanish American War. Others who later isolated dysentery bacilli from epidemic or sporadic cases were González Martínez in 1912, Costa Mandry in 1927 and Costa Mandry and Garrido Morales in 1928 and 1930. Jordan and McBroom in 1930 isolated Flexner bacilli from a diabetes case not suffering with diarrhea.

VIII. The clinical course of bacillary dysentery as it has occurred in Puerto Rico in the last decade is characteristic,

The disease, as a rule, is benign, the salient symptoms being generalized abdominal pain, tenderness to deep pressure over the abdomen, diarrhea with blood and mucus in the stools, fever and burning and tenesmus during defecation. The symptoms persist for an average of 5 or 6 days, the bowel movements numbering from 4 to 12 in twenty-four hours.

IX. During the post-cyclone epidemic of dysentery in 1932, a considerable number of bacilli belonging to the dysentery group were isolated at the Biological Laboratory of the Health Department of Puerto Rico, and together with others obtained from sporadic cases of the same disease after the epidemic had subsided, were typed and classified according to their cultural and serologic characteristics. The organisms were classed into four main groups: the Schmitz (A) which comprised two subtypes; the Flexner (B) with six serologic varieties and one non-agglutinable strain; the Sonne (C) with only one type; and the Dispar (D) with three serologic types and a non-agglutinable strain.

X. Bacillary dysentery is a specific disease entity in Puerto Rico, of paramount importance from the public health standpoint of the Island as a whole. In the cases studied it has been found to be produced by a number of bacilli of the dysentery group, the Flexner type being by far the most common, the Shiga being rare or absent. The disease occurs as sporadic cases, small endemic focci or epidemics localized in institutions, or widespread throughout the Island as a sequela of severe hurricanes.

XI. Since the year 1890, the generic term "dysentery" has been recorded in vital statistics as an important cause of death in the Island of Puerto Rico. Undoubtedly, under this denomination have been included a number of acute and chronic diarrheal conditions of obscure etiology, but if careful bacteriological studies of the feces had been carried out in all the cases, dysentery bacilli would have been found to account for a considerable number of them. There is a decided increase in the computed annual death rate from dysentery in the years when hurricanes have occurred, demonstrating that epidemic dysentery (bacillary) caused that decided increase in the mortality rate in those years when that specific disease was of frequent occurrence in the Island, and was thus readily recognized.

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TABLE No. 11

RESULTS OF FERMENTATION TESTS AND OTHER MISCELLANEOUS PROCEDURES IN STOCK STRAINS

Group	Culture Number	Milk	Indol (Tryp)	Indol (Pep)	Hydrogen Sulphide	Gelatin Liquefaction	Glucose	Lactose	Sucrose	Mannite	Maltose	Xylose	Salicin	Sorbitol	Rhamnose	Arabinose	Dulcitol	Trehalose	Inosite	Levulose	Galactose	Dextrin	Melezitose	Inulin	Glycerine
English	W	+	+	+	—	—	+	—	—	+	+	—	—	—	—	—	—	+	—	+	+	+	—	—	—
	Y	+	+	+	—	—	+	—	—	+	7	—	—	+	+	—	—	+	—	+	+	—	—	—	—
	Z	+	+	+	—	—	+	—	—	+	—	—	—	+	6	4	—	+	—	+	+	—	—	—	—
	V	+	+	+	—	—	+	—	—	+	+	—	—	—	—	+	—	+	—	+	+	+	+	—	—
	X	+	+	+	—	—	+	—	—	+	+	—	—	—	—	5	—	+	—	+	+	+	—	—	—
American	6Y	+	+	+	—	—	+	—	—	+	—	—	—	—	—	+	—	12	—	+	+	—	O	—	8
	FN	+	—	—	—	—	+	—	—	+	+	—	—	—	—	+	—	+	—	+	+	+	O	—	—
	FD	+	+	+	—	—	+	—	—	+	+	—	—	+	+	+	—	+	—	+	+	+	—	—	—
	HD	+	+	+	—	—	+	—	—	+	—	—	—	+	—	—	—	+	—	+	+	—	O	—	—
	SB	+c	—	—	—	—	+	+	+	+	+	—	—	—	+	+	—	+	—	+	+	+	—	—	+
	D	+c	+	+	—	—	+	+	+	+	+	+	—	+	+	+	—	+	—	+	+	+	—	—	+
	SH	+	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	O	—	—	—

\+ Acid Production within 4 days
— Negative Reaction
c Coagulation

Figure — Days when Reaction became Evident
O — Initial Acidity with Reversion To Neutrality

TABLE No. 12

AGGLUTINATION TITRE OF THE VARIOUS SERA WITH REPRESENTATIVE HOMOLOGOUS AND HETEROLOGOUS CULTURES

Group	Test Suspension	Agglutinating Serum									
		61	88	P_4	305	P_3	17	M	151	146	129
A.....	61	1..000	—	—	—	—	—	—	—	—	—
B–1...	88	200	2,000	2,000	5,000	2,000	10,000	2,000	—	500	100
B–2...	P_4	500	500	15,000	10,000	5,000	2,000	15,000	200	100	100
B–3...	305	500	1,000	15,000	10,000	5,000	1,000	15,000	100	200	500
B–4...	P_3	200	1,000	5,000	1,000	5,000	500	500	—	—	500
B–5...	17	—	500	5,000	10,000	1,000	10,000	2,000	100	200	—
B–6...	M	500	1..000	15,000	10,000	2,000	200	15,000	—	—	100
B–7...	33	—	—	—	—	—	—	—	—	—	—
	79	—	—	—	—	—	—	—	—	—	—
C.....	151	500	200	200	100	200	—	500	2..500	—	200
D–1 ..	129	—	—	—	—	—	200	—	100	.....	1..000
	170	—	—	—	—	—	—	—	—	—	200
D–2 ..	83	—	—	—	—	100	—	—	—	—	200
D–3 ..	146	200	—	200	—	—	—	—	—	2..000	—
D–4 ..	Rob.	—	—	—	—	—	—	—	—	—	—
	189	—	—	—	—	—	—	—	—	—	—
English	V	200	2,000	5,000	2,000	5,000	5,000	15,000	500	200	200
	W	200	1,000	10,000	10,000	5,000	2,500	10,000	100	200	200
	X	200	1,000	1,000	5,000	5,000	5,000	10,000	200	200	200
	Y	200	500	1,000	10,000	15,000	500	15,000	200	100	200
	Z	—	200	1,000	1,000	2,000	5,000	500	—	—	—

TABLE No. 14

SHOWING SEROLOGIC RELATIONSHIP OF THREE NATIVE SUBGROUPS WITH ENGLISH TYPES OF MANNITE FERMENTING (FLEXNER) ORGANISMS

Serum	Absorbing Culture	Testing Culture	Agglutination Titre
B–1.....	V	V	0
		B–1	0
Eng–V....	B–1	V	200
		B–1	0
B–5.....	Z	Z	0
		B–5	0
Eng–Z....	B–5	Z	200
		B–5	0
B–6.....	Y	B–6	0
		Y	0
Eng–Y....	B–6	B–6	0
		Y	500

TABLE No. 15

SEROLOGICAL GROUPING OF NATIVE DYSENTERY STRAIN

A—No Action on Lactose or Mannite		B-(Flexner) Acid production in Mannite but not in Lactose							C (Sonne) Acid Production in Lactose. No Indol Production	D-Acid in Lactose and Xylose. Indol Production					
										1				4*	
1	2*	1	2	3	4	5	6	7*		A	B	2	3	A	B
61	55	88	90	305	P_3	17	M	33	151	129	170	83	146	Rob	189
47	195	98	P_4	309	238	.....	.....	79	221	.....	.....	.....	.....	.....	.....
127	.....	13	215	303	16	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	279	P_2	288	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	286	233	202	250	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	160	.....	283	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	175	.....	40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	77	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	264	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

*-Inagglutinable Strains.

TABLE No. 16

SOURCE OF INDIVIDUAL CULTURES STUDIED

	SERIAL NUMBER OF CULTURES OF EACH TYPE													
	A-1	A-2	B-1	B-2	B-3	B-4	B-5	B-6	B-7	C	D-1	D-2	D-3	D-4
San Juan	.....	195	286	P^4 215	288	P_3	17	.....	.....	.....	.....	.....	.....	Rob
Arecibo	.....	.....	77	.....	283	.....	.....	.....	.....	.....	.....	.....	.....	.....
Bayamón	.....	.....	.....	.....	.....	238 250	.....	.....	.....	.....	.....	.....	.....	.....
Caguas	61	.....	.....	.....	.....	.....	.....	.....	79	.....	.....	.....	.....	.....
San Lorenzo	47	55	88	90	.....	16	.....	.....	.....	.....	.....	.....	.....	.....
Gurabo	127	....	160 98	....	....	....	....	....	....	....	 129	....	 146	 189
Humacao	.....	.....	279	.....	202	.....	.....	.....	.....	.....	.....	.....	.....	.....
Naguabo	.....	.....	.....	233	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Juncos	.....	.....	175	.....	.....	.....	.....	.....	.....	151	170	.....	.....	.....
Río Piedras	.....	.....	264	.....	40	.....	.....	.....	.....	221	.....	.....	.....	.....
Canóvanas	.....	.....	.....	P^2	.....	.....	.....	.....	33	.....	.....	.....	.....	.....
Cayey	.....	.....	.....	.....	.....	.....	.....	M	.....	.....	.....	83	.....	.....
Manatí	.....	.....	.....	.....	309 305 303	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	3	2	8	5	7	4	1	1	2	2	2	1	1	2

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