THE ANTIGENIC RELATIONSHIPS WITHIN THE SHIGELLA GROUPS AND BETWEEN SHIGELLA AND ESCHERICHIA

by

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I. Introduction

The genus Shigella as defined by Topley and Wilson (1) consists of "gram-negative non-motile rods, 2-3u long by 0.5-0.7u broad, non-spore-forming. Ferment a variable number of carbohydrates with the production of acid. Lactose is not attacked except by a few species and then not for two days or more. Reduce nitrates to nitrites, form ammonia but not hydrogen sulphide, are Voges-Proskauer negative and fail to grow in Koser's citrate. Facultative anaerobes, some species are antigenically related. At least one species produces a toxin. Most species are pathogenic to man, giving rise to dysentery or sometimes acute gastroenteritis. Found, as a rule, in the intestinal tract of human dysentery patients and contacts."

The name "Shigella" is commonly used today. Bergey's Manual (2) places the genus Shigella in the tribe Salmonella and the family Enterobacteriaceae.

Because of the constantly changing nomenclature and frequent discoveries of new strains, the classification and nomenclature of Shigella has been very confused for a long time, particularly those of the Shigella flexneri group. Boring (3) has proposed a scheme for the classification and nomenclature of the Shigella groups which was accepted by the Shigella Commission of The International Congress of Microbiology in Rio de Janeiro in 1950 (4). In this study the International Shigella Commission classification and nomenclature are used.

TABLE I. SHIGELLA NOMENCLATURE. (9)

	Subgelia Commission 1950	Ewing 1949		Kauffmann & Ferguson 1947	Wheeler 1944a	Boyd 1946	Boyd 1933	Weil et al. 1944	English[1] (older)	German[2]	Other
Group A	1	I								Shiga-Kruse	Bacterium shigae
	2	II								I	S. ambigua, S. schmitzii, S. ambigua
	3	III									[illegible] type 8525, S. arabinoteria[4]
	4	IV									[illegible], S. arabinoteria[4]
	5	V									[illegible]
	6	VI									[illegible]
	7	VII									[illegible]
Group B		Ewing's Antigenic Formula									
	1a	I	I:4	1b	I	I	V	I	V	B, C	Flexner
	1b	I	I:4,6	1a	I			I,VII	VI	A	
	2a	II	II:4	2a	IIa	II	VI	II	VI	D	
	2b	II	II:3,7,8,9	2b	IIb			II,VII	VIX	DX	
	3	III	III:6,7	3	III	III		III	X	H	
	4a	IV	IV:4	4a	IV	IV	VI3	IV		F	
	4b	IV	IV:6	4b	IV	IV	VI2	III,IV		J	
	[illegible]									[illegible]	
	5	V		5	V	V	P.119	V(V.VII)		G	
	6	VI		6	VI	VI	68	VI		L	S. newcastle, Newcastle and Manchester bacilli
	[illegible]	X	—7,8,9		X			VII	X		
	[illegible]	Y	—4		Y			VIII	Y	M1,M2	Hiss-Russell
Group C	1	I				I	P.70	IX			
	2	II				II	P.285	X			
	3	III				III	P.1	XI			
	4	IV					P.274	XIV		K	H
	5	V					P.183	XIII			
	6	VI					P.19	XII			
	7	VII								M	Lavington, type T, S. etousae
										P	Type 1296/7 (Boyd, 1946)
Group D	[illegible]		S. sonnei							M	Somme-Laval, Somme XII S. cpylonnensis-A

1 English: Andrewes & Inman (1919), Murray (1913), Gettings (1917).
2 Germans: Kruse (1900), Sartorius and Reploh (1932), Seeliger (1949).
3 Ceber, et al. (1944).
4 Christensen and Gowan (1944).

Biochemical studies have not lost their important position in recent years. Though many strains are variable in their biochemical reactions, the method is of value in dividing the shigellae into two main groups:

(a) The mannitol-negative group consists of:

Shigella dysenteriae (Group A, types 1-7).

(b) The mannitol-positive group consists of:

Shigella flexneri (Group B, types 1-6).

Shigella boydii (Group C, types 1-8).

Shigella sonnei (Group D).

Shigella alkalescens-dispar.

Since biochemical studies can distinguish only two main groups, the final classification of these five groups depends upon serological analysis. All members of the five groups possess distinctive antigens, though numerous serological cross reactions occur within the Shigella flexneri group. Each type within the boydii, sonnei and dysenteriae groups has its own distinctive antigens to separate it from the others, but rarely minor cross reactions may occur between certain strains within these groups. Boyd (5) stated that variation occurs commonly in strains of mannitol-fermenting dysentery bacilli when they have been maintained on artificial culture media for some time.

The alkalescens-dispar group is chemically and antigenically related to both the Shigella and Escherichia groups. These organisms ferment glucose and mannitol with acid and no gas, and vary in their fermentation of lactose. The indole and methyl red reactions are positive and Voges-Proskauer reaction is negative. Antigenically they are related to

Escherichia through their antigens and to some strains of Shigella. The position of the alkalescens-dispar group within Enterobacteriaceae has been the subject of considerable discussion. Most authors have referred them to the Shigella group. Kauffmann (6) classified the alkalescens-dispar group as belonging to the tribe of Escherichiae, which he divides into the following groups:

Escherichia.

Alkalescens-dispar.

Klebsiella.

Serratia.

Since the position of the alkalescens-dispar group is not yet decided, it will not be included in this study.

According to Kauffmann (4) the family Enterobacteriaceae is made up of a series of interrelated bacterial types which do not lend themselves to sharp division into tribes or into groups. Therefore in addition to the serological cross reactions among the shigellae numerous serological cross reactions have been found between them and other Enterobacteriaceae groups. Kauffmann (7) reported on antigenic relationships between Salmonella and Shigella strains. Wheeler, Stuart, and Ewing (8) reported antigenic relationships between Shigella boydii, type 4, and alkalescens, O group 1. Edwards and Ewing (9) noted antigenic relationships between Shigella boydii type 1 and alkalescens O group 1, Shigella boydii type 5 and alkalescens O groups 3 and 4, and Shigella boydii type 6 and alkalescens O groups 1, 3 and 4. Ferguson and Wheeler (10) reported on two paracolon cultures

related antigenically to the entire Shigella flexneri group and to Shigella boydii type 5. Madsen (11) described serological relationships between Shigella boydii types 1 and 5 and alkalescens O groups 1, 2, and 3, and Shigella flexneri types 3 and 4 and alkalescens O groups 2 and 3.

The serology of Escherichia coli is based on the analysis of H, O and K antigens. The H antigens are thermolabile flagellar antigens. However, this study deals with the non-flagellated Shigella group; therefore, we will not discuss the H antigens in detail.

The O antigens are thermostable somatic antigens, which are resistant to heat to 100°C, and not distroyed by treating with alcohol. The first diagnostic Escherichia O group antigenic schema, which was established by Kauffmann (19) consisted of 20 O groups, representatives of which had been repeatedly found. Later Kauffmann investigated 30 additional O groups, and Knipschildt found 52 different ones. A new O group 111 has recently been established by Kauffmann and Dupont (12), who found it associated with infantile diarrhea, and another O group 112, which contains the organisms originally called Shigella guensbere, was established by Ewing and Kauffmann (4). The O antigenic relationships between Escherichia and Shigella were first reported by Vaesie (13) who stated that strong O relationships exist between Shigella boydii type 4 and Escherichia coli O group 53, and Shigella boydii type 5 and Escherichia coli O group 79.

The K antigens are a group of different envelope or capsular antigens, which were designated as L, B and A antigens by Kauffmann (14). All of the K antigens inhibit O agglutination of living or formalin-killed antigens, but the three differ in respect to the degree of their thermolability,

antibody-binding properties after boiling, and certain other characteristics.

Kauffmann (15) (16) and Frantzen (17) described the thermolabile envelope antigen in coliform bacteria which later was called the L antigen. Organisms containing L antigen will be O-inagglutinable, but after being heated to 100°C. for one hour, the O inagglutinability will disappear and a strong O agglutination is obtained. The monospecific L antibody can be prepared by the agglutinin absorption method, using OL antiserum absorbed by O antigen (OL antigen heated at 100°C. for 1 hour). This procedure will remove the O antibody and leave the L antibody in the serum.

Knipschildt (18.) described a second variety of envelope antigen which was designated as B antigen. This antigen is similar to the L antigen in being thermolabile, but its antibody-binding properities are different from those of the L antigen, in that an OB antiserum cannot be absorbed by OB antigens, because the antibody-binding property, unlike its ability to inhibit O agglutination, is not destroyed by heat. However, the specific B antibody can be demonstrated by absorbing an antiserum with a suspension of organisms containing the same O antigen but no B antigen. This removes the O antibodies, and leaves the B antibodies in the serum.

Kauffmann (19) and Knipschildt (20) found a thermostable capsular substance, and designated it as A antigen. This antigen is thermostable, is contained in the capsule and results in O inagglutinability, which is removed by heating to 120°C. for 2 1/2 hours. The antibodies can be demonstrated by the capsule-swelling technic, and by absorbing an AO antiserum with an "A minus" suspension as in the B antibody absorption test.

The purpose of the present study was to investigate the antigenic relationships between the Shigella and Escherichia groups, with respect to

their O and K antigens (the H antigens were excluded from this study). Many investigators have thoroughly worked out the relationships between the Shigella flexneri and Escherichia groups; therefore, this study was limited to the Shigella groups dysenteriae and boydii.

As mentioned previously biochemical studies can divide the shigellae into two main groups. Therefore, a brief biochemical study was made to check the reactions of these two major groups, mannitol-negative and mannitol-positive. The main part of the study, the serological investigation, was divided into the following five parts:

(1) Antigenic relationships within the Shigella dysenteriae group and the Shigella boydii group.

(2) Investigation of O-inagglutinability of the Shigella dysenteriae group and Shigella boydii group.

(3) Antigenic relationships between Shigella groups dysenteriae and boydii and Escherichia coli O groups 1-112.

(4) Antigenic relationships between Shigella groups dysenteriae and boydii and living Escherichia coli O groups 1-112, to investigate the K antigen relationships among them.

(5) Absorption tests to investigate the three different degrees of antigenic relationships between Shigella and Escherichia.

  (a) Complete identity, as demonstrated by reciprocal absorption tests.

  (b) Unilateral relationships.

  (c) Minor interrelationships.

II. Materials

The antigenic relationships between the Shigella groups and between Shigella and Escherichia coli strains were serologically studied. The materials came from several sources:

(1) The Escherichia coli strains belonging to O groups 1-112 were from the culture collection of the Department of Bacteriology of the University of Oregon Medical School, and came originally from F. Kauffmann of the Serum Institute of Copenhagen, Denmark.

(2) A number of Shigella strains were from the collection of the Department of Bacteriology of the University of Oregon Medical School.

(3) Many strains of Shigella were received from the Communicable Disease Center, Public Health Service, Atlanta, Georgia.

(4) One strain of Shigella was from the American Type Culture Collection.

The immune serums were prepared for this study. One antiserum for Shigella dysenteriae type 1 was from the stock of the Department of Bacteriology of the University of Oregon Medical School.

A list of the Shigella strains which were used in this study with their sources is given in the following table:

Groups	Types	Sources	Original Strain Number	Earlier designation
A	1.	Dept.	F.M.S.	Sh. shiga, Kruse shiga bacillus, etc.
	2.	Dept.		Sh. ambigua, schmitzii
	3.	C.D.C.	2424/49	Q.771
	4.	C.D.C.	47	Q.1167
	5.	C.D.C.	78	Q.1030 Large-Sachs group
	6.	C.D.C.	77	Q.454
	7.	N.T.C.	9752	Q.902
C	1.	Dept.	565	170
	2.	Dept.	564	P.288
	3.	C.D.C.	1050/50	D.1
	4.	Dept.	563	P.274
	5.	Dept.	573	P.113
	6.	C.D.C.	70	D.19
	7.	C.D.C.	1130	Lavington, Sh. etousae
	8.	C.D.C.	3072/50	Provisional Type 8

C.D.C. = Communicable Disease Center.
N.T.C. = American Type Culture Collection.
Dept. = Department of Bacteriology of the University of Oregon Medical School.

Besides the groups A and C, a few other strains which had been classified as Shigellae by some investigators were also used for this study. These were:

Wheeler 1831 (coli 022) from C.D.C.
Sachs A12 (coli 032) from C.D.C.
MacLennan P25 Providence group 03 from C.D.C.

III. Methods

The strains were transferred from the stock cultures to meat-infusion broth and incubated at 37°C. for 18 hours, then inoculated to MacConkey agar media and incubated for 18 hours. The colorless, tiny, and smooth colonies were transferred to meat-infusion broth again for further investigation.

1. BIOCHEMICAL REACTIONS:

A. Sugar fermentation tests:

The following culture media were used for this study.

a) Monosaccharides:

Pentose group
- Arabinose
- Xylose
- Rhamnose

Hexose group
- Glucose

b) Disaccharides:

- Saccharose
- Maltose
- Lactose

c) Alcohols:

Monovalent:
- Mannitol
- Dulcitol
- Sorbitol

The above media in Durham fermentation tubes were inoculated with a loopful of the broth culture about 18 hours old. The tubes were incubated at 37°C. for one month with daily reading. Each tube was closed with a rubber stopper to prevent evaporation.

B. The Indole reaction:

A loopful of broth culture was transferred to tryptophane broth and incubated at 37°C. for 24-48 hours, after which a few drops of Bohme's reagent (1 ml. p-dimethylamino-benzaldehyde, 95 ml. 95% ethyl alcohol, 20 ml. Conc. HCl.) was let run down the side of the tube to form a layer on top of the media. If indole was present a red ring appeared between the two layers.

C. The Voges-Proskauer reaction:

The specimen was inoculated into glucose phosphate broth and incubated at 37°C. for 48 hours. The test was made by adding an equal quantity of O'Meara's reagent (Creatine hydrate 0.3 gm., KOH 50% 100 ml.). A pink color appearing first at the surface denotes a positive reaction. Shaking the tube will hasten the reaction.

D. Methyl-red test:

Inoculation and incubation was the same as for the Voges-Proskauer reaction. A few drops of 0.25% Methyl-red solution was added and the reaction was read immediately. A positive reaction is red, a negative reaction is yellow. The reaction was due to fermentation of the glucose with production of acid. The reaction became positive at a pH under 4.5.

E. Urea decomposition test:

A tube of urea broth was inoculated and incubated at 37°C. for 24 hours. Decomposition of urea was indicated by the change of the phenol-red indicator to a deep reddish purple color.

F. Motility:

A loopful of specimen was transferred to the surface of a soft agar tube (motility agar medium) and incubated at 37°C. for 24 hours. If

the organism was motile the agar media turned evenly cloudy down to the bottom. If the organism is not motile the medium remains clear and the organism grows only around the inoculated area.

2. PREPARATION OF ANTIGENS:

In preparing antigens only smooth colonies were selected and these were tested to make sure that spontaneous agglutination did not occur when suspensions were mixed with equal parts of physiological saline and incubated 20 hours in the water bath at 50°C.

A. "O" group antigen:

Smooth organisms were inoculated either into meat-infusion agar or meat-infusion broth and incubated at 37°C. for 20 hours. The agar cultures were suspended in physiological saline and boiled in the Arnold-steriliser for one hour. 0.3% formalin was added to the antigens for preservation, and they were stored in a refrigerator. These antigens may be used for 2 or 3 months.

B. Formalinised antigen:

Cultures were inoculated in the same way as for the O antigen. After incubation at 37°C. for 20 hours, 0.3% formalin was added to the antigens and they were left again at 37°C. for 20 hours, then stored in a refrigerator in the same way as the O antigen.

C. Living antigen:

The Escherichia coli group was inoculated in the same way as for the O antigen and incubated at 37°C. for 20 hours. Living antigens must be used immediately, or they will overgrowth.

3. PREPARATION OF IMMUNE SERUM:

Because normal rabbit serum may have the capacity to agglutinate a wide variety of gram negative intestinal bacilli, each rabbit's normal serum has to be tested for alpha agglutination against a living suspension of B. wakefield organisms. Rabbits weighing from six to eight pounds, with negative alpha agglutination, were immunized with increasing doses of formalinized antigen (as mentioned in the preparation of antigen). They were injected into the marginal vein of the ear. The doses were as follows:

Injections	Days	Doses	Method
1st.	1st.	0.2 ml.	I.V.
2nd.	3rd.	0.5 ml.	I.V.
3rd.	5th.	1.0 ml.	I.V.
4th.	7th.	1.5 ml.	I.V.
5th.	9th.	2.0 ml.	I.V.

Eight days after the last injection, the rabbits were bled to death from the heart and blood collected in a sterilized bottle. The serum was poured off, centrifuged and decanted. An equal amount of pure glycerine was added to each serum, the bottles were labeled, and stored in the refrigerator.

4. AGGLUTINATION TESTS:

The method of testing for agglutination was to set up serial dilutions of testing serum from 1:10 to 1:5120 or higher in normal saline, the final amount being 0.5 ml. To each of these tubes was added 0.5 ml. of the antigen to be tested. The antigens were either living, formalinized or boiled. The

formalinised and the boiled antigens were placed in the water bath at 50°C. for 20 hours. The living antigen was incubated at 37°C. for 2 hours and stored in the refrigerator for 20 hours, after which the reactions were read by naked eye and recorded by plus signs to represent the various degrees of agglutination.

5. AGGLUTININ ABSORPTION TECHNIC:

The cultures used for absorptions were chiefly those killed by formalin or boiling. The organisms were inoculated in Blake bottles and incubated for 18 to 24 hours at 37°C. and the resultant growth taken up in physiological saline. This thick bacterial suspension was killed by adding formalin or by boiling. If the antigen was for O group agglutinin absorption, the antigen was killed by boiling, then the sediment was spun down and washed with sterilised saline to remove the soluble excess substances in the suspension. The suspension was centrifuged at high speed until the organisms were firmly packed, the supernatant fluid was poured off, the serum which was to be absorbed was added, mixed well, and placed in a water bath at 50°C. for 2 hours and kept overnight in the refrigerator at 4°C. It was centrifuged again at top speed for 20 to 30 minutes, and the supernatant serum was decanted into a sterilised bottle. It was then tested against the absorbing strain to ascertain that the desired agglutinin had been absorbed completely. Reabsorption was done if necessary.

IV. BIOCHEMICAL REACTIONS

All Shigella types can ferment glucose within 24 hours. Some strains also ferment mannitol within 24 hours. The biochemical classification of the Shigella group is based on this point for dividing the mannitol-negative group from the mannitol-positive group.

1. THE MANNITOL-NEGATIVE GROUP (Table 2)

a) Shigella dysenteriae 1 (Group A, type 1).

Shigella dysenteriae type 1 ferments glucose within 24 hours without gas production, and ferments lactose after 3 days' incubation. Madsen (11) stated that some Shigella dysenteriae strains did ferment lactose sooner or later, and they all fermented glucose the first day without gas production.

b) Shigella dysenteriae 2 (Group A, type 2).

Table 2 shows that type 2 fermented glucose within one day without gas and fermented rhamnose late, and sorbitol after 2 days, and formed indole. According to MacLennan (21) the ability of Shigella schmitzii to ferment rhamnose and produce indole distinguishes it from type 1 (shiga).

c) The Large-Sachs group.

The Large-Sachs group contains the 5 "Q" strains originally identified by Large et al. (22) and later confirmed by Sachs (23). All the strains fermented glucose rapidly without gas and also attacked sorbitol with acid and no gas in from one to six days. Regarding the fermentation of arabinose by this group, there have been disagreements. Sachs (24) considers the

fermentation of arabinose of great importance for differentiation of this group. MacLennan (21) and Wheeler and Stuart (25) did not agree with Sachs, and found some variation in arabinose fermentation. In this present study only one strain of each type was used. They were all negative in arabinose.

TABLE IX. BIOCHEMICAL REACTIONS OF THE MANNITOL-NEGATIVE GROUP.

Group	Types	Glucose	Mannitol	Lactose	Dulcitol	Sorbitol	Rhamnose	Arabinose	Xylose	Indole	Motility
Shigella dysenteriae A	1	A	-	a^{2}	-	-	-	-	-	-	-
	2	A	-	-	-	a^{2}	a	-	-	+	-
	3	A	+	-	-	a^{2}	-	-	-	-	-
	4	A	-	-	-	-	-	-	-	-	-
	5	A	-	-	-	-	-	-	-	+	-
	6	A	-	-	-	a	-	-	-	-	-
	7	A	-	-	-	-	a	-	-	-	-
	X12	AG	-	-	-	X[a]	-	-	$a^{[2]}$	-	-
	8870	A	-	-	$a^{[2]}$	A	A	a^{2}	a^{2}	+	-
	P25	A	-	-	-	-	-	-	-	-	+

Key: A = acid after one day.
AG = acid and gas.
a = acid late.
a^{2} = acid after 6 days.
- = negative after 30 days incubation.

2. THE MANNITOL-POSITIVE GROUP (Table 3).

Shigella boydii (Group C).

Shigella boydii types all fermented glucose and mannitol without gas production. All these types fermented sorbitol rapidly or slowly. All types attacked xylose, either rapidly or slowly, except types 2 and 3, which were negative. Madsen (11) stated that types 1, 3, 5, 6, and 7, were rapid or late xylose fermenters, whereas types 2 and 6 were negative. Kauffmann (4)

described types 3, 4, 6, as being slow fermenters of dulcitol, the rest as negative and all types as fermenting arabinose within one day without gas. In this present study types 3, 4, 6, 7, fermented dulcitol late and 1, 2, 5, and 8 were negative. Only type 6 attacked arabinose, after 6 days incubation, the rest being negative.

TABLE XXI. BIOCHEMICAL REACTIONS OF THE MANNITOL-POSITIVE GROUP.

Group	Type	Glucose	Mannitol	Lactose	Dulcitol	Sorbitol	Rhamnose	Arabinose	Xylose	Indole	Motility
Shigella boydii c	1	A	A	-	-	a^{6}	-	-	a^{30}	-	-
	2	A	A	-	-	a^{30}	-	-	-	-	-
	3	A	A	+	a^{6}	A	-	-	a^{30}	-	-
	4	A	A	-	a^{30}	a^{30}	-	-	-	-	-
	5	A	A	-	-	A	-	-	a^{30}	+	-
	6	A	A	-	a^{42}	A	-	-	a^{30}	-	-
	7	A	A	-	a^{30}	A	-	a^{6}	A	+	-
	8	A	A	-	-	A	-	-	a^{21}	-	-

Key: A = acid after 1 day.
a_{30} = acid late.
a^{30} = acid after 30 days.
- = negative after 30 days incubation.

V. EXPERIMENTS AND RESULTS

1. AGGLUTININ TITER OF UNABSORBED SERUMS OF SHIGELLA DYSENTERIAE FOR THEIR OWN GROUP.

Most investigators agree that the 7 types within group A Shigella dysenteriae are not serologically related to each other or to other Shigella groups. Welch and Biddle (25) stated that Shigella dysenteriae 1 has no antigenic relationship to the other Shigella groups. Madsen (11) found that Shigella dysenteriae 1 and 2 contain no group antigen and bear no serological relationship to the other groups. Wheeler and Stuart (24) and Weil (26) concluded that all types of the Large-Sachs group are serologically distinct. Regarding the antigens that inhibit O agglutination in the Shigella dysenteriae group, Madsen (11) reported that Large-Sachs group contains thermolabile antigens which show higher titers with boiled antigens and lower titers with formalinized antigens. Kauffmann (4) stated that the Large-Sachs group give stronger agglutination with boiled than with formalinized cultures. He suggested the presence of both O and K antigens.

The results of the present study are shown in Table 4. The immune serums were prepared with formalinized cultures and cross-agglutination tests were made between all types of the Shigella dysenteriae group using formalinized antigens and boiled antigens. The results showed no serological relationships within the group. However, O inagglutinability occurred in each type, since higher titers were obtained with boiled antigens and lower titers with formalinized antigens.

TABLE IV. THE AGGLUTININ TITERS OF UNABSORBED ANTISERUMS OF THE SHIGELLA DYSENTERIAE GROUP.

		Antiserums prepared with formalinized cultures.						
Types		1	2	3	4	5	6	7
Antigens								
1.	F.	640	0	0	0	0	0	0
	B.	2,560	0	0	0	0	0	0
2.	F.	0	1,280	0	0	0	0	0
	B.	0	2,560	0	0	0	0	0
3.	F.	0	0	2,560	0	0	0	0
	B.	0	0	20,480	0	0	0	0
4.	F.	0	0	0	320	0	0	0
	B.	0	0	0	5,120	0	0	0
5.	F.	0	0	0	0	5,120	0	0
	B.	0	0	0	0	5,120	0	0
6.	F.	0	0	0	0	0	640	0
	B.	0	0	0	0	0	5,120	0
7.	F.	0	0	0	0	0	0	1,280
	B.	0	0	0	0	0	0	5,120

Key: F. formalinized cultures.
B. boiled cultures (100°C. for 1 hour.)
0 no agglutination in 1/20 dilution of antiserum.

2. AGGLUTININ TITERS OF UNABSORBED SERUMS IN SHIGELLA BOYDII FOR THEIR OWN GROUP.

Most types of the Shigella boydii group have been proved by many investigators to have no serological relationships with each other, each type containing an independant antigen of its own. Wheeler (27), Boyd (5), and Weil (26) stated that there were no serological relationships within the Shigella boydii group. Recently, a few investigators have found that a few exceptional strains may show minor cross reactions within the Shigella boydii group. Madsen (11) found some weak cross agglutinations between Shigella boydii types 1 and 4, and types 1 and 6. Edwards and Ewing (13)

have found minor cross reactions between Shigella boydii types 3 and 6. However, these different results may be due to strain variation.

O-inagglutinability also occurred in the Shigella boydii group. In the present study, as shown in Table 5, no serological cross reactions were found within the group. O-inagglutinability occurred in every type, higher titers being obtained with boiled antigens and lower titers with formalinized antigens.

Additional immune serums were prepared, using antigens boiled for 2 hours for immunizing the rabbits, in an attempt to get O immune serums to prove the presence of thermolabile antigens in the organisms. It appears in Table 6 that the titers of living and formalinized antigens were far lower than those of boiled antigens, which may be due to the fact that some antigens had been destroyed by boiling. A comparison of the titers in Tables 5 and 6 show that the formalinized antigens were agglutinated by O immune serums to much lower titers than by the immune serums prepared with formalinized antigens. The results show that the Shigella group does contain substances which prevent full O agglutination and that these substances are thermolabile. They also suggest, but do not prove, that these substances may be antigenic.

TABLE V. THE AGGLUTININ TITRES OF UNABSORBED ANTISERUMS OF THE SHIGELLA BOYDII GROUP.

		Antiserums prepared with formalinized cultures.							
Types		1	2	3	4	5	6	7	8
Antigens									
1.	F.	1,280	0	0	0	0	0	0	0
	B.	2,560	0	0	0	0	0	0	0
2.	F.	0	640	0	0	0	0	0	0
	B.	0	5,120	0	0	0	0	0	0
3.	F.	0	0	1,280	0	0	0	0	0
	B.	0	0	5,120	0	0	0	0	0
4.	F.	0	0	0	320	0	0	0	0
	B.	0	0	0	5,120	0	0	0	0
5.	F.	0	0	0	0	1,280	0	0	0
	B.	0	0	0	0	20,480	0	0	0
6.	F.	0	0	0	0	0	320	0	0
	B.	0	0	0	0	0	5,120	0	0
7.	F.	0	0	0	0	0	0	2,560	0
	B.	0	0	0	0	0	0	5,120	0
8.	F.	0	0	0	0	0	0	0	320
	B.	0	0	0	0	0	0	0	2,560

Key: F. formalinized cultures.
B. boiled cultures (100°C. for 1 hour).
0 no agglutination in 1/20 dilution of antiserum.

TABLE VI. AGGLUTININ TITRES OF UNABSORBED "O" ANTISERUMS OF THE SHIGELLA BOYDII GROUP.

Shigella boydii type 1	O antiserum
Living culture	160
Formalinised culture	160
Heated 100°C. culture	5,120

Shigella boydii type 2	
Living culture	80
Formalinised culture	160
Heated 100°C. culture	5,120

Shigella boydii type 3	
Living culture	160
Formalinised culture	640
Heated 100°C. culture	5,120

Shigella boydii type 5	
Living culture	40
Formalinised culture	80
Heated 100°C. culture	5,120

Shigella boydii type 6	
Living culture	640
Formalinised culture	80
Heated 100°C. culture	2,560

Shigella boydii type 7	
Living culture	80
Formalinised culture	80
Heated 100°C. culture	2,560

Shigella boydii type 8	
Living culture	160
Formalinised culture	40
Heated 100°C. culture	2,560

TABLE VIIa. THE ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DYSENTERIAE GROUP AND ESCHERICHIA O GROUPS 1-112.

Antigens	Antisera of Shigella dysenteriae types													
	1		2		3		4		5		6		7	
E. coli O group	L	B	L	B	L	B	L	B	L	B	L	B	L	B
2						160					80			
4			40	160										
11							20				40	80		
13				20										
14		160								160				
15								160						
18				320										
26			20	320										
27						20						160		
28														40
31			20											
36							20	160						
38														320
43			320					20						
46		20									40	2,560		
50			160	20		20								
51		80												
52	20						80							
56									20	2,560				
59			320											
62	20				20		20		20		20			
64														160
66			320	20										
69				20		160				640				
70					20	20								
72					20		20							
73					40		40		80				80	
74														320
76												20		
84								80						
87			40											
88														320
102	40			40			80		80		160	2,560		
105			320											
106	40				160		160		160					
107					160		320		80					
109			20											
112			80	160										

Key: L = living antigens of E. coli O groups.
B = heated antigens of E. coli O groups. (at 100°C for 1 hour)
Antiserums dilutions started 1:10.

TABLE VIIb. THE ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DISENTERIAE GROUP AND ESCHERICHIA O GROUPS 1-112.

Antiserum of E. coli O groups 1:100	O-Antigens of Shigella dysenteriae types						
	1	2	3	4	5	6	7
3			100				
4		100					
14	100				100		
15				100			
18		200					
26		200					
27						100	
36				100			
38							100
46						1,600	
58					6,400		
64							100
69					400		
71							100
88							200
102						800	
112		100					

Antiserums of E. coli O groups were prepared with O antigens (at 100°C for 1 hour) and the dilutions started 1:100.
O-Antigens of Shigella dysenteriae types heated at 100°C for 1 hour.

TABLE VIIIa. THE ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA BOYDII GROUP AND ESCHERICHIA O GROUPS 1-112.

Antigens of E. coli O groups	Antisera of Shigella boydii types 1 L	1 B	2 L	2 B	3 L	3 B	4 L	4 B	5 L	5 B	6 L	6 B	7 L	7 B	8 L	8 B
1								160								
2								160								
3																80
6												25				
13								80								
14												80				
15			80													
24			80					160								
50								640								
51							160	80								
53							20	1,280								
62									20		20		20		20	
64	80										20					
68									20							
69	80	80														
73									40							
79									640	1,280						
90											20					
93										80						
96			160													
105							80		160						80	

Keys: L = living antigens of E. coli O groups.
B = heated antigens of E. coli O groups (at 100°C for 1 hour)
Antisera dilutions started 1:10.

TABLE VIIIb. THE ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA BOYDII GROUP AND ESCHERICHIA O GROUPS 1-112.

O-Antiserum of E. coli O groups	O-Antigens of Shigella boydii types							
	1	2	3	4	5	6	7	8
2				100				
3				100				
34				100				
50				200				
53				3,200				
79					3,200			
96		100						

The antiserums of E. coli O-groups were prepared with O-antigens (at 100°C for 1 hour) and the dilution started 1:100.
O-antigens of Shigella boydii types heated at 100°C for 1 hour.

3. ANTIGENIC RELATIONSHIPS BETWEEN THE SHIGELLA DYSENTERIAE GROUP AND THE SHIGELLA BOYDII GROUP.

Studies on the relationships between Shigella dysenteriae and Shigella boydii were made by reciprocal agglutination tests. No cross reactions were found between those two groups, either with formalinised antigens or with boiled antigens.

4. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA GROUPS AND ESCHERICHIA COLI O GROUPS 1-112.

The present study was made to investigate the antigenic relationships between the Shigella dysenteriae group and the Shigella boydii group and Escherichia coli O groups 1-112. The agglutination tests were set up with living cultures and broth suspensions of cultures heated at 100°C. for one hour. The results in tables 7 and 8 show that there were large numbers of antigens which are related to each other. Antigenic relationships are classified as four different conditions, examples of which are given below.

a) Thermolabile antigenic relationships.

TABLE IX. THE THERMOLABILE ANTIGENIC RELATIONSHIPS.

Antigens	Antiserum S. dys. type 2
S. dysenteriae type 2	
Formalinised culture	1,280
heated culture	2,560
E. coli O13	
living culture	320
heated culture	0
E. coli O59	
living culture	320
heated culture	0

In Table IX the agglutination tests were made with a Shigella dysenteriae type 2 antiserum which had been produced with a formalinised broth culture,

against antigens of Escherichia coli groups 43 and 59, both living and heated to 100°C. for 1 hour. The titers with living antigens were as high as 1:320 and were negative in a dilution of 1:10 with heated antigens.

b) Thermostable antigenic relationships.

TABLE X. THE THERMOSTABLE ANTIGENIC RELATIONSHIPS.

Antigens	Antiserum S. dys. type 5
S. dysenteriae type 5	
formalinized culture	1,280
heated culture	2,560
E. coli O11:	
living culture	0
heated culture	160
E. coli O69	
living culture	0
heated culture	640

The results of Table X illustrate O-inagglutinability, since the living cultures gave no O agglutination, but heating removed the thermolabile inhibitory substance and O agglutination then occurred. Olitski, Shelubsky and Koch (28) demonstrated that Shigella dysenteriae 1 contains a thermolabile substance, which prevents agglutination of living bacteria. Madsen (11) found two thermolabile antigens, O1 and Oj, which could both act as antigens and inhibit or entirely prevent agglutination of living bacteria. An attempt was made to demonstrate K antigens in Shigellae by absorbing antisera of Shigella dysenteriae type 2 and Shigella boydii types 2, 4, and 5, all produced with formalinized cultures, with antigens heated to 100°C. All antibodies were removed from the sera by this treatment, showing that the inhibitory substance can not be an L antigen. It could, however, be a so-called B

antigen, as the D antigen is thermolabile but its antibody-binding property is thermostable.

c) Minor O antigen relationships.

TABLE XI. MINOR O ANTIGEN RELATIONSHIPS.

Antigens	Antiserums	
	E. coli 050	S. boydii 4
E. coli 050 heated culture	10,240	320
S. boydii 4 heated culture	200	5,120

Table XI shows an example of the numerous instances in which there was a marked difference in titer between the homologous and the heterologous antigen in each antiserum. The antigens were related to each other only through minor fractions.

d) Strong O antigen relationships.

TABLE XII. STRONG O ANTIGEN RELATIONSHIPS.

Antigens	Antiserums	
	E. coli 079	S. boydii 5
E. coli 079 heated culture	5,120	1,200
S. boydii 5 heated culture	1,200	20,480

The results of Table XII show reciprocal agglutination tests in which both titers were very high. The antigens were strongly related to each other.

In summary, living antigens of Escherichia coli O groups 43 and 59, but not heated antigens, were agglutinated by a Shigella dysenteriae type 2 antiserum made with a formalinized culture. On the other hand a Shigella dysenteriae type 5 antiserum agglutinated heated antigens of Escherichia coli O groups 44 and 69 but not the living antigens. Shigella boydii types 4 and 5 antiserums, produced with formalinized cultures, agglutinated

Escherichia O groups 79 and 53 (Table VIII), respectively to a lower titer with living cultures and to a higher titer with heated cultures. From these results we might conclude that the Shigella organisms do have two types of antigens, one thermolabile and one thermostable, the thermostable antigen being so-called the O antigen, the thermolabile one possibly a K antigen such as Kauffmann (29) described in Escherichia coli strains.

5. RECIPROCAL AGGLUTININ ABSORPTION TESTS.

The antigenic relationships between the Shigella groups and Escherichia coli strains have been discussed previously. To determine the degree of relationships reciprocal absorption tests were performed. Absorption tests were applied only in cases in which titers as high as 1:640 were obtained on direct agglutination. Otherwise the relationships were considered minor and absorption tests were not applied.

TABLE XIII. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DYSENTERIAE TYPE 5 AND ESCHERICHIA O GROUP 58.

Antigens heated 100°C for 1 hour	Antisorums			
	Unabsorbed		S. dys. 5 absorbed by E. coli O58	E. coli O58 absorbed by S. dys. 5
	S. dys. 5	E. coli O58		
E. coli O58	2,560	5,120	0	0
S. dysenteriae type 5	4,480	5,120	0	0

Table XIII shows the reciprocal agglutinin absorption tests applied to Shigella dysenteriae type 5 and Escherichia coli O58. The results show that both O antigens were completely identical. The absorbed serum of Shigella dysenteriae 5 was tested for fractional agglutination tests with E. coli O69 and O14. They were all negative, confirming the fact that the

agglutinins had been completely absorbed by the Escherichia coli 058 O-antigens. Since these results were obtained, Odden has published her work in the Acta. Path. et Microbio. Scand. (30) in which she made the same observation concerning the O identity of Shigella dysenteriae type 5 and Escherichia coli 058.

TABLE XIV. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DYSENTERIAE TYPE 5 AND ESCHERICHIA O GROUP 69.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. dys. 5 absorbed by E. coli 069	E. coli 069 absorbed by S. dys. 5
	S. dys. 5	E. coli 069		
E. coli 069 -	640	1,280	0	320
S. dysenteriae type 5	5,120	400	640	0
E. coli 058	2,560		320	
E. coli 014	160		80	

Table XIV shows the results of absorption of the Shigella dysenteriae type 5 serum with Escherichia coli 069. Their O antigens were only partially related. The absorption tests lowered the titers to a certain degree but did not remove all agglutinins. The table also shows that agglutinins remained for E. coli 058 and 014. Therefore we would consider Shigella dysenteriae and E. coli 069 as strong related but not identical.

TABLE XV. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DYSENTERIAE TYPE 6 AND ESCHERICHIA O GROUP 102.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. dys. 6 absorbed by E. coli 0102	E. coli 0102 absorbed by S. dys. 6
	S. dys. 6	E. coli 0102		
E. coli 0102	1,280	10,000	0	2,560
S. dys. type 6	5,120	640	640	0
E. coli 0\6	2,560		320	

Table XV shows the reciprocal agglutinin absorption tests applied to Shigella dysenteriae type 6 and Escherichia coli 0102. The results show that they were strongly related but not identical. The absorption tests lowered titers to a certain degree but did not remove all agglutinins. Absorption of the Shigella dysenteriae serum with E. coli 0102 left agglutinins both for the Shigella and for E. coli 0\6. Therefore we would consider this relationship as similar to that shown in Table XIV.

TABLE XVI. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA DYSENTERIAE TYPE 6 AND ESCHERICHIA O GROUP 102.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. dys. 6 absorbed by E. coli 0\6	E. coli 0\6 absorbed by S. dys. 6
	S. dys. 6	E. coli 0\6		
E. coli 0\6	2,560	6,400	0	640
S. dys. type 6	6,400	1,600	0	0

Table XVI shows that the entire antigenic complex of Shigella dysenteriae type 6 is found in Escherichia coli 0\6, but that Escherichia coli 0\6 contains in addition to the Shigella dysenteriae type 6 antigen, a minor amount of another distinctive antigen. As the results showed, Shigella dysenteriae

type 6 serum can be absorbed completely by Escherichia coli 086. The Escherichia coli 086 serum absorbed by Shigella dysenteriae type 6 retains a titer of 1:640 for the homologous organism.

TABLE XVII. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA BOYDII TYPE 4 AND ESCHERICHIA O GROUP 53.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. boydii 4 absorbed by E. coli 053	E. coli 053 absorbed by S. boydii 4
	S. boydii 4	E. coli 53		
E. coli 053	2,560	6,400	0	0
S. boydii 4	5,120	3,200	0	0

TABLE XVIII. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA BOYDII TYPE 5 AND ESCHERICHIA O GROUP 79.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. boydii 5 absorbed by E. coli 079	E. coli 079 absorbed by S. boydii 5
	S. boydii 5	E. coli 079		
E. coli 079	1,280	5,120	0	0
S. boydii 5	2,560	3,200	0	0

Antigenic relationships of the S. boydii group to the other Enterobacteriaceae group. As first shown by Boyd (5) a few members of the Shigella boydii group are to some extent antigenically related to the alkalescens-dispar group. Veasie (13) first reported strong O relationships between Shigella boydii type 4 and Escherichia coli 053, and between Shigella boydii type 5 and Escherichia coli 079. This study as shown in Tables XVII and XVIII by reciprocal absorption tests confirms the previous reported of O-identity of Shigella boydii type 4 with Escherichia coli 053 and of Shigella boydii type 5 with Escherichia coli 079.

TABLE XIX. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA BOYDII TYPE 4 AND ESCHERICHIA O GROUP 50.

Antigens heated 100°C for 1 hour	Antiserums			
	Unabsorbed		S. boydii 4 absorbed by E. coli 050	E. coli 050 absorbed by S. boydii 4
	S. boydii 4	E. coli 050		
E. coli 050	640	10,240	0	1,280
S. boydii 4	5,120	80	640	0
E. coli 03	160		160	
E. coli 053	2,560		320	0

Table XIX shows reciprocal absorption tests between Shigella boydii type 4 and Escherichia coli 050. The results show that the O antigens were partially related. The absorption tests only lowered the titers but did not remove the agglutinins completely. The absorbed serum of Shigella boydii type 4 retains a titer of 1:640 for its own homologous antigen, 1:160 for E. coli 03 and 1:320 for E. coli 053, and the absorbed serum of E. coli 050 retains a titer as high as 1:1,280 for its own antigen.

The mannitol-negative types were described by Sach (23) and by Wheeler and Stuart (24) under the designations of A12 and 1831. The position of these types have not been decided yet, as to whether they should belong to the Shigellas or must be classified with the Escherichia group. However, in this present study absorption tests have been done one those two types. The type A12 contains a somatic antigen identical with that of Escherichia coli 032, and type 1831 is identical with Escherichia coli 022.

VI. Discussion

The conclusions which were drawn from the results of the study recorded in the body of this paper will be discussed according to the topics listed in the introduction.

1. ANTIGENIC RELATIONSHIPS WITHIN SHIGELLA GROUPS A AND C.

In the classification of Shigella groups, biochemical reactions have not lost all interest. They have particular importance in separating them into two main large groups, mannitol-positive and mannitol-negative. The mannitol-positive group contains Shigella flexneri, boydii and sonnei, three subgroups, which could not be subdivided by biochemical reactions alone. Therefore, the chief basis of classification of these Shigella groups is antigenic analysis.

The Group A Shigella dysenteriae. Many investigators have agreed that the members of this group are not serologically related to each other or to other Shigella groups. This study has found that the Shigella dysenteriae 1 and 2 strains were serologically distinct from the other Shigella groups and that the Large-Sachs strains had their own type-specific antigens. The serological identification of these strains presents no diagnostic difficulties as they contain no group antigens and give no serological cross reactions with the group C Shigella boydii, or with the B. and D group.

The Group C Shigella boydii. Generally speaking this group also has only type-specific antigen, but some investigators have found some minor

relationships between them. As mentioned previously, Madsen (11) found such relationships between types 1 and 4, types 3,and 6, and types 5 and 6. Edwards and Ewing (9) found only between types 3 and 6 very minor reactions to a titer of 1:320. In the present study no relationships were found between the types. However, the results of different investigators may be due to strain variation. The complete lack of serological relationships between groups A and C agrees with the results of all previous investigators.

2. INVESTIGATION OF O-INAGGLUTINABILITY OF SHIGELLA GROUPS A AND C.

The question of whether O-inagglutinability may be due to thermolabile substances or K type antigens present in the Shigella group has become of some importance after the presence of L antigens have been proved in the alkalescens group by Praetsen (31). The thermolabile antigen is an envelope or capsular-like substance around the surface of the organisms, which has the ability to inhibit O agglutination, and this substance can be destroyed by heat. Therefore, most of the Shigella groups showed lower titers with formalinized cultures and higher titers with heated cultures. The fact is very clear that the Shigella groups do contain some substance around the surface area to prevent O agglutination and that this substance will be destroyed by heat of 100°C for 1 hour. The colonies of some Shigella boydii strains such as types 2 and 5 appeared more or less sticky and shiny. Attempts to demonstrate capsules by capsule swelling and capsule staining techniques were unsuccessful. Antiserums of Shigella boydii strains 1-5 were tested for agglutination with strains of 25 Klebsiella groups. They were all negative in 1:20 dilutions. Also as mentioned before agglutinin absorption tests were made to try to eliminate K type antibodies, but all

agglutinins were absorbed from the antisera. This might be due to the antibody-binding property of the inhibitory substance, suggesting that it may be a B antigen. However, this work needed further study.

3. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA GROUPS A AND C AND STRAINS OF ESCHERICHIA COLI O-GROUPS 1-112.

As mentioned previously, many investigators have found numerous serological cross reactions among the enteric organisms. Tables VII and VIII show that many strains were serologically interrelated, either strongly or weakly. If they are not carefully studied, it would be easy to make an incorrect diagnosis of the presence of pathogenic or non-pathogenic organisms. Therefore to know such relationships is of the utmost importance in diagnostic work. Serological studies of enteric bacteria must be as complete as possible and must always be confirmed by biochemical studies.

The classification of the serology of Escherichia coli by Kauffmann (14) has helped greatly in the determination of interrelationships of the family Enterobacteriaceae. In 1946 Ferguson and Wheeler (10) described two paracolon cultures related antigenically to the entire group of Shigella flexneri and to Shigella boydii type 5. These two paracolon cultures, as Ewing (32) stated, are now know to belong to Escherichia coli O group 4. He also stated that many paracolon bacteria similar to Escherichia which formerly could not be classified serologically now can be placed in one or another of the Escherichia coli O groups. Therefore the serological classification of the Escherichia coli O groups will be counted among fundamental antigenic studies of enteric bacteriology.

4. ANTIGENIC RELATIONSHIPS BETWEEN SHIGELLA GROUPS A AND C AND LIVING CULTURES OF ESCHERICHIA COLI GROUPS 1-112.

In the Tables VII, VIII, IX, and X are shown the antigenic relationships between Shigella and Escherichia strains. The Shigella antisera appear to contain two different antibodies: one agglutinates living Escherichia coli cultures and another one agglutinated heated Escherichia coli cultures, while in some cases Escherichia coli living cultures are agglutinated to a lower titer, and heated Escherichia coli cultures to a higher titer. The results showed very clearly that the Shigella antisera could agglutinate thermostable and thermolabile antigens of Escherichia coli strains, the thermolabile one being the so-called K-type antigens. We tried several times to demonstrate nonspecific K-type antibodies in the Shigella antisera, but failed to do so. Therefore, this problem leaves an opening for future studies.

5. RECIPROCAL AGGLUTININ ABSORPTION TESTS TO DETERMINE THE DEGREE OF RELATIONSHIPS BETWEEN SHIGELLA AND ESCHERICHIA.

The results indicate clearly that the O antigens of Shigella dysenteriae type 5 and Escherichia coli O59, Shigella boydii type 4 and Escherichia coli O53, and Shigella boydii Escherichia O79 are identical. This was proved by reciprocal agglutinin absorption tests. Shigella dysenteriae type 6 and Escherichia coli O46 are unilaterally identical, Escherichia coli O46 containing all the antigens of Shigella dysenteriae type 6 in addition to a specific antigen of its own. Shigella dysenteriae types 5 and 6 and Escherichia coli O69 and O102, and Shigella boydii type 4 and Escherichia coli O50 are strongly related but are not identical. These results agree with previous investigators. The family Enterobacteriaceae is a large

interrelated group which does not lend itself to sharp division into tribes or into groups. Therefore it is extremely important that interrelationships among members of the family Enterobacteriaceae be clarified.

Some relationships between Shigella and Escherichia which have not appeared in the literature were found in this study. They are as follows:

a) Antisera of Shigella groups have been found to contain two major antibodies which were demonstrated by agglutination tests with living and heated cultures of Escherichia. These results were mentioned in the discussion of the agglutination of the thermolabile and thermostable antigens of Escherichia coli. Since the K antigens of the Escherichia group have been established by Kauffmann, we will assume the presence of K antigens among the Shigella groups.

b) Shigella dysenteriae type 6 and Escherichia coli 0\6 are unilaterally identical, Escherichia coli 0\6 containing all the antigens of Shigella dysenteriae type 6 in addition to a specific antigen of its own (Table XVI).

c) Shigella dysenteriae types 5 and 6 and Escherichia coli 069 and 0102 are strongly related but are not identical (Tables XIV and XV).

d) Shigella boydii type 4 and Escherichia coli 050 are also strongly related but are not identical (Table XIX).

VII. Summary

1. The thermolabile and thermostable antigens of Shigella groups are described.

2. The antigenic relationships of Shigella groups and strains of Escherichia coli groups 1-112 are reported.

3. The degree of O antigenic relationships was demonstrated by reciprocal agglutinin absorption tests.

4. The importance of such relationships to diagnostic work is mentioned.

5. The biochemical reactions are considered also of importance to confirm diagnostic work.

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