ANTIGENIC STUDIES OF A GROUP OF PARACOLON BACTERIA (32011 GROUP)

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Thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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INTRODUCTION

The 32011 group of paracolon bacteria (Stuart et al 1943a) (Stuart and Rustigian 1943b) is composed of a number of organisms which show common biochemical reactions in basic media designed for the study of the enteric group. They belong to the genus Paracolobactrum (Borman et al 1944) (Breed et al 1948) and are members of the species eerogenoides. Preliminary studies revealed the presence of a number of antigenic groups but no further classification of the somatic or flagellar fractions was attempted (Stuart and Rustigian 1943b). With the increased importance of this group in the medical field as incitants of enteric disorders, it was felt that a further study of the antigenic relationships with respect to the various somatic and flagellar fractions would seem to offer a contribution to the epidemiology of the group, as has previously been demonstrated in the Salmonella group. With the above in mind. this paper will present an analysis by serological methods of the somatic and flagellar antigens present in this group. It will also present any interlocking relationships between the members of the group and the somatic and flagellar antigens of the Salmonella group.

HISTORICAL BACKGROUND

In the study of any members of the family Enterobacteriaceae it is well to bear in mind that here is a large group of closely related, highly integrated and somewhat unstable bacteria which actually form a continuous series of types, both physiologically and serologically (Parr 1939) (Wheeler et al 1943) (Saphra and Wassermann 1945) (Edwards et al 1948a).

Biochemically, the Enterobacteriaceae may be separated into broad groups (Kauffmann 1949). Early workers recognized the presence of intermediate groups and attempted to set up methods of differentiation by various combinations of biochemical characteristics. Mac Conkey (1905) studied coli and coli-like organisms on the basis of fermentation of came sugar and dulcite. and gelatin liquefaction. action on litmus milk and indol production. Bergey and Deehan (1908) studied the coli-aerogenes group with respect to fermentation of saccharose, dulcit, adonit and inulin; liquefaction of gelatin; Voges-Proskauer (VP) reaction; indol reaction and motility. Mac Conkey (1909) felt that the tests in use at that time did not allow a differentiation of the lactose fermenting bacilli adequately and suggested that certain other tests were necessary. Bronfenbrenner and Davis (1918) suggested the use of lactose concentrations above 1% in identification of late lactose fermenters. Nungester and Anderson (1931) noted considerable differences in the use of liquid and solid media containing lactose. Kennedy et al (1932) felt that some weight should be given to the fact that 21 out of 22 late lactose fermenting strains produced indol. Werkman and Gillen (1932) proposed a new genus of Citrobacter

for certain coli-aerogenes intermediates. Kriebel (1934) studied a group of non-lactose fermenting bacteria isolated from stools and concluded they were closely related to the colon group since they tended to dissociate into lactose fermenting organisms when cultured in 5% lactose solution. Tittsler and Sandholzer (1935) made a detailed study of the cultural characteristics of 29 methyl red (M.R.) positive and V. P. negative Escherichia-Aerobacter intermediate cultures. They found that on the basis of citrate utilization, H2S production and the fermentation of cellibicse and a-methylglucoside these cultures could be divided into 6 major groups. However, they found an additional 25 subgroups on the basis of motility, production of indol and the fermentation of other carbohydrates. Carpenter and Fulton (1937) found that the cultural and physiological characteristics of 125 intermediate strains showed such heterogenicity that it was impossible to classify them with methods in use then, but concluded that primary classification might be made on the basis of the V.P. reaction, citrate utilization and the M.R. reaction. Malcolm (1938) felt that on the basis of the V. P. reaction, citrate utilization, fermentation of inesitol and the indol reaction that all coliform types could be arranged into a series of well defined subgroups. Stokes et al (1938) studied 32 strains of the "paracoli" group and found that they produced some degree of lactose fermentation in 5% lactose broth. Parr (1938) felt that while a great many tests had been used to differentiate coliform intermediates up until that time, a good working basis was indol production, the M. R.-V. P. reactions and citrate utilization. Stuart et al (1939) gave a suggested grouping of slow lactose-fermenting coliform organisms on the basis of lactose fermentation and the Invic reactions and further suggested the term "aberrant coliforms" be used to describe all gram-negative, non-sporulating bacilli which ferment lactose slowly or weakly at 37° C. The general picture seems to be that biochemical reactions of this group only offer a broad classification and show no definite relationships.

Serological work in the classification of this grown of organisms seemed to offer much more toward the picture of where each group stood in the series. Gyorgy (1920) studied 47 cultures of paracolom bacilli, and while he was able to find 20 groups biochemically, he felt that their serological behavior depended entirely on the colony type. Trawinski (1924) studied 91 strains of paratyphoid B-like organisms isolated from feces and according to their biochemical reactions he placed them in 4 groups. He prepared an agglutinating serum in rabbits against a representative of each. Each serum agglutinated all the members of its own group to the same titer as its homologous organism but he apparently did not try the sera against the other groups. Fothergill (1929) studied a group of organisms called "atypical paratyphoid" bacilli from the stools of patients suffering from "summer diarrhea". Within the group, agglutination showed that they were not a homologous group like the typhoid bacillus. Havens and Irwin (1932) studied variants of Morgan's bacillus and found antigenic changes as different variants appeared. Sandiford (1935) studied 47 strains of paracolon bacilli of the coliform or intermediate type and found that the organisms did not agglutinate with

paratyphoid agglutinating serum. He also found that the group as a whole was serologically heterogeneous but it did show some small degree of common antigens between individual strains. Kriebel (1936) studied 19 strains of non-lactose fermenting variants of Escherichia coli and found that classification of the cultures by agglutination in diagnostic sera was impossible. Parr (1939) in a review of coliform bacteria states that certain authors early emphasized the serological heterogenity of the coliform group and concluded that "either the number of kinds of Escherichia coli is very considerable or the serological variability is very great". Paluffo et al (1942) studied 7 paracolon strains which they found to be serologically related to the Salmonella. These strains gave 5 serologic types and the antigenic composition was studied. This was the beginning of the work on the Arizona strains, studied more fully later on. Edwards et al (1943) studied 44 cultures of paracolon organisms and found that they had H and O antigens related to recognized Salmonella types. They divided this group into 14 types by antigenic analysis. Stuart et al (1932a) studied 465 cultures of paracolon organisms biochemically and serologically. They found that the paracolon Aerobacter group could be separated into 2 divisions on the basis of their Imvic reactions. Division I gave strong V.P. reactions, grew on citrate and fermented cellobiose in 24 hours. Division II gave week V.P. reactions and did not ferment cellobiose. In the strains of the second division they found that 47.84 of the cultures were closely related or identical serologically. Nearly all of these cultures had been isolated from human sources. Some contained Salmonella antigens.

In testing the cultures in normal Aerobacter and Salmonella antisera, it was found that they were no more closely related to normal Aerobacter than to Salmonella. No further attempt was made to fractionate these antigenic factors. In subsequent work Stuart and Rustigian (1943b) studied 149 strains of one of the more pathogenic of these paracolon Aerobacter cultures which they called 32011. The work was started with 35 strains and others added as isolated. A resume of their work with this strain follows: "Of the 35 strains isolated 32 were antigenically identical or closely related. As new cultures were obtained, however, it was evident that 32011 was serologically heterogenous. Several strains agglutinated in serum dilutions of 1-160 and a number of others as high as 1-1280 of 32011 antiserum, but failed to reduce the homologous titer when used to absorb the antiserum. Eight types were finally identified and 85.2% of the cultures fell into these groups. All eight possessed interlocking somatic and, in some instances, flagellar antigens. Three serotypes had minor unlabeled somatic antigens in common with Salmonella cholerasuis and Salmonella rubislaw. Twenty-two of the 149 strains did not belong to any of the 8 serotypes. These strains, however, possessed antigens in common with one or another of the 8 serotypes. Fourteen of them agglutinated to high titers with one or another of the 8 antisera (5 to the homologous titer) but in no instance was the homologous titer of any antiserum reduced upon abscrption." The present study is a continuation of the above work. Wheeler et al (1943) studied the Salmonella antigens of coliform bacteria and found that those that possessed complete or partial

Selmonella antigens belonged to various sections of the coliform group. Borman et al (1944) reviewed the taxonomy of the Enterobacteriaceae and suggested the new genus Paracolobactrum for certain members of the intermediate group. This term is now accepted by Breed et al (1948). Saphra and Wassermann (1944) studied the serological relationship of the Selmonella and other Enterobacteriaceae and concluded that there is considerable continuous individual variation in the group and that there are numerous biochemical as well as antigenic interrelations and transitional forms among the individual types, as well as between groups species and genera. Michael and Harris (1945) studied paracolon bacilli and found that on the basis of their Imvic reactions they fall into groups that are antigenically similar in themselves but dissimilar from their counterparts in the typical coliform groups. Stuart and Van Stratum (1945) studied the antigenic relationships of 254 Escherichia coli, paracolon and lactose negative cultures isolated from fecal specimens of infants in two institutions. They found 47.8% of 115 cultures from one institution to be antigenically identical with one or another of twelve cultures used to produce antiserums, while 69.8% of 139 cultures from the other institution were antigenically identical with 10 cultures used to produce antisera. It was interesting to note that the percentage of cultures from one institution antigenically identical with cultures from the other was small. Felsenfeld and Young (1945) studied 1200 paracolon organisms isolated from different sources and found that only 33 strains originating from 10 cases showed Salmonella O or Shigella antigens. Barnes et al (1946) studied a group of

paracolon organisms isolated from a small outbreak of mild gasteroenteritis and found them to be biochemically and serologically related. Edwards et al (1947) made a complete review of the Arizona paracolon group with a study of 373 cultures both biochemically and serologically. These cultures fell into 19 groups and 55 types by antigenic analysis. All are related to the Salmonella. West et al (1947) studied a group of diphasic paracolon cultures which had been included in the Arisona group at first. An antigenic table was worked out, showing the relationships to each other, to the monophasic Arizona strains and to the Salmonella. Kauffmann (1947) (1951) studied the serology of the coli group with respect to the O, K and H antigens and established a diagnostic schema. He concludes that in this group, "cultural tests play but a minor role, so the type division has to rest on a serological basis". Edwards et al (1948) studied 2 strains of an anserogenic sucrose-fermenting coliform bacteria which had Salmonella antigens. They were able to reverse the flagellar phases by cultural methods. Stuart et al (1948) studied the antigenic relationships of 765 Paracolobactrum intermedium cultures and observed that 59% were antigenically identical with one or another of 34 strains used to produce antisera. They also felt that antigenic continuity appears to be much greater in the Paracolobactrum intermedium group than in the Paracolobactrum aerogenoides and Paracolobactrum coliforme groups. Edwards et al (1948) studied 32 strains of paracolon organisms which had been studied earlier by Barnes and Cherry (1946). In an antigenic analysis they were able to divide them into 8 serologic types through the examination of their 0 and H

antigens. They designated the organisms as the Bethesda group. Buttiaux and Kesteloot (1948) discuss the biochemical and antigenic reactions of "B. paracoli" and find that it poses quite a problem in diagnosis, especially when the paracolon organisms possess Salmonella or Shigella antigens. Schwabacher (1949) studied 90 strains of paracolon bacilli, and found that there was no constant association between biochemical and serological groups. There also appeared to be no antigen common to all or even the majority of strains studied. Stuart and Carpenter (1949) studied the flagellar antigens of Escherichia coli and found that the continuity of H antigens in that group was far less than in the Salmonella group. Mushin (1949) studied 36 strains of paracolon organisms that had been isolated from various sources. Using 9 antisers prepared with these organisms she found that 23 of the strains were agglutinated. Seven of these cultures also reacted in Salmonella antisera and 16 reacted in Shigella antisera. Using the reverse procedure with 5 Salmonella cultures she found that none of them were agglutinated by the paracolon antisera, while 15 of 16 strains of Shigalla reacted. No titers are given so it is not known how complete the reactions were. Moran and Bruner (1949) made further studies on the Bethesda group of paracolons and found that 9 O groups could be divided into 25 serologic types on the basis of their H antigens. Bruner et al (1949) studied the Ballerup group of paracolon bacteria both biochemically and serologically. Antigenically the 45 cultures studied were divisible into 3 groups and 21 serological types. Nineteen of the cultures possessed Vi antigen. Edwards (1950) studied 8 cultures of an intermediate coliform organism which exhibited

stable. West (1951) in a review of the differentiation of paracolon flagellar variants from the same organism. All variants were quite studied groups of paracolog organisms has been very valuable in the considerable antigenic variation, in that he was able to obtain bacteria brings out that the use of antisera prepared for the diagnosis of enteric disease in the laboratory. It is thus evident that in the study of paracolom organisms much bacteriaceae. It is hoped that this study will play its small part work is needed to clarify their position in the family Entergin that respect.

EXPERIMENTAL METHODS

Biochemical studies. All the cultures used in this study conformed to the biochemical pattern established by Stuart and Rustigian (1943b). Cultures 26311 through C-7 (table I) were obtained from Dr. C. A. Stuart, Brown University, Providence, Rhode Island. Cultures 10-265 through SA-622 (table I) were obtained from Dr. L. A. Barnes of the Naval Medical Research Institute, Bethesda, Maryland. Cultures P146 through P352 (table I) were obtained from a culture collection at the Army Medical Service Graduate School, Washington, D. C.

This group of organisms ferments glucose, mannitol, maltose, and occasionally salicin, in 24 hours. Strong acid reactions and 20-30% gas are produced in glucose; a moderate to strong acid reaction and a bubble to 10% gas is produced in mannitol; a moderate acid reaction, with or without a bubble of gas, is produced in maltose and with salicin some strains ferment it rapidly, others slowly and some are negative within 18-24 hours. Lactose and sucrose are fermented slowly or not at all; some strains ferment one or another or both of these carbohydrates in about 6 days and other strains only after 3 to 4 weeks. Growth occurs on citrate agar in 1 to 5 days. The Voges-Proshauer reaction is positive, though it may be weak with freshly isolated strains. Only 2 of the strains used in this study were non-motile. Because of the relatively slow action on maltose and mannitol in 18-24 hours, this group of organisms almost always can be distinguished easily from other paracolon types. Table I gives the biochemical reactions of the organisms used in this study.

TABLE I

						Bi	Lochem	ical	Reac tic	ns o	f Para	colon (ultur	es Us	ed				Citrate	٠.	Motility
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ulture No.	214	দৈপ্ত	7DA	24	48	7DA	21,	ЦB	7DA	21,	Las.	70A	ध्य	48	7DA	ध्य	山西	7DA			
26311	-	**	***	10	⊕ 30	⊕ 30	-	*	444	⊕ 5	⊕ 20	®	⊕ 5	⊕ 50	⊕ 50		-	*	*	*	*
5011	-	-	440	⊕ 15	⊕ 20	⊕ 20	**	***	***	+ b	⊕ 10	®	+ b	⊕	⊕ 50	-	•	**	•	*	•
32611	-	***	•	⊕	€	⊕ 20	-	***	-	+ b	⊕ 30	⊕ 30	•	⊕ 25	⊕	-	-	-	•	+ #	-
37 711	-	•	•	⊕ 15	⊕ 60	©	-	*	•	+ b	⊕ 50	(3)	⊕	⊕ 25	⊕	-	***	-	+	•	+
Щ311	-	**	***	•	⊕ 30	⊕ 30		**	40	÷	⊕ 20	⊕	+ b	⊕ 50	⊕ 60	-		*	*	٠	•
56211	-	-	**	① 15	⊕ 30	⊕ 30	-	**	**	⊕ 5	⊕ 30	⊕	⊕ 5	⊕ 30	⊕ 50	-	***	•	•	•	+
53 511	-	-	***	+	⊕ 30	B	-	**	-	⊕ 5	⊕ 30	®	⊕	⊕ 60	⊕ 80	-	*	*	•	•	•
58 1 11	-	•	•	γ†0 ⊙	⊕	⊕ 60	-	*	•	•	⊕ 30	®	⊕	⊕	⊕	-	**	•	*	•	*
70811	-	-	•	①	⊕ 30	⊕ 30	-	*	* .	+ b	⊕ 30	⊕ 30	① 15	⊕ 60	⊕ 50	-	**	-			•
SRC	-	*	-	⊕	⊕ 50	90 ⊛ 60	-	**	•	⊕	7t0 ⊕	B	⊕ 30	⊕	ອບ • •	-	**	•	•	•	•
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-3	-	*	-	⊙ 20	&7 ⊕ 25	æ5 ⊕ 25	-	-	-	● 10	⊕ 15	®	•	•	®	•	•	•		•	+
	-	400	-	⊕	æ) ⊛ 20	20 ⊕	-	**	-	-	±5 ⊕ 5	®	20	1.0 ⊕ 1.5	ß	-	***	•	•		
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:- 6	-	* .	••	30 ⊕ 10	40 ⊙ 20	50 ⊕ †10	-	*		ъ -	15 • 15	③	+	70 ⊕	®	-	**	-	•	•	•
1- 7	-	-	-	10 15	20 ⊕ 30	20 30	-	-	.	b	15 ①	15 @	b	10 ①	3	_	-	_		*	
10-265	-	_	-	⊕ 25	γ ι ο ⊕	10 ⊕		•	****	⊕15		⊕	•	∯ ⊕	®	•	⊕	⊕	•		
10-305	-	-	-	⊕ 10	7₹0 ⊕	(E)	-	44 60;	**	+	25 ④ 25	25 B	15 ①	50 ⊕	®	30 -	1,0	<u>-</u>	•	+	+
10 - 845	-	***	-	10 ③ 30	±0 ⊕ 60	®	-	_	-	⊕ 5	25 ⊕ 25	æ	● 10	&0 ⊕	®	-	_	-	+		•
U-103		**	+	30 ⊕ 15	60 ⊕ 30	•		**	•	5 • 5	2 5 ⊕ 1 5	•	10 ①	æ ⊕ 60	•	_	*	•	•		
19-397	-	-	ъ 	15 • 15	30 ⊕ 25	30 ⊕ 25	-	•	10	5 • 5	15 ① 25	†t0 ⊕ 50	€0 (±0	60 ⊕ 60	60 ⊕ 60	***	**	•	•	+	•
19-406		-	•	15 ① 30	25 ⊕ 40	25 ⊕ 140	-	-	-	5 •	25 ⊕ 30	₽0 ₽0	40 ⊕ 10	60 ⊛ 50	60 ⊛ 50	_	+	⊕ 30	•	+	•
20-167	-	*	+	30 ⊕ 10	40 ⊕ 30	30 ⊕ ∏0	-	-	**	5 •• 5	30 ⊕ 15	®	10 (+) 60	50 ⊕ 60	50 ⊕ 60	-	⊕ 20	•		w +	
22 - L	-	•	b -	10 •	30 ⊕ 60	30 ⊕ 60	_	**		5		®	1	60 ⊕ 60	60 ®	•		20		•	
22-21	_	-	•			60 ®	_	**	•	b +	⊕	®	⊕ 30 ⊕		®	100	100	⊕ 100			
0-128		die	⊕	⊕ 15 ⊕	⊕ 40 ⊕			**	**	b +	⊕ 10	Œ	€5⊙	⊕ 30 ⊕	⊕		-	**	0		
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32-1 56	_	-	-	⊕ 25	⊕ 70			•	_	10	⊕ 30		⊕ 80	⊕ 100			_	_			
2-190B	_	_		⊕ 30		⊙ 50		-	-	ъ	⊕ 15	⊕ 20	b	⊕	⊕			-			
2-1905 59-22k		_		⊕ 20 ⊕	© No ⊕	& 1†0 ⊕			***	10		® 50 ⊕	&0 ⊕	⊕ 50	⊕ 50		***	ratio	•		•
100-658		 -	-	30	⊕ \$0			***		⊕ 5	⊕ 50		⊕ 30	⊕ 60	® •	*		•	•	•	*
		200		2 5	⊕ 50	®	**	-36	***	⊕ 20	⊕	(3)	10	⊕ 50	(3)	***		**	*	*	+
100-794	-	unit:		⊕ 30	⊙ 50	⊕ 90	710	168 *	- Annigo	10	⊕ 30	⊕ 30	⊕ 30	⊕ 5 0	⊕ 60	**	-	•	*	*	*
100-1305	-	-	-	10	⊙ 5 0	⊕ 5 0	-	*	•	⊕ 5	⊕ 15	⊕ 20	b b	₩ •	⊕ 60	-	G på	*	•	+	*

TABLE I (cont'd)

Biochemical Reactions of Paracolon Cultures Used Motility Citrate V. P. Dextrose Salicin Lactose Sucrose Maltose Mannitol Culture No. 48 24 48 7DA 24 48 7DA 5/1 7DA 24 ЦВ 7DA 24 48 7DA 24 45 7DA 103-57 € ⊕ • B • • 50 60 60 10 Ĭ,O 30 50 60 ⊕ 25 ⊕ 10 • € ④ 103-147 B ④ $^{\mathbf{R}}$ b 10 60 70 30 **⊕** 30 **⊕** 5 ⊕ 20 111-30A ⊕ 25 **①** ூ 30 30 ъ ⊕ 15 ⊕ 25 ⊕ 90 ⊕ 50 **⊕** 70 **⊕** 15 • SA-153 **(B)** ₿ 20 **ଟଠ ⊕** 50 ⊕ 60 ⊕ 10 ⊕ 30 ⊕ 30 **⊕** 60 • • SA-163A € 50 80 ⊕ 50 ⊕ 50 ① 10 **⊕** 60 50 ⊕ ⊕ 10 ⊕ 30 SA-569SS • 60 **⊕** 40 SA-599 **①** • ⊕ ூ • ⊕ 20 40 40 5 30 70 g0 **⊕** ∰ (Φ **⊕** 60 SA-622 ⊕ 25 **① ⊕** 15 **⊕** 35 P146 • ⊕ ⊕ Ð B B b 20 20 5 ⊕ 30 • ⊕ P173 B $^{\circ}$ ④ B 10 20 30 60 **⊕** 20 **⊕** 20 ① 10 **⊕** 10 ூ P189 • ₿ 30 **⊕** 60 **⊕** 100 **⊕ ⊕** 30 P206 ⊕ 90 ① 10 Œ ❻ B b **⊕** 70 **⊕** 30 P210 ⊕ 50 **⊕** 5 ⊕ 10 **⊕** 50 € **(B)** ➂ % ① 10 **⊕** 15 **(1)** P227 (R) B B **⊕** 35 ⊕ 15 **⊕** 35 P228 **⊕** 20 B ூ ④ 20 30 **⊕** 25 **⊕** 40 P246 • • B ĻО 10 ĭo 10 ъ **⊕** 30 ⊕ 15 P250 **⊕** 5 B **①** 5 ₿ (B) **⊕ ⊕** 5 ⊕ 15 € P253 **(B) (B)** • ➂ 10 b 15 P260 ⊕ ₿ ④ ④ ④ **B** 10 50 20 30 20 35 50 **⊕** 60 **⊕** 20 **⊕** 15 **⊕** 5 P281 Ø Œ æ 10 b P319 **⊕** 35 (B) ① 15 **⊕** 20 ➂ ➂ ĭo b **⊕** 50 P3144 50 ⊕ ⊕ 10 ⊕ B B b b 15 **⊕** 50 P352 € ₿ ④ B $^{\odot}$ 40 60 10 30

⁻ Negative; No reaction in carbohydrates

⁺ Acid only in carbohydrates; positive; + Positive, Weak reaction

Acid and gas, 10% gas 10

⁺ Acid, bubble of gas

[®] Reaction reversed - acid to alkaline

²⁴ Incubation for 24 hours

⁴⁸ Incubation for 48 hours

⁷DA Incubation for 7 days

The fermentation reactions in carbohydrate broths were studied using a 0.5% amount of the respective carbohydrate and brom cresol purple as an indicator (Bacto Purple Broth Base, dehydrated). Citrate reactions were observed on Simmon's citrate agar (Difco). Tests for the Voges-Proskauer reaction were made by growing the organism in Bacto M.R.-V.P. media for 24 hours. Three so of a 50% solution of potassium hydroxide which contained 0.3% creatin was then added to an equal amount of the incubated culture of the organism. It was shaken vigorously and observed for 24 hours. A positive reaction was indicated by the production of a red color. Motility was observed on the media recommended by Edwards (1942), using petri dish cultures.

<u>Serological studies</u>. Serological studies on this group of organisms followed the pattern set up by Edwards (1942) in the study of the <u>Salmonella</u> group and also that followed subsequently by Edwards, West and Bruner (1947), Edwards, West and Bruner (1948), and Moran and Bruner (1949).

Preparation of antisera. Somatic antigens were prepared for the immunization of rabbits by inoculation of a tube of Trypticase Soy Broth (Baltimore Biological Laboratories) and incubation at 37° C for 24 hours. The culture was then placed in a boiling water bath and held there for 2 hours. It was then cooled and standardized to a nephelometer # 3 reading with sterile 0.85% saline. This culture was then checked for sterility in Trypticase Soy broth and Thioglycollate media. If sterile, it was then ready for inoculation. Certain of the strains were found to be highly toxic to the rabbits when given intravenously, so it was necessary to alter the above

procedure by autoclaving the culture for $2\frac{1}{2}$ hours at 15 lbs instead of boiling it. Edwards et al (1948) had used broth cultures heated to 121° C for $2\frac{1}{2}$ hours for preparation of their sera, and found them to be equally satisfactory with sera prepared from cultures that were boiled at 100° C. The rabbits were then given the following amounts of the antigen in an ear vein at four day intervals: 0.5 cc, 1.0 cc, 2.0 cc and 3.0 cc. The rabbits were trial bled 6 - 8 days after the last inoculation. If this bleeding showed that the titer was not satisfactory (1-1280 or above) a final inoculation of 1 cc of a heavy suspension of the organisms was given. Six to eight days later the rabbits were bled aseptically from the heart, the blood was allowed to clot and the serum removed and preserved with aqueous merthiclate (1-20,000).

Flagellar antigens were prepared by first enhancing the motility of the culture by a number of passages through motility agar. Cultures were inoculated at the side of the petri dish of semi-solid agar and allowed to incubate at 30° C for 18 - 24 hours. The organism was not considered satisfactory unless it had spread to the opposite side of the plate within that period of time. Most of the organisms met that criteria after 2 - 4 passages in the semi-solid media. One loopful was then inoculated into a tube of Trypticase Soy broth which was allowed to incubate overnight at 30° C. One cc of this culture was then inoculated into 50 cc of Trypticase Soy broth which was incubated overnight. Next day an equal amount of 0.6% formalinized normal saline was added to the culture. This was allowed to stand overnight at room temperature and was then standardized to nephelometer # 3

standard with 0.3% formalinized normal saline. Sterility was then checked as above and inoculations made into rabbits as has been described. The rabbits were trial bled after 6 - 8 days and a titer of 1-5120 was considered adequate. Most titers ran 1-10,240 and higher but if an adequate titer was not reached a fifth injection of 3.0 cc was given. The rabbits were then bled and the serum preserved as has been described above.

Determination of presence of diphasic flagellar fractions. Tests were made to determine if these cultures contained diphasic flagellar antigens by using the technic of Gard (1937). One tenth of a cc of antiserum was added to 15 cc of melted, cooled, semisolid agar and the mixture poured into a petri dish. After the agar had hardened the medium was inoculated with a loopful of the homologous strain on one side of the plate. The dish was incubated in an upright position at 30°C for 48 hours. In case of the presence of a second phase a swarming of the organism takes place, while the homologous phase is suppressed. None of the cultures in this series showed diphasic fractions.

Agglutination tests. Somatic and flagellar antigens as prepared above for the production of antisera were used in the tube agglutination tests which demonstrated the homologous and heterologous fractions of the strains in this study. The somatic antigens were prepared in large tubes (40 cc) of Trypticase Soy broth, boiled for 2 hours and then standardized to nephelometer # 1 standard for the performance of the test. No dilution of the flagellar antigens was necessary when prepared as described above for the performance of the

agglutination tests, since the suspensions were of the proper density.

All normal saline for agglutination tests and for all procedures throughout the study was prepared using Merck reagent grade sodium chloride (Blue label).

Doubling dilutions of the antiserum in normal saline were made from 1 - 10 through 1-2560 for the somatic antigens and from 1 - 20 through 1 - 10,240 for the flagellar antigens. Five tenths of a cc amounts of the diluted serum was added to a small test tube and 0.5 cc of the standardized antigen added. Tests with the somatic antigens were incubated at 50°C in a water bath for one hour and then allowed to stand overnight at room temperature before reading. With the flagellar antigens, the tests were incubated at 50-52°C for one hour and then read. Titers were read as the highest dilution giving a two plus reaction.

Agglutinin absorption technic. Absorption of somatic antisera were carried out at first by using thickly poured petri plates of Bacto Heart Infusion agar, streaked with cotton swabs which were heavily impregnated with growth from a 24 hour agar slant of the culture. One swab was used for each three plates streaked. After 24 hours incubation at 37° C growth was removed with 2.0 cc. of sterile normal saline per plate and transferred to sterile 15 cc centrifuge tubes. These suspensions were then boiled for 2 hours. Organisms were packed by centrifugation at 3000 R.P.M. for 40-60 minutes, the supernatant discarded and a serum dilution of 1-10 was added to the packed cells. Growth from one plate was used for each 2.5 cc. of serum dilution. Three absorptions were carried out at 37° C, the first for one hour, the second for 1½ hours and the

third for 2 hours. After the third absorption the serum was checked for the complete removal of the homologous fraction by a tubeagglutination test. This was the criteria used throughout the study to determine if absorption had been complete. If it was found that all homologous fractions had been removed the serum was then used for tube agglutination studies with both the homologous organism and the heterologous strains which had shown titers of 1-160 or above, as antigens. It was later found that if absorption was carried out at 50° C, only two were generally necessary to remove the homologous somatic fraction. Most of the work was completed using the higher temperature. It was also noted at this time that the Trypticase Soy agar greatly enhanced the smoothness of the cultures so it was substituted early in the work for the Heart Infusion agar. Also by using Kolle flasks it was found that an equivalent amount of packed organisms could be obtained in the ratio of one Kolle flask to 3 petri plates. The flasks were inoculated with one to 2 cc of a 24 hour broth culture of the organism and the growth removed by the addition of 5 cc of saline. The procedure was then carried on as described above.

Absorptions for the flagellar antigens were carried out by inoculation of Kolle flasks containing 75 cc of Trypticase Soy agar with 5 cc of a Trypticase Soy broth culture of the organisms which had previously been passed through semisolid agar to enhance the motility, as has previously been described. These cultures were incubated at 30° C for 24 hours and the organisms were removed from the surface by gently tilting the plate back and forth. The organisms

were poured into a sterile 15 cc centrifuge tube and an equal amount of 0.6% formalinized 0.85% physiological saline added and the resultant mixture allowed to stand overnight at room temperature. The growth from one flask was used to absorb up to 10 cc of a 1-20 dilution of the serum after the organisms had been packed by centrifugation at 3000 R.P.M. for 40-60 minutes. One absorption was carried out at 50° C for one hour and the resultant serum was used to make dilutions for trial tube agglutination tests as described above. Certain of the strains required further absorptions with the packed organisms to remove all homologous flageller fractions. When complete removal was found the serum was used in tube agglutination tests with the heterologous strains which had shown reactions, as antigens.

Studies with Salmonella antisera. Using 24 hour Trypticase Soy agar slant cultures of the organisms and Salmonella somatic antisera all strains were tested for the presence of Salmonella fractions according to the technic of Edwards and Bruner (1942). This slide agglutination technic gave excellent results. To test for the presence of Salmonella flagellar fractions, the technic as described by the same authors was used. Here, with the tube agglutination tests, it was necessary to test blindly for both phase one and phase two fractions.

RESULTS AND DISCUSSION

Somatic antisera. Table II shows the results of the cross agglutination tests in 32 somatic antisera. From the first it was suspected that it would be necessary to prepare quite a number of antisera to obtain reactions to titer of all strains. Hence the first 10 antisera prepared were from the group of organisms supplied by Dr. C. A. Stuart. As it was found that all of these antisera did not give complete reactions with all the strains, other antisera were prepared using next the cultures supplied by Dr. Barnes and finally those obtained from the Army Medical Center. From the reactions shown in this table, all heterologous strains showing a titer of 1-160 or higher were used to absorb the homologous antiserum with somatic antigens. After absorption of all homologous agglutinins, each serum so prepared was used in a series of agglutination tests to determine if the heterologous strains had removed all or only a part of the fractions.

Antisera of cultures 10-848, SA-599, P206 and P210 gave no reactions above a titer of 1-160 except with the homologous organisms, so were considered to be single factor sera.

Tables III through XXIX give the results of the somatic agglutinin absorption studies of all other antisera prepared for this study.

	TABLE II RESULTS OF ACCLUTINATION IN SOMATIC ANTISERA																															
ANTIGENS	=	=	_		=	=			=		-				ITN 22		i -	:>:		a .	38	1	:53	5	83	97	90	0	9.	0.1		2
26311	1280	100% 2001	33611	57711	o 44311	1295 156211	o 6351	₩ 20	[1801] o	\$ ∭	(·)	0 [4	; C5	\$60 ×	5 14-103	- 19:397	5 19-406		2.21	S8:24	99-001 30-001 30	HF30A	SA-153	> SA-560	\$5.55 \$5.55	P.188	907·d <	P-210	3 P246	P-519	5 P3#	5 p.352
32011	0	2560	0	0	0	20	0	0	0	20	40	40	20 O	40 20	4 0 0	0	40 80	20	0 40	40	20 40	2 560 0	0	0 20	20	0	0	20	20 20	20 640	40 40	80 80
32611	0	640	1280	0	0	320	0	0	0	0	20	80	40	20	80	160	4 0	40	80	160	320	20	0	160	80	80	40	80	40	40	80	20
37711 44311	0 20	80	0	1280	0 1280	160	160	40	80	0	20	0 80	0	0	0	0	20	0	0	0	20	0	0	0	20	20	0	0	0 20	0	0	0 20
56211	40	0	40	0	0	1280	0	0	0	20	0	20	0	0	0	0	0	0	160	0	0	0	0	0	20	20	0	0	20	0	20	40
63511	0	80	0	0	0	0	5120	0	0	0	0	0	40	0	.0	0	0	0	0	0	0	0	0	320	0	20	0	0	0	0	0	0
68 1 11 70811	20	80	0	0	0	160 80	20	5120° 20	80 5120⁺	0	0	0	0 140	0	0	0 20	320	0	0	0	20	0	0 64 0	0	20	0	0	0	0 20	0	20	0 40
SRC	640	40	20	0	0	320	0	40	0	1280	640	0	0	20	160	20	80	0	20	ő	0	1280	0	320	20	0	0	0	0	80	0	40
C-1	160	40	0	0	160	0	0	0	0	1280	1280	0	0	0	0	0	40	0	0	0	0	160	0	0	20	0	0	0	0	40	0	0
C-2 C-3	0	40	0	0	0	80	40	20 2560	2560 160	0	0	0	0	0	0	0	160	0	0	0	0	0	1280	0	0	0	0	0	0	0	0	0
C-4	0	0	0	80	0	320	160	640	80	0	20	1280	20	0	20	0	80	0	80	20	0	0	40	0	Ö	40	0	0	40	0	0	0
C-5 C-6	0	20 320	0 320	0	0 640	80	20	20 80	0	0	0 20	0	2560	0	0	0	0	0	320	20	1200	0	0	0	0	0	0	0	40	20	0	0
C-7	0	320	320	0	640	١	20	40	0	0	20	0	40	40	0	2560 1280	0	160 160	160	40 20	1280 1280	20	0	0	20	40	20 20	20	20 40	0	20	40 20
10265	20	0	20	0	0	0	0	20	1280	0	20	0	0	0	0	0	160	0	0	40	0	0	640	0	0	0	0	0	0	0	0	0
10·305 10· 84 8	40	20	20	0	0	80 40	0 40	2560 20	40	20	0	0	20	0 2560	0	0	0	0	0	20	0	0	0	80	0	0	0	0	0	0	0	0
14.103	0	0	0	0	0	0	0	0	0	0	80	20	0	40	1280		0	0	0	40	0	40 2560	0	0	0	40	0	0	0	20 40	0	0
19:397	0	320	40	0	320	20	40	40	0	0	40	20	40	40	20	2560	0	320	160	40	1280	40	0	0	40	80	20	0	40	20	20	0
19*406 20:167	40	80	0	0	0 40	0	0	0	0	640	160	0	0	20	0	160	1280	0 2560	0	0	0 1280	2560	0	0	0	0	20	0	0	5120	0	0
22.4	80	20	40	0	0	80	80	20	2560	20	0	0	20	0	0	20	160	0	4 0 20	0	0	0	0 640	0	0	20	0	20	0	0	0	20
22.21	20	0	40) 0	40	80	20	20	0	0	80	80	20	40	20	0	80	0	1280	1	0	0	0	0	0	0	0	0	20	0	0	0
40·128 40·177	640 1280	20	2560 20	0	80	1280	40	20	0	0 2560	40 320	80	0	40	40	0	0 40	0 0	640 40	20	40	0 1280	0	20	20	20	0	0	40	10	0	0
82.125	40	80	40	0	640	40	80	0	40	40	20	20	160	20	0	320	20	80	20	80	1280	0	0	20	0	40	20	0	40	40 20	0	80 20
82:156	320	0	0	0	0	0	0	0	0	2560	320	0	0	20	160	0	320	0	20	0	0	1280	0	0	0	0	0	0	0	80	0	0
82·1908 89·224	640	0	0	0	0	320	0	0	0	1280	80 0	0	0	20	80	0	20	0	20	0 2560	0	80	0	20	0	0	0	0	0	160	0	20
100658	0	160	0	0	160	1	0	0	0	0	0	0	0	0	0	640	0	160	40	0	2560	0	0	Ö	ő	40	0	20	80	0	20	20
100-794	640		0	0	0	320	0	0	0	1280	320	0	0	20	80	0	20	0	20	0	0	5120	0	0	0	20	0	0	20	20	0	80
100.1305	20	2560 40	0	0 1280	0	0 40	20 40	20	20	20	0	20	0	0	20	20	4 0	0	20	20	20	0	0	0	0	20	0	0	0	640 0	80 0	160
103:147	160	40	160	0	0	640	20	0	0	40	0	0	0	0	0	0	0	0	320		0	0	0	0	0	0	0	0	0	0	20	40
114-30A	320	0	0	0	0	0	0	0	0 320	1280 0	320 0	0	0	80	160	0	320 0	0 80	0	0	20	5120 O	0 1280	0	0	0	0	0	0	640 0	0	0
SA:153 SA:163A	0 20	20 160	0	0	0 640	20	0	0	0	0	0	40	160	20	0	320	0	80	20	0	2560	0	0	0	0	40	20	20	40	20	20	20
SA 5693	1 20	0	0	0	0	80	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	1280		0	0	0	0	0	0	0
SA:599 SA:622	0	0 1280	0	0	0	40	20	0	0	20	20	40	320 0	20	0	20	40	20	40	20	0	0	0	0	2560 0	0	0	0	40 20	0 640	0 80	0 320
P-146	0	20	0	640		80	320	80	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Ö	0	20	0	0	0	0	ő	0
P-173	0	160	0	0	1280	20	0	20	20	0	0	20	40	0	0	160	0	40	20	160	640	0	0	0	0	40	20	0	20	20	20	20
P-189 P-206	0	20	0	0	0	0	0	0	0	0	20	1280 0	80	20	0	0	0	0	0	0	20	0	0	0	0	2560 0	20 1280	20	320 0	20	0	20 20
P-210	0	20	0	0	0	0	0	0	0	Ö	o	0	0	0	Ö	0	0	0	0	40	20	0	0	0	0	0	0	1280	0	0	0	0
P-227	0	20	0	0	1280		0	0	0	0	0	20	80	40	0	20	0	0	0	20	0	0	0	0	0	0	40	0	40	0	0	20
P-228 P-246	0	0	0	0	1280	0	0	20	0	0	20 20	40 1280	40 40	20	0	20	20	0	20 20	20	0	0	0	0	0	0 160	40	20	20 2560	0	0	0
P-250	0	2560		0	0	80	20	20	0	0	0	20	0	0	0	0	0	0	0	40	80	0	0	0	0	40	0	0	0	1280	80	160
P-253	0	40	0	1280		320	160	20	80	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	20	0	0	0	0	0	20
P-260 P-281	0	40 80	0	1280	1 0 1280	160 20	160	40 20	80	0	20	20	40	20	0	40	0	0 40	80	20	0 160	0	0	0	0	20 40	0 20	0	40	0	0 20	0 20
P-319	40	640		0	0	20	0	0	0	320	160	20	0	20	20	0	0	0	0	0	0	640	0	O	Ö	0	0	0	0	5120	0	0 (
P·319 P·344	0	20	0	0	0	80	20	40	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1280	
P-352	0	20	0	0	0_	40	20	40	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	[0	0	0	0	1280	<i>L</i> 500

Whole numbers represent reciprocals of titers obtained.

TABLE III

Results of Somatic Agglutinin
Absorption Study of Culture
26311

Serum					Antigens		T	·			· · · · · · · · · · · · · · · · · · ·
	26311	40-128	40-177	82-156	82-190B	100-794	103-147	114-30A	SRC	C-1	P3 19
26311 "0" Unabsorbed	1280	640	1.280	320	640	640	160	320	640	160	160
Absorbed by 26311	0	0	0	0	0	0	0	0	0	0	0
40-128	640	0	3 20	320	320	320	0	20	640	40	0
40-177	0	0	0	0	0	0	0	o	0	0	0
82-156	80	40	80	0	80	160	80	0	640	0	0
82-190B	0	0	0	0	0	0	0	0	0	0	0
100-794	0	0	O	0	0	0	0	0	0	0	0
103-147	320	0	320	32 0	32 0	640	0	40	1280	20	0
114-30A	160	160	160	0	80	160	20	0	320	0	0
SRC	0	0	0	0	0	o	O	0	40	0	0
C-1	320	80	320	40	160	160	0	0	160	0	0
P 319	32 0	80	320	160	320	640	0	20	640	20	0

TABLE IV

Results of Somatic Agglutinin
Absorption Study of Culture
32011

Serum				Antigen	3				
	32011	100-1305	SA-622	32611	C6	C-7	P 17 3	P250	P319
32011 "O" Unabsorbed	2 560	2 560	1280	640	320	320	160	2 560	64.0
Absorbed by 32011	0	0	0	0	o	0	0	0	o
100-1305	0	o	0	o	o	o	0	0	o
SA-622	0	0	0	0	0	0	0	0	o
32 611	160	160	160	0	0	0	0	80	40
c_6	160	320	320	0	o	0	0	640	160
C-7	320	320	320	0	0	0	0	320	160
P173	32 0	640	320	o	20	20	o	320	160
P2 50	0	0	0	o	0	0	0	0	0
P3 19	320	640	640	o	20	20	0	320	0

TABLE V

Results of Somatic Agglutinin
Absorption Study of Culture
32611

Serum		Y	Antigens	7	
	32611	40-128	103-147	C-6	C-7
32611 "0" Unabsorbed	1280	2560	160	320	320
Absorbed by 32611	0	0	0	0	0
40-128	40	0	o	40	40
103-147	160	o	0	40	40
C6	320	640	80	0	o
C-7	160	640	40	0	0

TABLE VI

Results of Somatic Agglutinin
Absorption Study of Culture
37711

Serum	Antigens										
	37711	103-57	G-4	P146	P253	P260					
37711 "0" Unabsorbed	1280	1280	80	640	1280	1280					
Absorbed by 37711	0	0	0	o	0	o					
103-57	0	0	0	0	O	0					
C4	640	640	0	640	640	640					
P 146	0	0	0	0	0	0					
P253	0	o	0	0	0	0					
P2 60	0	o	0	0	0	o					

TABLE VII

Results of Somatic Applutinin
Absorption Study of Culture
44311

Serum				A	ntigens	1	1	7			1	
	44311	19-397	82-125	100-658	SA-163A	C-1	C-6	C-7	P173	P227	P228	P2 81
44311 Unabsorbed	1280	320	640	160	640	160	640	640	1280	1280	1280	1280
Absorbed by 44311	0	0	0	0	0	0	0	0	0	0	0	0
19-397	40	0	40	0	320	0	0	0	80	160	160	160
82-125	0	0	0	0	o	0	0	0	0	0	О	0
100 -6 58	160	160	40	0	80	0	20	40	160	160	160	160
SA -163 A	o	0	0	0	0	0	0	0	0	0	0	0
C-1	80	160	40	0	160	0	80	80	80	160	160	160
0-6	40	0	40	0	160	0	0	0	40	80	160	160
C -7	40	0	40	0	160	0	0	0	40	160	160	160
P 17 3	0	0	0	o	0	0	0	0	0	0	0	0
P2 27	0	160	0	O	40	0	40	40	0	0	0	40
P228	0	160	0	0	40	0	40	40	0	o	0	40
P2 81	0	0	0	0	0	0	0	0	0	0	0	0

TABLE VIII

Results of Somatic Agglutimin
Absorption Study of Culture
56211

Serva	erus Antigens													
on de Circum	56211	40-128	40-177	82-190B	100-794	103-147	32611	37711	26311	68411	SRC	C-4	P253	P260
56211 Unabsorbed	2560	2560	6Ho	320	320	1280	160	160	640	320	320	1280	160	320
Absorbed by 56211	0	μo	. 0	o	O	0	0	160	.0	20	9	0	80	40
40-128	0	8	0	0	0	0	0	ණ	0	140	0	160	40	140
40-177	640	2560	0	.0	o	1280	0	80	0	0	0	20	5 0	140
82-1908	6 7 10	25 60	0	0	0	1280	0	60	0	ЦO	0	0	50	140
100-794	640	2560	0	0	o	1280	0	80	0	20	0	20	L _i O	40
103-147	0	o	0	0	•	0	0	80	0.5	Lo	0	0	80	20
32611	320	640	0	٥	0	320	0	160	o	20	0	0	20	20
37711	640	2560	160	20	ЦO	1250	0	o	L _I O	0	20	320	٥	0
26311	640	1280	0	0	0	6H0	0	80	0	20	0	40	140	20
68 L 11	640	2560	0	0	0	1280	0	80	0	0	140	0	140	20
SRC	1280	2560	0	0	0	1280	20	80	0	20	0	160	80	40
C-14	1280	2560	20	9	40	1260	20	80	0	L _I O	40	0	ජව	40
P253	1280	2560	40	0	5 0	1280	0	0	40	40	40	320	0	0
P260	640	2560	20	0	0	1280	0	0	6	0	20	50	0	0

TABLE IX

Results of Somatic Agglutinin
Absorption Study of Culture
63511

Serva	Antigens								
	63511	37711	C-4	P146	P253	P260			
63511 "0" Unabsorbed	5120	160	160	320	160	160			
Absorbed by 63511	o	80	0	20	80	40			
37711	2560	0	20	0	o	o			
G-4	1280	20	0	20	40	40			
P 146	1280	0	0	0	0	0			
P253	1.280	0	0	0	o	0			
P260	1280	0	0	0	0	0			

TABLE X

Results of Somatic Agglutinin

Absorption Study of Culture

68411

Serum	Antigens							
	68411	10-305	C-3	G-4				
68411 "O" Unabsorbed	5120 ⁴ (40,960)	2560	2560	640				
Absorbed by 68411	o	o	o	0				
10-305	o	o	0	0				
C-3	0	o	0	o				
C-4 2560		2560	1280	0				

TABLE XI

Results of Somatic Agglutinin
Absorption Study of Culture
70811

Serum	Antigens .							
	70811	10-265	22-4	SA-153	C-2	C-3		
70811 "O" Unabsorbed	5120	1280	2 560	320	2560	160		
Absorbed by 70811	0	0	0	0	0	0		
10-265	o	0	0	0	0	0		
22-4	0	0	0	0	0	o		
SA-153	640	640	640	0	1280	80		
C-2	0	0	0	o	О	o		
C+3	1280	640	1280	320	80	0		

TABLE XII

Results of Somatic Agglutinin
Absorption Study of Culture
14-103

Serum	Antigens							
	14-103	82-156	114-30A	SRC				
14-103 "0" Unabsorbed	1280	160	160	160				
Absorbed by 14-103	0	0	0	0				
82-156	1280	0	0	0				
114-30A	0	0	0	0				
SRC	1280	0	0	o				

TABLE XIII

Results of Somatic Agglutinin
Absorption Study of Culture
19-397

Serum	Antigens								
	1 9- 397	20-167	82-125	100-658	SA-163A	32611	c <u>-</u> 6	G-7	P 17 3
19-397 "O" Unabsorbed	2560	160	320	640	320	160	2560	1280	160
Absorbed by 19-397	0	0	0	0	0	0	0	0	0
20-167	2 560	o	160	160	160	40	1280	1280	80
82-125	1280	0	0	160	40	20	640	640	0
100-658	1280	o	80	0	160	20	640	640	0
SA-163A	1280	o	О	160	0	40	640	640	0
32611	1280	20	160	160	80	0	1280	1280	80
c -6	320	0	0	20	20	20	0	О	0
C -7	640	0	0	40	20	40	0	o	0
P1 7 3	640	0	0	80	0	20	640	640	0

TABLE XIV

Results of Somatic Agglutinin
Absorption Study of Culture
19-406

Serum				Antigens .		, 	,	
	19-406	10-265	22-4	82-156	114-30A	70811	C-2	P319
19-406 "0" Unabsorbed	1280	160	160	320	320	320	160	320
Absorbed by 19-406	0	0	0	0	o	0	0	0
10-265	320	0	0	80	160	0	0	160
22-4	320	o	0	80	160	0	0	160
82-156	160	80	80	О	0	160	20	80
114-30A	160	80	160	0	0	160	20	40
70811	320	0	0	160	160	o	0	160
C_2	160	0	o	80	160	0	0	160
P3 19	80	80	80	80	80	160	20	0

TABLE XV

Results of Somatic Agglutinin
Absorption Study of Culture
20-167

Serum		Antigens						
	20 –167	19-397	100-658	C-6	C-7			
20-167 "0" Unabsorbed	2 560	320	160	160	160			
Absorbed by 20-167	0	0	0	0	0			
19-397	640	0	0	0	0			
100-658	640	40	0	20	20			
C-6	640	20	o	0	0			
G-7	640	20	0	0	0			

TABLE XVI

Results of Somatic Agglutinin
Absorption Study of Culture
22-21

Serum		T	Antigo	na			
	22-21	19-397	40-128	103-147	56211	C-6	C-7
22-21 "0" Unabsorbed	1280	160	640	320	160	320	160
Absorbed by 22-21	0	0	0	0	o	0	0
19-397	320	0	160	160	80	20	20
40-128	320	80	0	40	0	80	80
103-147	320	160	20	0	0	160	160
56211	320	0	o	20	o	o	0
C6	320	40	320	160	320	o	o
C-7	320	40	320	320	320	0	0

TABLE XVII

Results of Sometic Agglutinin
Absorption Study of Culture
89-224

Serum			
	89-224	32611	P173
89-224 "O" Unabsorbed	2560	160	160
Absorbed by 89-224	0	o	0
32611	640	o	0
P173	320	0	0

TABLE XVIII

Results of Somatic Agglutinin
Absorption Study of Culture
100-658

Serum				Antig	ens				1	
	100-658	19-397	20-167	82-125	SA-163A	32611	C6	C-7	P 17 3	P281
100-658 "O" Unabsorbed	2560	1280	1280	1280	2560	320	1280	1280	640	160
Absorbed by 100-658	0	0	0	0	0	0	0	0	0	0
19-397	2 560	o	20	160	160	40	160	160	80	80
20-167	2560	320	0	320	320	320	320	320	320	160
82-125	1280	80	0	0	20	40	160	160	0	0
SA-163A	1280	40	0	o	0	40	160	160	o	0
32 611	2 560	160	20	160	160	0	320	160	80	40
C-6	12 80	80	0	80	160	40	0	0	80	40
C-7	2 560	80	0	80	160	40	20	0	80	80
P 173	640	40	0	0	0	0	80	40	0	0
P2 81	1280	80	0	0	20	40	160	160	0	0

TABLE XIX

Results of Somatic Agglutinin
Absorption Study of Culture
114-30A

Serum				A	ntigens						
	114-30A	14-103	19-406	40-177	82-156	82 -190 B	100-794	26311	SRC	C-1	P319
114-30A "0" Unabsorbed	5120 [≠]	2560	2560	1280	51204	2 56 0	51.20 [±]	2 56 0	1280	160	640
Absorbed by 114-30A	0	o	0	0	0	0	0	0	0	0	0
14-103	5120	0	1280	160	80	80	640	160	160	0	160
19–406	1280	40	o	80	80	40	160	80	80	0	0
40-177	640	0	160	0	80	o	320	0	o	0	80
82 - 156	0	0	0	o	0	0	0	0	0	0	0
32-190B	640	0	160	o	160	o	320	0	0	0	80
100-794	320	20	80	0	40	o	0	0	0	0	80
26311	640	0	160	o	320	0	320	0	0	0	80
SRC	640	0	80	0	320	o	320	0	0	0	80
;_1	640	0	160	80	80	80	320	80	40	0	160
2319	1280	0	40	160	160	80	320	160	40	0	0

TABLE XX

Results of Somatic Agglutinin
Absorption Study of Culture
SA-153

Serum		Antigens						
	SA-153	10-265	22-4	70811	G - 2			
SA-153 "O" Unabsorbed	1280	640	640	640	1280			
Absorbed by SA-153	0	0	0	0	0			
10-265	320	0	0	0	0			
22-4	320	0	0	o	o			
70811	320	0	0	o	0			
C-2	0	0	0	0	o			

TABLE XXI

Results of Somatic Agglutinin
Absorption Study of Culture
SA-569SS

Serum	Antigens					
	SA-569SS	32611	63511	SRC		
SA-569SS *0* Unabsorbed	1280	160	320	320		
Absorbed by SA-569SS	0	0	0	0		
32 611	320	0	0	0		
63 5 11	640	0	o	0		
SRC	640	0	0	0		

TABLE XXII

Results of Somatic Agglutinin
Absorption Study of Culture
C-1

Serum		Antigens							
	C-1	19-406	40-177	82-156	100-794	114-30A	26311	SRC	P319
C-1 "O" Unabsorbed	1280	160	320	320	320	320	640	64,0	160
Absorbed by C-1	0	0	o	0	o	o	0	o	0
19-406	320	0	160	40	80	40	80	8C	0
40-177	160	0	0	0	o	0	0	0	0
82-156	160	0	20	0	o	0	20	20	0
100-794	160	20	0	0	0	0	0	0	20
114-30A	320	0	80	0	40	0	80	40	0
26311	320	0	0	O	o	0	0	0	0
SRC	32 0	0	o	0	o	0	0	0	o
P3 19	320	0	80	80	80	80	160	160	0

TABLE XXIII

Results of Somatic Agglutinin
Absorption Study of Culture
C-4

Serum	Antigens					
	C-4	P189	P246			
C-4 "O" Unabsorbed	1280	1280	1280			
Absorbed by C-4	0	0	0			
P189	20	0	80			
P246	20	0	0			

TABLE XXIV

Results of Somatic Agglutinin
Absorption Study of Culture
C-5

Serva				
	C-5	82-125	SA-163A	SA-599
C-5 "O" Unabsorbed	2 560	160	160	320
Absorbed by C-5	0	0	o	320
82-125	1280	0	0	320
SA-163A	640	0	0	160
SA-599	2560	80	80	0

TABLE XXV

Results of Somatic Agglutinin
Absorption Study of Culture
P189

Serum	Antigens				
	P189	P246			
P189 "0" Unabsorbed	2560	160			
Absorbed by P189	0	o			
P246	320	o			

TABLE XXVI Results of Somatic Agglutinin Absorption Study of Culture P246

Servm	A	tigens
	P246	P189
P246 "O" Unabsorbed	2560	320
Absorbed by P246	0	0
P 189	160	0

TABLE XXVII

Results of Somatic Agglutinin
Absorption Study of Culture
P319

Serum				Anti	zens		Y		
	P319	19-406	89-224	100-1305	114-30A	SA-622	32011	P250	
P319 "0" Unabsorbed	₅₁₂₀ ≠	5120 /	160	640	640	640	640	1280	
Absorbed by P319	0	0	0	o	20	0	0	0	
19-406	1280	0	o	1280	20	80	1280	640	
89-224	1280	1280	o	1280	1280	80	640	640	
100-1305	320	1280	160	О	1280	0	o	o	
114-30A	320	80	0	320	o	160	160	160	
SA-622	320	320	160	0	1280	o	0	0	
32011	320	640	160	0	640	0	0	0	
P250	320	640	160	0	640	0	0	0	

TABLE XXVIII

Results of Somatic Agglutinin
Absorption Study of Culture
P344

Serum		An	tigens		1
	P344	100-1305	SA-622	P250	P352
P344 "O" Unabsorbed	1280	80	80	80	1280
Absorbed by P344	0	0	o	0	o
100-1305	320	0	0	О	640
SA-622	320	o	О	0	640
P2 5 0	320	o	0	0	640
P3 5 2	o	o	0	0	0

TABLE XXIX

Results of Somatic Agglutinin

Absorption Study of Culture

P352

Serum		Ar	tigens		
	P352	100-1305	SA-622	P250	P344
P352 "O" Unabsorbed	2560	160	320	160	1280
Absorbed by P352	0	0	0	0	0
100-1305	640	o	0	o	640
SA-622	640	0	0	0	320
P2 5 0	640	o	0	o	640
P344	0	o	o	0	О

From the tables here presented it is thus possible to indicate the presence of a very definite number of somatic groups. If, for instance, we take culture 26311 (Table III) and arbitrarily assign it the number I as representing the somatic fraction, then cultures SRC, 40-177, 82-190B and 100-794 would also have the same major fraction number since each one completely absorbs the agglutinins from the antiserum of 26311. It is noted however that all of these strains show cross agglutination with other strains. In some instances agglutinin absorption tests show that the organism will remove a sizable fraction of that antibody from the antiserum with which it reacted. Thus it is necessary to take these secondary factors or interlocking antigens into consideration in any classification schema. There are present, also, and common to all the organisms in group 1. two major somatic fractions of the Salmonella group, namely XXVIII and XXX. Continuing, in a like manner, with all the organisms studied and determining the presence of minor fractions by the ability of the heterologous organism to reduce the titer of the homologous antiserum by at least two dilutions, the tenative grouping as is shown in Table XXX may be reached.

TABLE XXX

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac Major	olon fractions Interlocking	Salmonella fraction	
26311	1	6,18,22	XXVIII; XXX	
SRC	1	18,22	XXVIII; XXX	

TABLE XXX (cont'd)

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac Major	olon fractions Interlocking	Salmonella fractions
40 –177	1	18,22	XXVIII; XXX
82-190B	1	6,18	XXVIII; XXX
100-794	1	6,18,22	XXVIII; XXX
32011	2	29	none found
100-1305	2	29,30	none found
SA-622	2	29,30	none found
P2 50	2	29,30	none found
3 2611	3	2,6,17,20 .	I,II,XII; IV,V,XII; VI,
			VII; VI,VIII; I,III,
			XIX; XIII,XXII; I,XIII,
			XXIII; (I),VI,XIV,XXV;
			(VIII),XX; XXX
37711	4	6.	none found
103-57	4	none found	none found
P146	4	•	none found
P253	4	•	none found
P2 60	4	•	none found
44311	5	none found	I,II,XII; VI,VII; I,III,
			XIX; I,XIII,XXIII; (1),
			VI,XIV,XXV; XXX

TABLE XXX (cont'd)

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac <u>Major</u>	olon fractions Interlocking	Salmonella fractions
82-125	5	• • •	I,II,XII; VI,VII; I,
			III,XIX; I,XIII,XXIII;
			(I),VI,XIV,XXV; (XXX)
SA-163A	5	2,24	I,II,XII; VI,VII; I,
			III,XIX; I,XIII,XXIII,
			(I), VI, XIV, XXV
C6	5	3,12,14,15 .	I,II,XII; VI,VII; I,
			III,XIX; XIII,XXII;
			VXX,VIX,IV,(I)
C-7	5	2,3,12,14,15	I,II,XII; VI,VII; I,
			III,XIX; XIII,XXII; (1),
			VI,XIV,XXV
P173	5	12,16,17	I,II,XII; VI,VII; I,
			III,XIX; I,XIII,XXIII;
			(I),VI,XIV,XXV; (XXX)
P227	5	none found	XXX
P228	5	none found	XXX
P 281	5	nene found	VI,VII; I,III,XIX; I,
			XIII,XXIII; (1),VI,XIV,
			XXV; XXX
56211	6	15	none found

TABLE XXX (cont'd)

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac Major	colon fractions Interlocking	Salmonella fractions
40-128	6	2,3,15 .	none found
103-147	6	1,3,15	III,XV
63511	7	2, .	none found
68411	8	2,6	none found
10-305	8	none found	none found
C-3	8	9	none found
70811	9	13,19	none found
10-265	9	13,19	none found
22-4	9	13,19	none found
G-2	9	13,19	none found
10-848	10	none found	none found
14-103	11.	•	XXX
19-397	12	14,15	I,II,XII; VI,VII; III,
			X,XXVI; III,XV; I,III,
			XIX; I,XIII,XXIII; (1),
			VI,XIV,XXV; (XIII,XXII)
19-406	13	18,22,29	XXX

TABLE XXX (cont'd)

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac Major	olon fractions Interlocking	Salmonella fractions
20-167	14	none found	1,11,X11; 1,111,X1X;
			VXX,VIX,IV,(I)
22-21	15	none found	I,II,XII; VI,VII; I,
			III,XIX; I,XIII,XXIII
89-224	16	29	VI,VIII
100-658	17	5,14	I,II,XII; VI,VII; I,
			III,XIX; I,XIII,XXIII;
			VXX,VIX,IV,(I)
114-30A	18	1,11,13,22,29	XXVIII; XXX
82-156	18	1,13,22	XXVIII; XXX
SA-153	19	9	none found
S A- 569SS	20	none found	none found
SA-599	21	none found	XVII
G-1	22	1,5,18	VI,VII; XIII,XXII; (I),
			VI,XIV,XXV; XXX
C-4	23	2,7,8	none found
C-5	24	none found	none found
P189	25	23,28	XXI,XXVI
P206	26	none found	none found
P210	27	none found	none found

TABLE XXX (cont'd)

Results of Grouping Organisms by Somatic Agglutinin Absorption Tests

Culture	Parac Major	olon fractions Interlocking	Salmonella fractions
P246	28	25, 23	XVI; XVIII; XXI, XXVI
P31 9	29	2,13,18,22	XXX
P344	30	none found	none found
P352	30	none found	XXX

⁽I) Fraction may or may not be present

. or .. or ... Indicates number of fractions present which do not remove homologous antibodies below one dilution drop in titer.

It is felt that this schema is a broad one and that a much more simplified diagnostic schema could be evolved and developed through the study of close relationships by slide agglutination tests. For example, group 9 and group 19 are very closely related. However, group 19 organisms do not completely remove the homologous fractions from group 9 antiserum and the reverse is also true. Other groups that are closely related are group 25 and group 28, group 2 and group 29, groups 5, 12, 17 and 22.

It is felt, however, that such a schema is not within the immediate scope of this study.

⁽XXX) Weak reaction - under 2/

Flagellar antisera. Table XXXI shows the results of cross agglutination tests in 29 flagellar antisera. The same method of selection of cultures for preparation of antisera and the same criterium for the performance of agglutinin absorption tests was followed here as was carried out in the somatic studies. Again it is noted that there are many major fractions and many interlocking antigens present.

Antisera of cultures 14-103, 19-406, 20-167, 40-128 and P210 gave no reactions above a titer of 1-160 except with the homologous organisms, so were considered to be single factor sera.

Tables XXXII through LIV give the results of the flagellar agglutinin absorption studies of all other antisera prepared for this study.

					RF	<u>'S</u>	TS	, nr	7 A	LC!				3L					} Δ I	۲ <i>ا</i>	\ \!7	ואוי	ER/	\					
ANTIGENS	S				ı YE		ں ا د	. UI	/1	اںں	ا ب	11.		1 UI 1T1SI		4 1	4∕س	JUL	 /∖.	\ /	W		_ y יר	`					
	3311	IIO	711	1311	5211	3511	<u>F</u>	9811	S	ن	3848	.103	3397	3:400	291.0	0.128	3.125	3-224	10.794	5147	1:153	1,034	56933	.599	983	`210	546	344	.325
26311 32011 37711 44311 56211 63511 68411 70811 SRC C-1 C-2 C-3 C-4 C-5 C-6 C-7 10-265 10-305	10241 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	10240 0 0 0 0 256C 0 0 0 2560 0 0 2560 0 0 0 2560 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 160 0 0 0 160 0 0 0 0 1250 0 0 0 0 0 1280 0 1280 0 0 0 0 0 1280 0 0 0 0 0 0 1280 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	\frac{11}{377}	11033 0 0 0 0 0 1600 0 0 0 0 0 0 0 0 0 0 0 0	1[529] 40 80 40 320 0 5120	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	11802 0 40 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	\$\cdot \cdot	40 0 0 1280 0 0 0 1280 0 0 0 0 1280 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0				ERA	$\emptyset \oplus 0 \circ 0$	11.11 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 2560 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	₩₩ 6°00000000000000000000000000000000000	0 0 1280 0 640 0 0 1280 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		2560 0 0 0 0 5120 0 0 0 0 0 5120 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 640 0 160 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	₩\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	₩d000000000000000000000000000000000000	₩2d 0008080000000000080000000000000000000	₩d 000000000000000000000000000000000000	0 0 1280 40 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 1280 0 80 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Whole numbers represent reciprocals of titers obtained.

TABLE XXXII

Results of Flagellar Agglutinin Absorption Study of Culture 26311

Serum				Antigens	90			
	26311	10-128	11 1- 01	82-1908	100-794	SA-153	9 0	C-7
26311 "H" Unabsorbed	10,240	320	10,240	1280	1280	oilo	10817 02	20,450+
Absorbed by 26311	0	0	0	0	0	0	0	0
40-128	10,240	0	10,240	0/19	OH9	0	10,240	5120
12-ot	0	٥	0	0	0	0	0	0
82-190B	10,240	0	10,240	0	0	0	10,240	5120
100-794	10,240	٥	10,240	0	0	0	10,240	5120
SA-153	10,240	0	10,240	0179	Offo	•	10,240	5120
Ą	0	0	0	0	0	OH7	٥	0
C=7	0	0	0	•	٥	8	0	0

TABLE XXXIII

Results of Flagellar Agglutinin Absorption Study of Culture 32011

Serum			Antigens	zens				
	32011	10-305	100-1305	54-622	11/89	J	P250	P319
32011 *H*								
Unabsorbed	10,240	2560	10,20	5120	2560	2560	25,60	1280
Absorbed by 32011	o	OŞ	0	0	0	0	0	0
10-305	320	0	9	0	0	0	9	0
100-1305	8	%	0	0	0	0	0	0
SA-622	8	8	0	0	0	o	0	0
11/189	320	Š	3	9	0	0	0	0
6-3	9/19	8	97	0	0	0	0	0
P250	5120	0479	10,240	10,240	10,240	10,240	•	2130
P319	350	8	0	0	0	0	0	0

TABLE XXX

Results of Flagellar Agglutinin Absorption Study of Culture 57711

Serum								Anti	ntigens										1
	37721	10-305	10-84,8	7-8	12-23	82-125	469-22h	100-658	103-57	103-147	SA-56988	56211	SRC	77	P206	P228	P253	P260 P352	352
PH ed	10,240	o 1 79	760	1280	O [†] TS	320	920	160	2560	1280	320	160	2560	320	160	2560	2560	Of PO	9
Absorbed by 37711	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10-305	1260	0	0	•	997	0	1280	1280	1280	0 1 /9	Off9	8	7280	160	0	1280	97	0†79	9
10-848	1280	0	0	0	997	0	1280	1280	1280	320	Off9	160	1280	160	0	offo	88	9	9
n-23	1260	•	0	0	760	0	Office (Proposition)	12%0	1280	320	380	S	1280	8	0	Off9	1280	OH)	160
22-21	1260	0	0	0	0	0	O419	1280	1280	97	389	0	1280	0	0	Offo	1280	38	38
62-125	25,60	0	0	0	160	0	1286	1250	1260	offo	Of To	160	2560	991	0	1280	1280	9	9
1723-68	973	0	G	0	0	0	0	380	38	0	160	0	9	0	0	160	04	160	997
100-658	38	0	6	6	0	0	•	۰	3	0	0	0	380	0	0	0	38	0	0
103-57	88	0	0	0	0	•	0	0	0	0	٥	0	380	0	0	0	380	0	0
103-147	1280	0	0	0	0	•	979	1280	1280	0	388	0	1280	0	0	offo	1280	0179	380
88-56938	1280	•	0	•	2	0	380	otho	GIAD	80	0	9	1280	9	0	160	1280	0	8
11296	1260	0	0	0	0	0	380	O [†] IS	0,79	æ	9	0	997	3	0	OT19	1280	93	997
SEC	0	0	0	0	0	0	0	0	9	0	0	0	0	•	0	0	3	0	0
3	1280	9	0	0	0	0	OH79	1280	Offio Offio	160	320	0	1280	0	0	0179	2560	9	979
P206	0475	•	0	0	160	0	977	0387	5560	O#19	Offo	991	1280	93	0	64,0	2560	380	380
P226	0 1 9	0	0	0	04	O	360	330	8	£	017	9	0179	9	0	0	OHD	9	9
P253	9	9	0	0	•	0	0	0	0	0	0	0	9	0	0	0	O 1	0	0
P260	1280	0	0	0	3	0	320	Ot 79	Q175	160	0	9	1280	8	0	320	1280	0	8
P352	1280	0	0	0	160	0	OH)	1260	1280	320	160	160	1280	8	0	380	1280	350	0

Results of Flagellar Agglutimin
Absorption Study of Culture

Serum			Antigen				
	44311	82-125	114-30A	SA-163A	C-5	P173	P227
14311 "H" Unabsorbed	5120	160	160	6H0	160	320	640
Absorbed by	0	0	0	0	0	40	0
52-12 5	1280	0	හ	0	0	0	3 20
114-30A	1280	0	0	160	0	o	160
SA-163A	1280	0	80	o	80	0	320
C-5	6H0	0	0	40	0	0	160
P 173	1250	0	ŢΦ	o	0	0	160
P 227	64 o	0	0	o	0	0	0

TABLE XXXVI

Results of Flagellar Agglutinin
Absorption Study of Culture
56211

Serum			Anti	ens	·		Y	
	56211	22-21	40-125	103-147	SA-153	SRC	C-1	P352
56211 "H" Unabsorbed	10,240	10,240	5120	5120	640	160	5120	160
Absorbed by 56211	0	0	0	0	0	o	0	0
22-21	0	0	0	0	0	0	0	0
40-128	2560	2560	0	2560	0	0	0	0
103-147	6H0	6 40	0	o	0	0	0	0
SA-153	2560	256 0	0	2560	0	140	0	80
SRC	2560	25 60	0	25 60	80	0	0	0
C-1	0	0	0	0	140	o	0	0
P352	5120	5120	0	5120	80	0	0	0

TABLE XXXVII

Results of Flagellar Agglutinin
Absorption Study of Culture
63511

Serum				Antigens						1
	63511	82-125	82-156	89-224	114-30A	14311	C-5	C-6	P227	P344
63511 "H" Unabsorbed	5120	160	160	64 0	320	320	640	160	160	1280
Absorbed by 63511	o	o	o	0	0	0	0	0	0	0
82-125	6l ₁ 0	0	160	o	320	160	80	0	6H0	0
82- 156	640	0	0	0	0	40	0	0	320	0
89-22l ₁	64o	0	50	o	o	40	40	0	6Ho	O
114-30A	320	o	0	o	0	40	0	0	320	0
44311	320	0	0	0	0	0	0	0	640	0
C -5	640	0	О	0	0	40	0	0	6H0	140
C 6	320	0	160	o	160	160	160	0	640	0
P227	80	0	o	o	0	o	0	0	0	0
P344	64 0	0	160	0	160	160	80	0	640	0

TABLE XXXVIII Results of Flagellar Agglutinin Absorption Study of Culture 68111

Serum		Antigen			
	68411	10-305	89-224	C=3	P3ld4
68411 "H" Unabsorbed	10,240	ما2,0	6Lp0	10,240	160
Absorbed by 68411	o	కం	0	o	0
10-305	o	0	0	0	0
89-224	2560	1250	0	5120	0
C -3	0	80	0	0	0
P-344	2560	1280	0	2560	0

TABLE XXXIX Results of Flagellar Agglutimin Absorption Study of Culture 70811

Serum		Antigens			
	70811	10-265	14-103	22-4	C-2
70811 "H" Unabsorbed	5120	10,240	160	5120	160
Absorbed by 70811	0	50	0	0	0
10-265	o	0	o	40	0
14-103	1280	1280	0	2560	0
55-11	o	160	0	0	0
C-2	1280	2560	o	2560	0

TABLE XL

Results of Flagellar Agglutinin
Absorption Study of Culture
10-848

Serva			Intigens				
	10-848	82-156	111-30A	44311	63511	C-5	P227
10-848 "H" Unabsorbed	10,240	1280	1250	1250	5120	1280	5120
Absorbed by 10—848	0	0	0	0	0	0	0
82- 156	2560	0	0	320	2560	o	640
111-30A	5120	0	0	320	5120	0	2560
I.J.311	5120	320	320	0	5120	640	2560
63511	640	80	ήο	160	0	හ	80
C-5	2560	0	0	320	2560	0	2560
P 227	320	0	0	80	0	0	0

TABLE XLI

Results of Flagellar Agglutinin
Absorption Study of Culture
19-397

Serum	Antigens	
	19-397	P 206
19-397 "H" Unabsorbed	10,240	160
Absorbed by 19-397	0	0
P 206	5120	0

TABLE XLII

Results of Flagellar Agglutimin
Absorption Study of Culture
82-125

Serve		Antigens			
	. 82-125	SA-163A	Pl46	P173	P280
82-125 "H" Unabsorbed	2560	160	2560	2560	2560
Absorbed by 82-125	o	0	0	0	0
SA-163A	2560	o	2560	1280	2560
Р146	0	0	0	o	0
P173	0	0	0	0	0
P281	0	0	0	0	0

TABLE XLIII

Results of Flagellar Agglutinin Absorption Study of Culture 89-224

Serva						A	ntige ns							
	89-224	22-21	100-658	103-57	103-147	SA-56988	37721	56211	SRC	P228	P250	P253	P260	P352
39-224 "H" Inabsorbed	5120	160	2560	5120	1280	1280	2560	160	2560	1280	1280	1280	1280	1280
lbsorbed by 59—224	0	0	0	0	o	0	0	0	0	0	0	0	0	0
32 -21	2560	0	1280	2560	80	1280	2560	0	2560	640	1280	1280	1280	1280
100-658	6H0	0	0	0	0	40	0	0	0	0	40	•	40	140
L0 3- 57	1280	0	0	0	0	80	0	0	o	40	80	0	80	40
103-147	2560	0	1280	2560	0	640	1280	0	2560	مب ا	1280	1280	1280	1280
BA-56988	2560	0	640	640	o	o	640	0	640	160	160	640	0	160
57711	1280	0	80	50	0	160	0	8	0	5 0	8 Q	0	160	80
56211	2560	0	1250	1280	40	1280	1250	0	1280	640	1280	1250	1280	1280
SRC	6 40	0	Lio.	0	o	80	6	Ö	0	0	ήο	•	80	Į40
P225	2560	80	1280	1250	o o	64 0	1280	0	1280	0	640	1280	640	640
P250	5 120	80	1280	1250	80	320	1280	80	1280	160	0	1280	320	0
P253	1250	0	50	50	0	160	0	0	.00	40	80	6	160	80
P260	2560	0	640	61 10	0	0	640	6	640	160	160	640	,0	160
235 2	2560	80	1280	640	40	320	640	10	64o	160	0	1280	320	0

TABLE XLIV

Results of Flagellar Agglutinin
Absorption Study of Culture
100-794

Serva			Antigens	 				
	100-794	40-177	52-190B	8A-153	26311	C-2	C-6	C-7
100-794 "H" Unabsorbed	10,240	640	10,2h0	6H0	640	640	6Ho	640
Absorbed by 100-794	0	o	0	0	0	0	0	0
40-177	5120	0	5120	0	0	0	0	0
82- 190B	0	0	0	0	0	0	0	0
SA-153	5120	640	5120	0	640	0	320	320
26311	2560	0	5120	0	0	0	0	0
c-2	2560	640	5120	0	6Ho	0	320	320
C-6	5 120	0	5120	320	0	320	0	0
C-7	5120	0	5120	320	0	320	0	0

TABLE XLV

Results of Flagellar Agglutinin
Absorption Study of Culture
103-147

Serum								Antige	18	<u> </u>							
	103-147	22-21	89-22L	100-658	103-57	SA-56988	37711	56211	SRC	C-1	C-2	P228	P250	P253	P260	P3lulu	P352
Dabsorbed	5120	6hp	1250	640	640	1250	1250	640	1260	1280	320	2560	64o	1280	1280	640	6H0
Absorbed by 103-147	0	0	0	9	0	0	0	0	6	0	0	0	0	6	0	o	0
22-21	2560	0	1280	640	6Ho	1250	1250	0	640	0	160	2560	640	1280	1280	6H0	6kc
89-224	1280	640	0	0	0	80	o	640	0	320	160	1280	0	0	5 0	0	0
100-658	1250	320	80	0	0	80	0	320	0	320	80	1280	0	0	80	0	0
103-57	2560	6Ho	0	0	0	80	o	640	0	640	160	640	0	0	140	0	0
SA-569SS	2560	6 1 /0	80	కం	40	0	50	640	140	640	320	1280	40	80	0	10	40
37711	2560	640	0	0	0	80	0	6H0	0	640	320	1280	0	0	80	0	0
56211	2560	0	640	64,0	1280	1280	640	0	640	0	0	2560	640	640	1250	6H0	640
SRC	2560	640	40	0	0	50	0	640	0	640	160	2560	0	0	0	140	0
C-1	5120	0	1280	1250	1280	1280	1260	0	1250	0	160	2560	640	1280	1250	640	6ly0
G- e	5120	640	1280	1250	1250	2560	1280	640	1280	6110	0	2560	640	1280	1280	640	64o
P226	0	0	0	0	0	0	0	ميا	0	0	0	0	0	0	0	0	0
P250	2560	640	320	160	160	ميلة	320	640	320	640	160	2560	0	320	6H0	160	0
P253	2560	640	0	0	0	కం	0	640	0	640	160	2560	0	0	40	0	0
P260	2560	640	50	80	80	0	80	6 L IO	50	640	160	2560	ණ	80	0	ЦO	80
P3144	2560	640	8 0	0	0	160	40	640	ΙΦ	640	160	1280	0	70	160	0	0
P352	2560	320	320	160	160	320	320	320	160	320	160	1280	0	320	640	160	0

Results of Flagellar Agglutinin Absorption Study of Culture SA-153

Serum	A		
	S A-153	C-2	P206
SA-153 "H" Unabsorbed	5 120	5120	2560 (?)
Absorbed by SA-153	0	0	0
C=2	o	o	o
P206	2560	25 60	o

TABLE XLVII

Results of Flagellar Agglutinin Absorption Study of Culture SA-163A

Serum			Antigens					
	SA-163A	10-305	100-1305	SA-622	32011	68411	C-3	P319
SA-163A "H" Unabsorbed	5120	5120	5120	5120	2560	5120	5120	5120
Absorbed by SA-163A	0	0	0	0	0	0	0	0
10-305	0	0	o	0	0	0	0	0
100-1305	160	40	o	0	0	ħΟ	710	0
SA-622	160	740	0	0	0	80	80	40
32011	67to	320	320	320	0	320	320	320
६ द्योग	80	0	0	0	0	0	0	0
C-3	0	0	0	0	0	0	0	0
P319	50	0	0	o	0	0	0	0

TABLE XLVIII

Results of Flagellar Agglutinin Absorption Study of Culture SA-56988

Serum							Antigen								
	SA-569SS	22-21	69-22L	100-658	103-57	103-147	37711	56211	SRC	C-1	P228	P250	P253	P260	P352
A-569SS "H" Inabsorbed	2560	160	1280	320	640	320	бцо	160	320	320	640	1280	640	2560	1280
Absorbed by SA-569SS	0	0	0	0	0	6	0	0	0	0	0	0	O	0	0
22-21	1250	0	640	320	1280	6	320	0	320	0	640	6H0	640	1280	640
59- 221,	320	0	40	6 0	40	0	40	0	μo	0	40	40	40	6L 0	Ļρ
100-658	640	80	160	0	40	160	0	160	0	ණ	80	320	80	1250	160
103-57	6 1 /0	୫୦	320	0	0	160	0	80	0	0	80	160	0	शक	0
L03-147	1280	0	6H0	320	520	0	320	0	320	0	0	320	640	1280	640
1773	320	80	320	LiO	10	80	0	40	0	40	40	160	80	640	320
6211	6 i ,0	0	640	320	320	0	320	0	320	0	320	320	320	610	320
SRC	610	80	320	ħΟ	140	80	0	හ	0	140	160	160	0	610	160
3-1	610	0	640	320	640	0	320	0	320	0	67 1 0	320	320	1280	320
228	6H0	LIO .	640	160	160	40	40	ŁΦ	80	40	0	160	320	6110	320
P250	610	0	320	160	160	0	320	0	160	0	40	0	සං	640	0
? 253	640	80	61 10	μo	j o	160	0	80	0	0	හිට	320	0	640	160
?260	0	0	0	o	0	0	0	0	0	0	0	0	0	0	0
?352	6H0	0	320	160	160	0	80	0	160	0	0	0	80	640	O

TABLE XLIX

Results of Flagellar Agglutinin

Absorption Study of Culture

SA-599

Serve	Antigers							
	SA-599	10-265						
SA-599 "H" Unabsorbed	2560	160						
Absorbed by SA-599	0	0						
10-265	320	o						

TABLE L

Results of Flagellar Agglutinin
Absorption Study of Culture
C-5

Serus			Ā	ntigens					
	C-5	10-848	40-128	62-156	114-30A	44311	67511	P206	P227
C-5 "Ha Unabsorbed		320	160	2560	2560	160 °	640	160	640
Absorbed b	0	0	0	0	0	0	0	0	0
10-848	2560	0	ÌΦ	1280	2560	0	න	320	160
10-128	2560	320	0	2560	2560	80	320	320	320
82-156	0	0	0	0	0	0	0	0	0
114-30A	0	0	0	0	0	0	0	0	0
44311	2560	160	80	2560	2560	0	320	320	320
63511	2560	0	0	2560	2560	0	0	160	o
P206	2560	320	o	1280	1280	හ	320	0	160
P227	640	0	o	640	640	0	0	80	0

TABLE LI

Results of Flagellar Agglutinin
Absorption Study of Culture
P206

Serva		Antigens	
	P 206	SA-153	0-2
P206 "H" Unabsorbed	5120	640	320
Absorbed by P206	0	o	o
SA-153	2560	0	0
C-2	5120	0	6

TABLE LII

Results of Flagellar Agglutinin
Absorption Study of Culture
P216

Serve	Antigen	
	P21,6	C-4
P2L6 wha	5120	5120
Unabsorbed by P216	0	20
C-4	0	0

TABLE LIII

Results of Flagellar Agglutinin
Absorption Study of Culture
P344

Serva		Amtigene											
	РЗіціц	89-224	100-658	103-57	103-147	SA-569SS	37722	SRC	P228	P250	P253	P260	P352
P314 WHW	2560	2560	2560	2560	64,0	2560	1280	1280	1250	1250	2560	2560	2560
Absorbed by P344	0	0	0	0	0	0	0	0	0	0	0	0	0
89- 224	320	0	320	320	0	5 0	160	320	140	0	160	80	6
100-658	0	0	0	0	0	0	0	0	0	0	0	0	o
103- 57	0	0	0	0	0	0	0	40	0	0	140	0	0
103-147	2560	1280	1280	2560	o	640	640	1280	0	640	1280	640	1280
SA- 5 69 SS	6Ho	160	320	320	0	0	320	320	160	160	320	0	160
37731	320	80	80	160	o	80	0	0	0	80	0	160	80
ERC	320	80	160	160	0	80	0	0	0	80	. 0	8 0	80
P228	1280	6H0	6H0	64 0	0	320	320	640	0	320	6H0	320	6HD
P250	1250	6110	6H0	64 0	80	320	160	640	320	0	640	320	0
P253	160	40	8 0	80	0	80	0	0	0	80	0	80	jto.
P260	640	160	320	320	0	0	160	320	80	5 0	320	0	50
P352	6H0	320	320	320	50	160	320	320	160	0	320	160	0

TABLE LIV

Results of Flagellar Agglutinin
Absorption Study of Culture
P352

Serve						An	tigens						
	P352	89-224	100-658	103-57	103-147	SA-56988	37721	SRC	P228	P250	P253	P260	Р344
P352 WHW Unabsorbed	5120	2560	1280	2560	640	1250	1280	1280	1280	2560	2560	1280	640
Absorbed by P352	0	0	0	0	0	0	O	0	0	0	0	0	0
89-224	640	0	0	0	40	80	40	40	140	320	LO	140	140
100-658	640	160	0	40	80	160	80	80	160	640	80	160	80
103-57	640	160	•	0	40	5 0	0	0	160	320	0	80	0
103-147	1280	610	शक	6H0	0	91 10	6110	64o	8	1280	640	1280	1280
8 A- 56988	640	160	80	80	8 0	0	80	80	80	320	80	o	80
37711	1280	160	80	80	0	80	0	0	1.60	320	0	160	5 0
SRC	640	160	80	80	10	320	0	0	160	640	0	80	5 0
P228	1250	320	320	320	0	3 20	320	320	0	640	320	640	64o
P250	0	0	0	0	0	0	0	0	0	0	0	0	0
P253	6H0	160	0	0	0	160	0	0	80	320	6	160	Цо
P260	320	80	ήο	40	50	0	140	ΡΦ	80	320	10	0	40
P314	هل6	160	0	0	40	80	0	0	160	320	0	80	0

From the results obtained in these agglutinin absorption studies it is possible to indicate a number of flagellar groups. Again, as was done with the somatic fractions, and by following the same order with the key strains, the other organisms can be arranged as shown in Table LV. Only one organism shows any flagellar fractions of the Salmonella group and none of them show any reactions with a polyvalent Arizona group flagellar antiserum.

TABLE LV

Results of Grouping Organisms by Flagellar Agglutinin Absorption Tests

Culture	Paracol Major	on fractions Interlocking	Salmonella fractions	Arizona paracolon fractions
26311	1	17	none found	none found
40-177	1	•	none found	none found
C6	1	•	none found	none found
G-7	1	•	none found	none found
32011	2	19	none found	none found
100-1305	2	19	none found	none found
SA-622	2	19	none found	none found
37711	3	16,21,27,28, .	none found	none found
SRC	3	5,16,21,27,28, .	none found	none found
P2 53	3	16,21,27,28, .	none found	none found
44311	4	6, .	none found	none found

TABLE LV (cont'd)

Results of Grouping Organisms by Flagellar
Agglutinin Absorption Tests

Culture	Paraco Major	olon fractions Interlocking	Salmonella fractions	Arizona paracolon fractions
56211	5	3,21,	none found	none found
22-21	5	3,	none found	none found
G-1	5	3,21, .	none found	none found
63511	6	9, •	none found	none found
P227	6	4,9	none found	none found
68411	7	2,20	none found	none found
10-305	7	2,3,20	none found	none found
C-3	7	2,20	none found	none found
70811	8		none found	none found
10-265	8	22	none found	none found
22-4	8	3	none found	none found
10-848	9	3, •	none found	none found
14-103	10	8	none found	none found
19 - 39 7	n		g,m; g,m,s; g,p; g,p,u; m,t	none found
19-406	12		none found	none found
20-167	13		none found	none found

TABLE LV (cont'd)

Results of Grouping Organisms by Flagellar
Agglutinin Absorption Tests

Culture	Paraco Majo r	lon fractions Interlocking	Salmonella fractions	Arizona paracolon fractions
40-128	14	5, 4 •	none found	none found
82-125	15	3,4,6	none found	none found
P146	15		none found	none found
P173	15	4	none found	none found
P281	15		none found	none found
89-224	16	3,6,7,18,21, 27,28	none found	none found
100-794	17	•	none found	none found
82-190	17	•	none found	none found
103-147	18	3,5,28,	none found	none found
P228	18	3,16,28,	none found	none found
SA-153	19	5,	none found	none found
0-2	19	8,17, .	none found	none found
SA-163	20	2,4, .	none found	none found
P319	20	2	none found	none found
SA-569SS	21	3,27,28	none found	none found
P260	21	3,27,28	none found	none found

TABLE LV (cont'd)

Results of Grouping Organisms by Flagellar
Agglutinin Absorption Tests

Culture	Paraco Major	lon fractions Interlocking	Salmonella fractions	Arizona paracolon fractions
SA-599	22		none found	none found
C-5	23	4,6,9	none found	none found
82-156	23	6,9	none found	none found
114-30A	23	4,6.	none found	none found
F206	24	3,	none found	none found
P510	25		none found	none found
P246	26		none found	none found
C-4	56		none found	none found
P344	27	6,7,28	none found	none found
100-658	27	3,16,18,21,28	none found	none found
103-57	27	3,16,21,28, .	none found	none found
P352	28	3,21,27	none found	none found
P250	28	21,	none found	none found

[.] or .. or ... Indicates number of fractions present which do not remove homologous antibodies below one dilution drop in titer.

Here again as in the somatic groups the flagellar schema merely indicates a method of grouping and it is felt that a simplified diagnostic schema could be evolved by the use of dilute sera and tube

agglutination studies.

It is apparent when a comparison of Table XXX and Table LV is made that not all the groups are "simple" strains. For instance, somatic group 1 and flagellar group 1 contain only two organisms which show the same somatic and flagellar fractions, namely 26311 and 40-177. Organisms 82-190B and 100-794 of the somatic group 1 constitute a separate flagellar group (17) and it shows only a weak interlocking fraction with flagellar group 1. Organism SRC of somatic group 1 fits into flagellar group 3 and organisms C-6 and C-7 of somatic group 5 fit into flagellar group 1.

Somatic group 2 is a more homogeneous group since only one organism is lost from the group (P250) to form flagellar group 2 and the interlocking antigens are comparatively simple in both groups.

This type of variations of interlocking antigens is not new since it was found to be present to a considerable degree in the Bethesda and Ballerup groups of paracolon organisms.

From Tables II and XXXI it is noted that a number of one-sided reactions occur. For example with the somatic groups, culture 14-103 agglutinates in antiserum 114-30A to a titer of 1-2560, but in the reverse, culture 114-30A only agglutinates in antiserum 14-103 to a titer of 1-160. A much more striking example is with culture 19-406 which agglutinates in antiserum P319 to a titer of 1-5120 while culture P319 shows no agglutination in antiserum 19-406 at all. A number of these reactions also occur among the flagellar groups, too. Culture 63511 agglutinates in antiserum 10-848 to a titer of 1-5120 but culture 10-848 shows no agglutination in antiserum 63511.

According to these charts there are 22 of these reactions occurring among the somatic groups and 18 among the flagellar. These reactions do appear quite frequently in coliform cultures in both the 0 and H antigens and have been reported by Wheeler (1944), Edwards et al (1947) and Moran and Bruner (1949).

The instances of serologic variation which have been noted above indicate that the classification of this group will certainly require further study as other strains are found. Since there are no sharp biochemical or serological boundries to the members of the Enterobacteriaceae, it must be recognized that, from time to time, key organisms will be isolated which will help to fill in certain of the gaps present now. However, just as among the Salmonella and Shigella, there are among the paracolon organisms certain outstanding groups that possess uniform biochemical characters. It is with these groups that a start must be made.

SUMMARY AND CONCLUSIONS

An antigenic study of 58 cultures of <u>Paracolobactrum aerogenoides</u> belonging to a definite biochemical group (32011) is presented.

This series of organisms is divided broadly into 30 somatic and 28 flagellar groups according to agglutinin absorption studies.

Many of these strains show interlocking antigens which are not consistently true to form between the somatic and flagellar groups in that organisms belonging to one somatic group may contain the flagellar components of an entirely different somatic group.

Many of the organisms in the group show from one to ten somatic fractions of the <u>Salmonella</u> group. These help further to divide the paracolon groups in that, when present, nearly all the organisms of the same paracolon groups contain identical <u>Salmonella</u> fractions.

Only one organism in this group showed the presence of <u>Salmonella</u> flagellar fractions.

None of the organisms in this group showed any reaction with a polyvalent Arizona group antiserum.

None of the organisms used in this study showed diphasic flagellar fractions.

It is felt that a simplified diagnostic schema could be evolved through the study of strains which show closely interlocking fractions in both the somatic and flagellar groups.

Further study is needed to see where these strains fit serologically in other studied and recognized groups of paracolon organisms. Such a schema involving the Arizona group, the Bethesda group, the Ballerup group and this group would be invaluable epidemiologically in the study of enteric infections.

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ACKNOWLEDGEMENTS

Appreciation is deeply expressed to the following people for their many helpful suggestions which served as the necessary stimulus to bring this work to a conclusion:

Dr. C. A. Stuart, Brown University, Providence, Rhode Island.

Dr. K. S. Wilcox, Connecticut State Health Department, Hartford, Connecticut.

Dr. W. R. Hinshaw, Camp Detrich, Maryland.

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