

# The Ballerup Group of Paracolon Bacteria

By D. W. Bruner

P. R. Edwards

Alice S. Hopson



Kentucky Agricultural Experiment Station  
University of Kentucky  
Lexington

## CONTENTS

	Page
Sources of Cultures .....	4
Definition .....	4
Methods .....	6
Somatic Antigens .....	6
Flagellar Antigens .....	9
Phase Variation .....	13
Serological Classification .....	15
Pathogenicity .....	16
Discussion .....	17
Summary .....	19
References .....	20

# The Ballerup Group of Paracolon Bacteria<sup>1</sup>

By D. W. Bruner, P. R. Edwards<sup>2</sup>, and Alice S. Hopson

The first culture of the group of paracolon bacteria characterized here was the organism described by Kauffmann and Moeller (1940) as *Salmonella ballerup*. This culture seemed to possess the usual biochemical characteristics of the genus *Salmonella*, but had no antigens in common with other members except Vi antigen which is present also in *Salmonella typhi* and *Salmonella paratyphi C*.

Monteverde and Leiguarda (1944) described *Salmonella hormaechei*, which had O antigens similar to those of *S. ballerup* and which also possessed Vi antigen. These authors and Monteverde (1944) discussed a peculiar variation which involved the H antigens of cultures of *S. hormaechei* and of *S. ballerup*. This variation, largely induced by exposure to agglutinating serums, consisted of a mutual transformation of the H antigens of the two types and was called "zeta variation." Edwards (1946) noted a natural segregation of antigens in *S. hormaechei* which was essentially a loss variation similar to that encountered in paracolon cultures by Edwards, West, and Bruner (1948). He also confirmed the finding of Monteverde that *S. ballerup* could be induced to produce H antigens identical with those of one of the H components of *S. hormaechei*.

Shortly after this work a number of cultures, including some lactose fermenters, having O antigens of *S. ballerup* and possessing a variety of H antigens were recognized by several workers. Some of these cultures were described by Harhoff (1949). Thus the opinion that cultures of this group were true *Salmonella* strains no longer was tenable. Rather they constituted a group of paracolon bacteria which, like other bacterial groups, formed a discontinuous series of related types connected through the possession of common O or H antigens. The present bulletin reports an inquiry into the serological properties of 45 of these cultures and constitutes a beginning in the classification of the group.

---

<sup>1</sup> This research was supported in part by a grant from the U. S. Public Health Service.

<sup>2</sup> The Enteric Bacteriology Laboratory, Communicable Disease Center, United States Public Health Service, Atlanta, Georgia.

## Sources of Cultures

Forty-five cultures were examined during the investigation. Dr. J. J. Monteverde sent cultures LC54, M and Horm to this laboratory. LC54 was isolated from sewage and M was supposed to be Kauffmann's original *S. ballerup* strain. The culture labeled Horm was a selected colony of Monteverde's (1944) *S. hormaechei* which was isolated from the ovary of a hen. Culture 7-39 was received from Mr. W. E. Deacon and was isolated from a human case of gastro-enteritis. Culture Fels was obtained by Dr. O. Felsenfeld from a case of diarrhea in man. Cultures 79, 5096, 8382, 294, 17016, 16385, 17776, 695, 3796, 5818, 14671 were received from Mrs. Mildred Galton. All her strains were isolated from human feces, except number 79, which was derived from oysters. Culture 17776 and *Salmonella derby* were isolated from a case of gastro-enteritis in man. Cultures 17016 and 14671 were obtained from human patients suffering from diarrhea, and the remainder were isolated from asymptomatic carriers. Culture 223 was received from Dr. James Watt who secured it from a case of diarrhea in a child. Cultures 48203 and 48204 were received from Dr. G. W. Cox. Both came from human cases of gastro-enteritis. Culture N153, received from Dr. K. S. Wilcox, was isolated from a case of gastro-enteritis. Culture 3153F came from the feces of a normal hog and was sent to the laboratory by Dr. H. W. Schoening. Culture Dyer, received from Major P. R. Carlquist, was derived from a human case of gastro-enteritis. Cultures 3205, 4924, H1-H10 inclusive, 1938, 1939, 2058, 6699, 8665, 59, 527, Lille, 20964, L81b, 107 were received from Dr. F. Kauffmann. Many of these cultures were isolated by various European workers and sent to Dr. Kauffmann. Cultures 59 and 527 were sent to him by Dr. A. Giovanardi; cultures 3205 and 4924 by Dr. E. Mustakallio; culture Lille by Prof. Ch. Gernez-Rieux; culture L81b by Dr. A. Clarenburg, who isolated it from the feces of a duck; cultures H1-H10, 1938, 1939, 2058, 6699 and 8665 by Dr. N. Harhoff. Harhoff's cultures probably were derived from man. The source of culture 20946 was not known. Culture 107 was the standard *S. ballerup* strain of Kauffmann.

## Definition

Inasmuch as study of the Ballerup group is in a formative stage, and since subsequent investigations may bring about the inclusion of cultures of diverse biochemical characteristics, it is difficult to frame an exact definition of the group. Experience with other groups indicates that this group will have no exact biochemical or serological

limits but will merge with other groups of similar cultures. Assignment of particular cultures to this or other groups undoubtedly will depend upon arbitrary agreement. For the present the bacteria may be described as follows: Motile or nonmotile coliform bacteria which produce abundant  $H_2S$  but fail to form indol; usually are methyl red positive; are Voges-Proskauer negative; promptly produce acid and gas from glucose, arabinose, maltose, rhamnose, xylose, trehalose, mannitol, or sorbitol. Attack lactose, sucrose, dulcitol, or salicin with varying avidity, but fail to ferment inositol. Utilize d-tartrate, usually utilize sodium citrate, and rarely liquefy gelatin.

One culture (294) liquefied gelatin. All of the strains except two (7-39 and H10) fermented salicin within 1-19 days. These two refractory strains failed to ferment salicin upon prolonged incubation. Thirteen strains (8665, 527, 79, 5096, 59, Dyer, H2, H6, H10, 20964, 5818, L81b, and 14671) did not attack dulcitol. Three strains (H2, H6 and 20964) did not utilize sodium citrate and 9 strains (7-39, 223, 3153F, LC54, 17776, H1, H8, Lille, and L81b) were methyl red negative.

The majority of the 45 strains fermented lactose or sucrose, or both, when transferred serially in the presence of these sugars for periods of 1 to 6 months. Strains 527, 3205, 7-39, 79, 59, Dyer, 695, H6, 3796, 5818, L81b, and 14671 fermented lactose in unsealed fermentation tubes, while 4924, 6699, 5096, 8382, 294, 16385, 2058, H1, H3, H4, H5, H7, H8, H9, Lille, 48203 and 48204 did not attack this sugar in sealed tubes. It was necessary to seal the fermentation tubes with paraffined corks in order to induce the remaining 16 cultures to ferment lactose. Two strains (2058 and H10) fermented sucrose in unsealed tubes and 20 strains (6699, 1938, 1939, 3205, 5096, Horm, 8382, M, 17016, N153, 3153F, LC54, 16385, Dyer, H6, 20864, 5818, L81b, 48203, and 14671) were induced to attack this sugar in sealed tubes. Strains 4924, 294, H3, H4, H5, H7, H8, Lille and 48204 could not be induced to ferment lactose or sucrose.

All of the 45 strains investigated were related through somatic or flagellar antigens. The first described member of this group, *S. ballerup*, was assigned the *Salmonella* symbols XXIX, [Vi]: z<sub>14</sub> (Kauffmann and Moeller, 1940). As additional types were added more symbols were needed to express the newly discovered antigens. It soon became evident that the Ballerup group of bacteria formed a rather closely knit unit and should be assigned symbols which did not tie them into the *Salmonella* group. Since the precedent was established in assigning Arabic numerals to designate both the somatic (O) and flagellar (H) antigens of the Arizona and Bethesda

paracolony groups (Edwards, West and Bruner, 1947 and 1948) the same procedure was followed in assigning antigenic formulas to members of the Ballerup paracolony group. In addition to the Arabic numerals small Roman letters were used in designating the minor antigens which distinguish the somatic subgroups. A complete delineation of the antigens was not attempted, but those fractions whose recognition was necessary for differentiation and identification of the types were assigned symbols.

### Methods

Edwards and Bruner (1942) described the methods used in identifying *Salmonella* cultures. Similar procedures were used in this study. Antiserums were prepared by injecting actively motile formalinized broth cultures into rabbits. In most cases these antiserums were used to determine both the H and O antigens of the culture. In a few instances antiserums were prepared which contained only agglutinins for somatic antigens. This was accomplished by injecting broth cultures of bacteria that had been heated in a boiling water bath for 2 hours, or by injecting formalinized broth cultures of O forms of the organisms which were totally lacking in flagellar antigens. Alcohol-treated bacteria were used in tests for O antigens and formalinized broth cultures in H agglutination tests. The relationship among the O antigens were established by tube agglutination tests and agglutinin absorptions. Once this was done it was possible to identify the O somatic groups by slide agglutination with alcohol-treated antigens, but it was not possible, in all cases, to determine the subgroups of somatic group 1 by relying on slide agglutination tests.

### Somatic Antigens

*S. ballerup* was assigned the somatic formula of XXIX, [Vi] by Kauffmann and Moeller (1940), and was said to contain Vi antigen serologically identical with that of *S. typhi*. The culture possessed the V-W variation described by Kauffman (1936). Colonies which contained Vi antigen were called V colonies, and those which lacked it, W colonies. V colonies were agglutinated by Vi antiserum, but not by pure O antiserum, while W colonies were agglutinated by O antiserum, but not by pure Vi antiserum. He also found that on continued transfer each form gave rise to the other, Vi antigen like H antigen is inactivated by heat and a culture in the Vi form can be rendered agglutinable in pure O antiserum by suspending the Vi growth in saline and heating it before exposing it to the test antiserum.

Of the 45 cultures studied, Vi antigen was detected in 19. These included cultures 107, Fels, 8665, 3205, 79, Horm, 223, 294, 17016, N153, LC54, 16385, 17776, 2058, Dyer, 695, H1, Lille, and L81b. Since cultures maintained in the usual manner frequently lose Vi antigen, it is quite possible that additional cultures possessed Vi on primary isolation. In fact, culture LC54 had lost its Vi antigen through serial transfer over a period of several years. Vi antigen was detected by plating and examining single colonies. V colonies of the Ballerup group appear more opaque to the naked eye than do W colonies and they agglutinate readily on a slide when exposed to pure Vi antiserum.

Somatic relationships were established by using each culture in agglutinin-absorption tests. Form variation occurs in serological types of the *Salmonella* bacteria (Kauffman, 1940 and 1941; Edwards and Bruner, 1946). The plating of a culture containing mixed O and Vi antigens and fishing of a single O colony might lead to the selection of a form deficient in O antigens. For this reason it was desirable to use the growth from whole cultures in absorbing the O antisera rather than to rely on the results obtained by employing growth from a single O colony. To obtain a satisfactory O antigen for use in testing the absorbed antisera, the growth from a culture possessing both O and Vi antigens was placed in absolute alcohol and heated. Although the Vi antigen was temporarily preserved by this procedure (Peluffo, 1941), it soon was destroyed after the specimen was centrifuged, the alcohol decanted, and the residue suspended in phenolized saline. Upon standing in the latter solution for 2-3 days the Vi antigen disappeared, leaving a satisfactory O antigen. Cultures lacking Vi antigen were treated as recommended by Edwards and Bruner (1942) in preparing O antigens. After preliminary agglutinin-absorption tests were made, cultures which did not possess Vi were used to represent each group in studying their somatic relationships.

In Table 1 are shown the agglutination reactions of the somatic groups. One strain of each O type and their respective sera are included. Ballerup cultures LC54, H8, 527, 4924, and 3153F possess common O antigens and form an agglutinative group. No cross reactions occurred between the three major somatic groups. Table 1 also lists the Arabic symbols and Roman letters assigned to the O factors of the various somatic groups and subgroups.

Table 2 presents a summary of agglutinin-absorption tests showing somatic relationships in somatic group 1. Since antiserum prepared from any one of the five involved strains will agglutinate all the cultures, the Arabic numeral "1" and Roman letter "a" were used to express this common antigen. Roman letters were assigned according

Table 1.—Agglutination Reactions of Somatic Groups.

(Figures indicate highest dilution at which agglutination occurred; "O" indicates no agglutination at 1-100; "( )" indicates part of antigen lacking)

Cultures	Antigens O Factors	Antiserums						
		LC54 1a,1b	H8 1a,1b,1c	527 1a,1b,1d	4924 1a,1d,1e	3153F (1a),1f	3796 2	5818 3
LC54	1a,1b	2000	2000	2000	500	1000	0	0
H8	1a,1b,1c	2000	2000	1000	500	1000	0	0
527	1a,1b,1d	2000	2000	2000	1000	200	0	0
4924	1a,1d,1e	1000	1000	2000	2000	500	0	0
3153F	(1a),1f	1000	1000	1000	200	2000	0	0
3796	2	0	0	0	0	0	2000	0
5818	3	0	0	0	0	0	0	2000

to the results of the agglutinin-absorption tests. Antiserum H8 absorbed by LC54, 527, 4924, or 3153F agglutinated the homologous strain. It was assigned symbols 1a, 1b, 1c. Antiserum 527 absorbed by LC54, H8, 4924, or 3153F was not exhausted of agglutinins for antigen 527, but absorption by both LC54 and 4924 removed all agglutinins. Accordingly, antigen 527 was assigned symbols 1a, 1b, 1d. Antigen H8 or antigen 527 was able to remove all the agglutinins from antiserum LC54 and antigen LC54 was assigned symbols 1a, 1b. Antigen 4924 was unable to absorb all of the agglutinins from LC54, H8, or 527 antiserum. Each of these antisera absorbed by antigen 4924 agglutinated LC54, H8, and 527. In turn antiserum 4924 absorbed by LC54 or H8 agglutinated antigens 527 and 4924 and antiserum 4924 absorbed by antigen 527 agglutinated the homologous strain. The antigens of 4924 were assigned the symbols 1a, 1d, 1e. Although culture 3153F reacted with somatic group 1 antiserum, it possessed a factor of its own and only a fraction of antigen 1a, since it was unable to remove all the agglutinins for this factor from antisera LC54, H8, 527, or 4924. The antigens of culture 3153F were assigned the symbols (1a), 1f. The parenthesis indicates that part of antigen 1a is lacking. Unilateral agglutinative relationships exist in the colon bacteria (Kauffman, 1944) and the inability of culture 527 to remove all the agglutinins from antiserum 3153F is not accounted for in the symbols assigned to the subgroups of somatic group 1.

The O antigens of culture 3796 were assigned figure 2 and those of culture 5818 were assigned figure 3. Although it readily was possible to distinguish the 3 somatic groups by slide agglutination it was necessary in most cases to use tube tests to determine the 5 serological types of somatic group 1. Single factor 1c of culture H8 could be



Table 2.— Summary of Agglutinin-absorption Tests Showing Somatic Relationships

(Figures indicate highest dilution at which agglutination occurred; "O" indicates no agglutination at 1-100)

Antiserums	Antigens and their O Factors					
	LC54	H8	527	4924	3153F	
	1a,1b	1a,1b,1c	1a,1b,1d	1a,1d,1e	(1a),1f	
LC54 absorbed by						
(1a,1b) .....	H8	0	0	0	0	0
	527	0	0	0	0	0
	4924	100	100	100	0	0
	3153F	1000	200	1000	1000	0
H8 absorbed by						
(1a,1b,1c) .....	LC54	0	500	0	0	0
	527	0	500	0	0	0
	4924	200	200	100	0	0
	3153F	500	500	200	500	0
527 absorbed by						
(1a,1b,1d).....	LC54	0	0	100	100	0
	H8	0	0	100	100	0
	4924	200	100	100	0	0
	3153F	500	100	1000	1000	0
	LC54 + 4924	0	0	0	0	0
4924 absorbed by						
(1a,1d,1e) .....	LC54	0	0	100	1000	0
	H8	0	0	200	500	0
	527	0	0	0	100	0
	3153F	100	100	500	500	0
3153F absorbed by						
[(1a),1f] .....	LC54	0	0	0	0	200
	H8	0	0	0	0	200
	527	200	100	0	100	200
	4924	0	0	0	0	200

demonstrated by slide agglutination. Apparently the other factors were too weak to be manifested in this less sensitive test.

### Flagellar Antigens

Of the 45 cultures under investigation 9 proved to be non-motile. These cultures were 4924, 6699, 1938, 1939, H3, H4, H5, H7, and H9. A non-motile strain of *Salmonella aberdeen* which flocculated well in i serum was used by Kauffmann (1939) to prepare diagnostic i serum, and Edwards, Moran and Bruner (1946) described other non-motile *Salmonella* cultures which possessed flagellar antigens. Accordingly

antisera were prepared using formalinized non-motile cultures 4924 and H7. These antisera would not flocculate the H antigens of any member of the group at a dilution of 1 to 50. The microorganisms apparently lacked the ability to stimulate the production of flagellar agglutinins effective against any of the 36 motile Ballerup strains, and they were not flocculated by any Ballerup H antiserum.

In the agglutination tests for H factors, formalinized broth cultures of actively motile bacilli were used as antigens. Often it was necessary to pass the cultures through tall tubes of semisolid medium to insure maximum motility with an excellent development of H antigens. Cultures possessing a large amount of Vi antigen usually required several passages through the semisolid medium to render them actively motile. The tubes were incubated in a water bath at 50°C. and read after 1 hour.

Table 3 shows the agglutination reaction of the H antigenic types. One representative of each serological H type and its respective antiserum are shown. Two groups of related strains were established. The other 9 types gave minor reactions with antisera other than the homologous antisera. Table 3 shows a number of unilateral relationships. For instance antiserum 48203 agglutinates antigen N153, but antiserum N153 does not agglutinate antigen 48203 in the lowest dilution employed in the tests. However, the use of type antisera at 1 to 1000 was satisfactory in spotting the serological types and final confirmation was made by employing single-factor sera.

The symbols assigned to the H factors of the Ballerup group are given in Table 4. The antiserum strains and the absorbing strains used to determine the single factors also are given. It will be noted that culture 5818 is agglutinated by antisera possessing factor 3, but it does not remove all of this fraction from 3 antiserum. Furthermore a culture possessing factor 3 removed all the agglutinins for this factor from 5818 antiserum, but left a strong fraction for the homologous strain. Accordingly, culture 5818 is assigned the figures (3), 4; the parenthesis indicating the presence of a part of 3 antigen. Culture 3153F lacked part of antigen 2 and was listed (2),3. Cultures H10 and 20964 both possess part of antigen 5 but not the complete antigen, and each culture has a strong diagnostic antigen of its own. The H factors given in Table 4 are those of diagnostic importance and do not in all cases represent the complete antigenic pattern of the cultures. The absorbed sera indicating these fractions were selected from a large number of tests, and if comparable results are to





that this antigen is not shared by other H types of the group. Arabic numbers 2,3 represent the *Salmonella* symbols assigned to the H antigens of *S. hormaechei* (XXIX, [VI]:  $z_{30}, z_{31}$ ) of Monteverde (Edwards, 1946). Figure 2 accordingly represents symbol  $z_{30}$  and figure 3 takes the place of symbol  $z_{31}$ .

### Phase Variation

Edwards (1946) in investigating the variation of *S. hormaechei* of Monteverde described the occurrence of two loss variants represented by the formulas XXIX, [VI]:  $z_{30}$  and XXIX, [VI]:  $z_{31}$ . The two variants had no major H antigen in common, but in combination possessed all the antigens of the parent culture. Culture LC54 contained H antigens  $z_{30}, z_{31}$  designated 2,3 in the new Ballerup formulas. Eleven strains of the 36 motile cultures studied agglutinated in 1 to 1000 or in higher dilution of the LC54 antiserum. Agglutination reactions of members of the 2,3 H group are shown in Table 6. When tested with single factor 2 and 3 antisera, cultures 8382, 223, 294, M, 17016, N153, and 695 reacted only in the former, while cultures Horm and 5818 reacted only in the latter serum. Cultures LC54 and 3153F reacted in both 2 and 3 single factor antisera. Culture 5818 also possessed a specific factor of its own (Factor 4). It seems that bacteria belonging to this serological pattern appear in nature in the antigenic 2 phase; 3 phase; 2,3 form; and in combination with other antigens.

The peculiar type of phase variation exhibited by the 2,3 types was

Table 6.—Agglutination reactions of members of the 2,3 H group in single factor antisera.

Antigens	Antisera — Single H factors		
	2	3	4
Horm .....	—	+	—
5818 .....	—	+	+
M .....	+	—	—
N153 .....	+	—	—
8382 .....	+	—	—
223 .....	+	—	—
17016 .....	+	—	—
695 .....	+	—	—
3153F .....	+	+	—
LC54 .....	+	+	—
294 .....	+	—	—

investigated by plating and selecting single colonies. Thousands of colonies were examined with the following results: All single colonies examined of cultures Horm and 5818 reacted with single factor 3 serum. All of those fished from plates of M, N153, 8382, 223, 17016 and 695 reacted only with single factor 2 serum. Of 300 colonies examined, culture 294 yielded one colony which was flocculated by single factor 3 serum; the others agglutinated only in single factor 2 serum. Cultures LC54 and 3153F readily yielded both 2 and 3 type colonies.

Culture LC54 was used to prepare an antiserum containing agglutinins for H factors 2,3. A portion of this antiserum was absorbed with the 2 phase of LC54 and a second portion was absorbed with the 3 phase. These absorbed serums were used according to the method of Edwards and Bruner (1942) in inducing variation within the *Salmonella* group. Absorbed serum possessing agglutinins for single factor 2 was placed in tubes containing semisolid medium. Each tube of this medium was inoculated with a member of the 2,3 H group. Likewise tubes of semisolid medium containing agglutinins for single factor 3 antigen were inoculated. Culture Horm readily migrated through semisolid containing 2 antiserum, but would not migrate through 3 antiserum, indicating that it was stable in the 3 phase. Culture 5818 migrated unchanged through the factor 2 serum, but lost its relationship to the 2,3 H group upon migration through factor 3 serum. It continued to agglutinate strongly in single factor 4 serum. Cultures M, N153, 8382, 223, 294, 17016 and 695 all migrated through semisolid medium containing single factor 2 serum as well as through the single factor 3 containing medium. In each case it was possible to isolate the 2 phase and the 3 phase. When these induced phases were returned to the appropriate antisera in the hope of reversing them it was found that 2 phases readily yielded 3 phases, but the 3 phases were much more stable. Finally the 3 phases of cultures 294, 17016 and 695 were induced to revert to the 2 phase. Accordingly, in working with whole cultures it was possible to reverse the phases of three of the cultures belonging to the 2,3 H group.

Since culture 294 was shown to possess both the 2 phase and the 3 phase through selection of isolated colonies it is quite possible that all of these 7 cultures that split readily into both phases contained minimal amounts of the suppressed phase even though it was not evident in agglutination tests. When single colonies were used in attempts to reverse the phases of these cultures it was found that phase 3 could be induced from a single colony of phase 2, but could not be reversed. Repeated attempts to induce phase 2 from phase 3 made

with cultures 294, 17016, and 695 also failed when single colonies were used. As would be expected, it easily was possible to split cultures LC54 and 3153F into the 2 phase or the 3 phase. Furthermore, it was possible to induce H antigen 3 ( $z^{31}$ ) from H antigen 1 ( $z^{14}$ ). This induction was not reversible nor was it possible to change H antigen 1 to H antigen 2 ( $z_{30}$ ) by the methods employed. Monteverde (1944) stated that through induced variation brought about by the technic of Gard (1937) the H antigens of culture XXIX, [Vi]:  $z_{30}$  could be changed to  $z_{14}$ , i.e., the H antigens of *S. ballerup*. This  $z_{14} \longleftrightarrow z_{30}$  variation was named "zeta variation".

### Serological Classification

After establishing the O and H antigens of the Ballerup group it was necessary only to correlate the results of the examination of the O and H antigens of each of the 45 cultures studied in order to determine the serological types. This is done in Table 7. When all the cultures which possessed O antigens 1a, 1b, 1c were brought together it was found that their H antigens fell into 9 groups represented respectively by H factors 1; 2; 5,6; 5,7; (5),8; (5),9; 10; 11; and 12. Thus 9 serological types are present in O subgroup 1a, 1b, 1c. Somatic subgroup 1a, 1b, 1d was represented by H factors 3; 5,7; 13; and 14. Within this O subgroup are 4 serological types. Somatic subgroup 1a, 1b contains 5 types as well as a group of nonmotile cultures, and H factors 2; 2,3; 15; 16; and 17 are present. Somatic subgroup 1a, 1d, 1e is represented by only one nonmotile culture, while subgroup (1a) If was found with H factors (2),3 and contains a single serological type. The O antigens 1a, 1b, 1c; 1a, 1b, 1d; 1a, 1b; 1a, 1d, 1e; and (1a), 1f all are placed in O group 1 because of the common factor in their O complexes. Somatic group 2 is assigned O antigen 2 and was found only in combination with H factor 1. Somatic group 3 contains O antigen 3 and H factors (3),4. Both groups are represented by single serological types. As in the genus *Salmonella* H antigens recurred in more than one somatic group. There were 3 O groups divisible into 21 types on the basis of their H antigens. H factors 2 and 3 were most widely distributed throughout the group. The presence of the Vi antigen is signified by adding symbols Vi to the somatic formula. No Vi antigen was found in somatic groups 2 and 3.

Table 7 also lists the cultures representing the serological types. In many cases these types were represented by a single culture. In three instances cultures isolated in Europe and in the United States belonged to the same serological type.

**Table 7.—Proposed Diagnostic Schema for Bacteria Belonging to the Ballerup Group**

“( )” indicates part of antigen lacking

Group	O	H	Cultures classified	
1	1a,1b,1c	1°	107, Fels, H1, Lille	
		2°	8382, 223, 294, 17016, 685	
		5,6°	8665, 3205, 7-39, H2, H6, L81b	
		5,7°	79	
		(5),8	H10	
		(5),9	20964	
		10°	16385	
		11°	Dyer	
		12	H8	
		1a,1b,1d	3°	Horn
			5,7	527, 5096
			13	59
	14		14671	
	1a,1b	2°	M, N153	
2,3°		LC54		
15°		17776		
16°		2058		
17		48203, 48204		
—		6699, 1938, 1939, H3, H4, H5, H7, H9		
	1a,1d,1e	—	4924	
	(1a),1f	(2),3	3153F	
2	2	1	3796	
3	3	(3),4	5818	

The presence of Vi antigen is indicated in the O formulas of members of the Ballerup group in the usual manner, as 1a,1b,1c[Vi]: 1 for cultures 107, Fels, H1 or Lille.

\* Vi antigen detected in at least one member of these types.

In order to designate the O and H antigens it is proposed that the antigenic formula be preceded by the letters “Bal.” to note they are members of the Ballerup group. Thus the original *S. ballerup* strain would become Bal. O-1a,1b,1c,[Vi]: H-1, or simply Bal. 1a,1b,1c,[Vi]: 1. Culture H8 which lacks Vi would be assigned the formula Bal. 1a,1b,1c: 12.

### Pathogenicity

Little is known about the pathogenicity of this group of paracolon bacteria. The original Ballerup strain was isolated from the feces of a woman who was suffering from gastro-enteritis. At least 11 of the 45 cultures typed were associated with gastro-enteritis in man. One culture came from sewage, one from the feces of a normal hog, one



from the feces of a duck, one from the ovary of a hen, and one from oysters. A number of the cultures were derived from asymptomatic human carriers. Since these microorganisms were isolated in some instances from cases of diarrhea in man in which no other inciting bacteria were found, it is possible that they were the causative agents. Animals apparently carry paracolon bacteria of this group and may be important in spreading them.

### Discussion

The original Ballerup strain was placed in the *Salmonella* group when it was described by Kauffmann and Moeller (1940). The culture possessed Vi antigen, an antigen shared by *S. typhi* and *S. Paratyphi C*. Neither its O antigens XXIX, nor its H antigens z<sub>14</sub> was found in other members of the *Salmonella* group. Later it was shown that Vi antigen occurred outside the *Salmonella* genus in certain coliform strains of enteric bacteria (Kauffmann, 1941). The microorganism conforms to the biochemical characteristics of the genus *Salmonella* when examined by the usual methods. However, it is a slow salicin fermenter and can be forced to ferment lactose with acid and gas production when cultured serially in the presence of this sugar in sealed fermentation tubes. Since members of the *Salmonella* group ordinarily are not rapid salicin fermenters, nor do they ferment lactose, the Ballerup strain does not conform entirely to the genus definition of *Salmonella*... The fact that it shares no antigens other than Vi with *Salmonella* types adds weight to the argument for establishing it in a paracolon group of its own.

Although it is impossible to pick out a list of biochemical characteristics that will fit all the microorganisms of a serological grouping, Table 8 depicts some reactions of the *Salmonella*, Arizona, Bethesda, and Ballerup groups which are reasonably uniform. The biochemical behavior of the Bethesda and Ballerup groups was essentially the same,

Table 8.—Some biochemical characters of the *Salmonella* and paracolon groups  
(“±” indicates variable reactions)

Test substance	Serological groups			
	<i>Salmonella</i>	Arizona	Bethesda	Ballerup
Lactose .....	—	+	+	±
Sucrose .....	—	—	±	±
Salicin .....	—	—	±	+
Gelatin .....	—	+	—	—
d-tartrate .....	+	—	+	+

although members of the Bethesda group showed more avidity for lactose and less avidity for salicin than did members of the Ballerup group. The final criterion for fermentation of lactose or sucrose was the use of the sealed fermentation tube. The practice of sealing fermentation tubes to induce refractory bacteria to attack a sugar is not uncommon and standard *Salmonella* strains which were carried as controls could not be induced to ferment either sugar.

In demonstrating the minor antigenic differences which differentiate the subgroups of O group 1 it would appear that the original Ballerup strain should be used as one of the type strains. H8 was selected instead of the original 107. Harhoff (1949) in his study of Ballerup bacteria stated that cultures 107 (original Ballerup strain of Kauffmann and Moeller, 1940) and H8 were identical and our mirror absorption results confirmed his findings. Furthermore, culture 107 possesses Vi antigen, whereas H8 lacks this antigen and the whole culture of H8 could be used as antigen without the necessity of destroying Vi antigen.

There is some evidence indicating that form variation occurs in the somatic antigens of culture 107. Irregularities observed in the results of absorption tests in which the progeny of a single colony was used to absorb serum derived from the whole culture suggested that type of variation. The problem of form variation in the Ballerup paracolony group is not definitely settled at this time. It may account for some discrepancies between this work and that of Harhoff (1949), who examined 24 strains of Ballerup bacteria. These strains were included in this study along with 21 additional cultures. Harhoff established three O types. The original Ballerup strain (culture 107) was assigned the O formula XXIX<sub>1</sub>,XXIX<sub>2</sub>,XXIX<sub>3</sub>. Cultures H1 and H8 belonged to this type. Culture 2058 became XXIX<sub>1</sub>,XXIX<sub>2</sub> and cultures Horn 40, LC54, N153, 1938, 1939, 6699, H3, H4, H5, H7, and H9 fitted into this type. Up to this point our results confirmed the findings of Harhoff and the O antigens of culture 107 were assigned the Arabic numeral and Roman letters 1a,1b,1c and the antigens of culture 2058 became 1a,1b (See Table 7). However, to culture 8665 Harhoff assigned the formula XXIX<sub>1</sub>,XXIX<sub>3</sub> and included cultures 3205, H2, H6, H10, 59, 527, Horn, and 4924 within this type. Our results showed that antigens 8665, 3205, H2, H6, or H10 were able to remove all agglutinins from O antisera 107 or H8. Both of these antisera were shown to possess O antigens 1a,1b,1c. Furthermore, O antiserum 8665 absorbed by LC54 (O-1a,1b) agglutinated the homologous strain; absorbed by H8 (O-1a,1b,1c) it was freed of all

agglutinins; and absorbed by 4924 (O-1a,1d,1e) it left agglutinins for LC54, H8, 527 (O-1a,1b,1d), and 8665,

As shown in Table 7 strains 8665, 3205, H2, H6, and H10 are placed in the 1a,1b,1c O subgroup. Harhoff stated that cultures 59, 527, Horm, and 4924 gave varying results when they were used to absorb 8665 antiserum. Our results indicated that cultures Horm, 59, and 527 possessed O factors 1a,1b,1d and that culture 4924 carried O factors 1a,1d,1e. Five cultures were placed in the 1a,1b,1d O subgroup (Table 7). It is true that factor 1b was less developed in 5096 and 14671 than in 527, but its presence could be demonstrated by use of single factor 1b serum in tube tests.

Harhoff stated that the results he obtained in studying the H antigens of 16 motile Ballerup strains were not clear-cut. Our findings agreed essentially with the results he published; however, Table 7 shows a much more complete antigenic analysis of the H antigens of the Ballerup paracolon group than he set forth.

Observed phase variation in the Ballerup group was limited to a form of dissociation into loss variants and induced transformation of H antigen 1 to H antigen 3. As mentioned above, culture LC54 (Bal. 1a,1b: 2,3) possessed flagellar antigens in a 2,3 form, a 2 form and a 3 form. Starting with a single colony Bal. 1a,1b: 2,3, it readily was possible to induce a Bal. 1a,1b: 2 phase or a Bal. 1a,1b: 3 phase. No evidence of diphasic Ballerup microorganisms, as seen in many types of the genus *Salmonella*, was noted. An attempt was made to split culture 8665 (Bal. 1a,1b,1c: 5,6) into H phases 5 and 6 or to change H phase 5,6 to 5,7. These changes were not accomplished.

### Summary

A group of 45 cultures of Ballerup paracolon bacteria was examined by biochemical and serological methods. The group was defined and antigenic formulas were assigned to the various types. The 45 cultures were divisible into 3 groups and 21 serological types. They were isolated from man, a hog, a hen, a duck, oysters, and sewage. Although many of the cultures were derived from asymptomatic carriers, at least 11 of the 45 were associated with gastroenteritis in man. Nineteen cultures possessed Vi antigen and 3 of these cultures were identical with the original Ballerup strain. All cultures were monophasic in character, but a form of loss phase variation was encountered.

## References

- EDWARDS, P. R.  
1945. Form variation in Group C *Salmonella* strains. Soc. Expt. Biol. and Med. Proc. 59: 49-52.
- EDWARDS, P. R.  
1946. The segregation of antigens in a bacterial culture by an undescribed form of variation. Jour. Bact. 51: 523-9.
- EDWARDS, P. R. AND BRUNER, D. W.  
1942. The serological identification of *Salmonella* cultures. Ky. Agr. Expt. Cir. 54.
- EDWARDS, P. R. AND BRUNER, D. W.  
1946. Form variation in *Salmonella pullorum* and its relation to X strains. Cornell Vet. 36: 318-24.
- EDWARDS, P. R., MORAN, ALICE B., AND BRUNER, D. W.  
1946. Flagellar and flagellar antigens in "Nonmotile" *Salmonella* cultures. Soc. Expt. Biol. and Med. Proc. 62: 296-8.
- EDWARDS, P. R., WEST, MARY G. AND BRUNER, D. W.  
1947. Arizona group of paracolon bacteria. A new group of bacteria pathogenic for animals and probably also for man. Ky. Agr. Expt. Sta. Bul. 499.
- EDWARDS, P. R., WEST, MARY G. AND BRUNER, D. W.  
1948. Antigenic studies of a group of paracolon bacteria. (Bethesda) group. Jour. Bact. 55: 711-9.
- GARD, S.  
1937. Ein Colistamm mit *Salmonella*-H-Antigen, zugleich ein Beitrag zur Frage der Definition der Salmonellengruppe. Ztschr. f. Hyg. u. Infektionskrank. 120: 59-65.
- HARHOFF, N.  
1949. Studies on bacteria of the Ballerup group. Acta. Path. et Microbiol. Scand. 26: 167-74.
- KAUFFMANN, F.  
1936. Untersuchungen über die Körperantigene in der *Salmonella*-Gruppe. Ztschr. f. Hyg. u. Infektionskrank. 117: 778-91.
- KAUFFMANN, F.  
1939. Die serologische *Salmonella*-Diagnose. Acta. Path. et Microbiol. Scand. 16: 278-302.
- KAUFFMANN, F.  
1940. Zur Serologie des I-Antigens in der *Salmonella*-Gruppe. Acta. Path. et Microbiol. Scand. 17: 135-144.
- KAUFFMANN, F.  
1941. Die Bakteriologie der *Salmonella*-Gruppe. Copenhagen, Einer Munksgaard.
- KAUFFMANN, F.  
1944. Untersuchungen über die Körper-Antigene der *Coli*-Bakterien. Acta. Path. et Microbiol. Scand. 21: 46-64.
- KAUFFMANN, F. AND MOELLER, E.  
1940. A new type of *Salmonella* (*S. ballerup*) with Vi-antigen. Jour. Hyg. [London]. 40: 248-51.
- MONTVERDE, J. J.  
1944. *Salmonella hormaechei*. Rev. facultad agron. vet. Univ. Buenos Aires. 2: 4-12.
- MONTVERDE, J. J. and LEIGUARDA, R. H.  
1944. A new antigen of *Salmonella*. Nature [London]. 153: 589-91.
- PELUFFO, C. A.  
1941. Stability of Vi antigen of *Salmonella typhi*. Soc. Expt. Biol. and Med. Proc. 48: 340-3.