Salmonelliasis in Infancy and Its Diagnosis[1]

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1. INTRODUCTION

IN NOVEMBER, 1934, we began systematic investigations[2] on the etiology of what is sometimes vaguely known as "Summer diarrheas of infancy." We arrived at the conclusion that "shigellosis" (bacilliary dysentery) constitutes approximately 30 percent of the cases in which the symptoms present themselves as acute inflammation in the intestinal mucosa.

In different successive publications,[3] we have discussed shigellosis in Montevideo and the part it plays in diarrheas of infancy. In the course of these investigations (1935) we came across species of the genus Salmonella in the feces of some children and, realizing that these bacteria are the most common agents in adult enteritis ("food poisoning"), we resolved upon systematic study of the part played by them in infantile dysentery. In 1936 we modified the methods we habitually used in fecal examinations, which had for their chief object the isolation of dysentery bacilli and adopted, after various experiments, the procedures of isolation and classification of the salmonellas.

In accord with our findings it is evident (at least, in Montevideo) that the salmonella groups play a role of great importance in infant pathology—much more so than in adult infections. In the literature we have found casual mention of the detection of salmonella in cases of different types of infantile diseases. The majority of these cases are intestinal, but many are "extra-enteric," septicemias, abscesses, bronchopneumonias, meningitis, arthritis, pleurisy, pyelonephritis, etc. In a recent publication dealing with non-intestinal localizations of the salmonellas, Ramón Guerra, Peluffo and Aleppo[4] cite numerous extant cases in the literature.

In adults, salmonella infection of animal origin may be due to accident, such as the ingestion of contaminated food; in children, especially in nursing infants, the illness is endemic and, although an increase in cases is noted in the summer, they are met with the year round.

Believing that diagnostic methods are of fundamental importance, we shall describe those used by us, especially the procedure followed in serological classification.

2. DIAGNOSTIC METHODS

At the present time the diagnosis is based on the isolation of the salmonellas taken from the different organs or excreta of patients, especially from fecal matter. The indirect methods, such as the determination of specific agglutinins in the sera of the patients may be used as auxiliary help and principally, in our opinion, as a differentiation between the carriers and the infected; but successful determinations can be arrived at only if the type of salmonella is recognized.

Isolation.

When one is dealing with the isolation of salmonella in the feces or in other materials contaminated with different species of bacteria which grow easily on culture media generally used for such purpose (lactose-litmus-agar, MacConkey, Endo, etc.) only exceptionally is success attained, and that is when the salmonella are present in large quantities. If one inoculates a large amount of material the colon bacilli overgrow all other organisms and, furthermore, the proteous sometimes invades the plates. Therefore, media must be selected which will inhibit the growth of this species. According to our experience the best is that of Kristensen, Lester and Jürgens, modified by Kauffmann. Furthermore, in using this media which may be thickly seeded, we find that the method of enrichment gives vastly superior results. The technique which we use for direct inoculation and enrichment is as follows:

Direct Inoculation.

The media of Kristensen, modified by Kauffmann[5,6] is prepared as follows: To one liter of the media is added and mixed 10 grams of Reidel's peptone; Liebig's Extract, 5 grams; NaCl, 5 grams; lactose, 15 grams; phenol red, 40 c.c.; agar, 25 grams. Adjust the pH to 7–7.2, and add to the resultant medium, before pouring it on to different plates, 1.5 c.c. to each liter of liquid solution of Hoechst's brilliant green stain at 0.5 percent. The formula of the solution of phenol red is as follows: Phenol red, 1 gram; soda, N/10.40 c.c.; distilled water, 460 c.c.

In place of Reidel's peptone we have used that of de Witte,[7] and for brilliant green, that of Grübler, with the same results. We usually add 10 percent saccharose to the medium, and then adjust the proportion of lactose to the same concentration. The addition of saccharose eliminates some of the colon bacilli which develop in the media, and which ferment this carbohydrate but not lactose, in

twenty-four hours. In order to avoid the production of glucose, the solution of saccharose must not be heated. We use distilled water heated to boiling point for several moments, and when the temperature has dropped to 60° C., we add the saccharose and, immediately on its dissolution, we add 20 percent chloroform to ensure sterility.

With the exception of a few types known to be extremely difficult (S. abortus equi, S. typhi suis and some others) the species develop well in these media. The colonies are red, circular, convex, transparent, with even borders. The proteus are inhibited though, occasionally, when their number is very great, they may produce very fine colonies, almost invisible, and chiefly recognizable by superficial zones of red on the media. The majority of the coli are inhibited and, if they develop, produce opaque colonies, yellowish green in color. The pyocyaneus, frequent in the pus of the ear and sometimes in pharyngeal exudates, develops well in this medium, and its colonies might be mistaken for those of the salmonellas. In general, they can be recognized by their flatness, irregular borders and, at times, a metallic sheen; others are a dirty red, inclining to dull gray. When its development is marked, the pyocyaneus may be identified by its characteristic aromatic odor, but sometimes its colonies are like those of salmonella and may give rise to errors if salmonella is coexistent. We have had success at different times in isolating the first from the latter by using plates poured with glucose-litmus-agar, because the pyocyaneus does not ferment the glucose in twenty-four hours. As to the dysentery bacilli and Proteus morgani (Morgan's bacillus), they do not develop in Kristensen's medium.

For direct inoculation with suspected materials we use one plate of Kristensen's medium and 3 to 4 of glucose-litmus-agar, or a similar medium, without inhibitives. This combination is of value because some colonies of salmonella are at times totally or partially inhibited in Kristensen's medium. On the other hand, this medium is convenient for the isolation of shigellas.

Kristensen's medium must be thickly inoculated, using a platinum loop, and streaking the whole medium without fresh addition. A reading should be made every twenty-four hours. If proteus develops on the glucose-litmus-agar plates, we do not directly pick those colonies developed in Kristensen's medium, even though they present the characteristics of salmonella and appear separate. We prefer to take 15 to 20 of them, make a suspension of physiological saline, and transplant again on one plate of Kristensen's medium and two of lactose-litmus. The reason for this procedure is that, although the proteus are inhibited in Kristensen's medium, they

often remain alive, and if one picks the colonies under these conditions one obtains contaminated cultures. The plates without inhibitives thus serve as a control, and for this reason we always start with the transplanted colonies which they contain.

Enrichment.

The procedure of choice is, in our opinion, that described by Kauffmann[8,9] under the name of "method of enrichment combined with tetrathionate." The medium K, used as a base, is prepared as follows:[10]

(*a*) Original tetrathionate: Place in a receptacle 50 grams of chalk (carbonate of calcium) and sterilize in the autoclave. Add 900 c.c. of sterile, plain broth, and then 100 c.c. of hyposulphite of sodium (sterilized by autoclave), 500 grams of hyposulphite of sodium plus 1 liter of water. Add 20 c.c. of solution of iodide-iodine (iodine, 20 grams, potassium iodide, 25 grams, water, c.s.p. 100 c.c.). Use aseptically, for this medium must not be heated.

(*b*) Medium K. To 1,000 c.c. of the original solution of tetrathionate add 10 c.c. of liquid solution of brilliant green at 1 percent, and 50 c.c. of sterilized ox-gall. Divide this into sterile tubes to the quantity of 10 to 15 c.c. to a tube, shaking continuously to avoid sedimentation of the chalk. Preserve sterility, leaving the test tubes several days in the incubator at 37° C.

The test tubes containing the medium K must be thoroughly streaked with the material to be examined. For fecal examination take a piece of material the size of a pea, or an equivalent amount or more if liquid or mucous materials are to be dealt with. For isolation, when they are removed from the medium K, we use two plates of Kristensen's medium and one of lactose-litmus-agar, which we then inoculate after one to five days of enrichment at 37° C. It is advisable to re-transplant, for as we have already said, proteus develops well in the K medium.

As an example of the value of the methods of isolation, we reproduce the results which we have obtained with the use of 384 positive tests in children and adults, exclusive of S. typhi.

It appears evident that if we had used the direct inoculation only we should have obtained 108 positive results, or it may be that the enrichment is superior in proportion of 3.5:1. It is noteworthy that in 5 cases only did we have success by direct inoculation. If we compare the results obtained by incubation of the medium K during one to five days, we see that the total positives were respectively 310 and 290. At present we cannot offer any explanation for this

Enriched Medium

Direct Inoculation	*1 Day*	*5 Days*	*Totals*
—	+	+	143
+	+	+	79
—	+	—	70
—	—	+	63
+	+	—	18
+	—	+	5
+	—	—	6

unexpected result i.e., why it sometimes happened that the salmonellas, present for twenty-four hours, disappeared from the medium. The enticing hypothesis of the existence of the bacteriophage might explain the fact, but various tests to discover it have always failed. It is possible that the co-existence of other bacteria which develop in the medium K, especially the pyocyaneus, causes the lysis of the salmonellas. As to the respective value of the two methods of procedure, it is to be noted that in spite of the fact that the total result of positives at twenty-four hours is superior to that obtained at five days, there are 65 cases in which the first was negative and the second, positive. One must add that these cases were in the majority carriers or convalescents.

For reasons of comparison with the medium K we use the enrichment medium Selenite or the medium "F" of Leifson.[11] Up to the present the results we have obtained with it are similar to those obtained with the medium "K" and, as far as S. typhi is concerned, they are superior, if for isolation Kristensen plates are used, and not those with desoxychotate-citrate, recommended by Leifson. This medium, doubtless excellent for the isolation of shigellas, and which we believe to be superior to that of Kristensen for the isolation of S. typhi, is not appropriate for salmonellas. In recent tests we have used either medium, in order to check results, obtaining in 29 positive cases only 24 in Kristensen's medium, 4 in both, and one negative in it and positive in desoxychotate-citrate. The types of salmonella were: 17 typhi murium; 3 newport; 2 anatum; 1 montevideo; 1 bredeny; 1 B. paratyphi; 1 munchen, and 1 panama.

Classification.

Biochemical properties: The limitation of the genus Salmonella was for many years based on biochemical properties, especially on the action on carbohydrates. Buchanan (1918) attributed to the

genus the property of fermenting glucose with the production of acid and gas, but not lactose. Castellani and Chalmers defined the genus as "fermenting glucose completely, but not lactose; partially or totally manitol, as well as other carbohydrates, and does not coagulate milk." According to Bergey[12] (1934) these properties limit the genus: ". . . forms motile or immotile. One meets with them in the intestinal tract of animals and in various types of acute inflamatory processes. They attack numerous carbohydrates with the production of acids or acid and gas, but not lactose, nor saccharose, nor salicin. Usually they do not produce acetyl-methyl-carbonol."

In 1935 the Subcommittee of the Salmonellas of the International Society of Microbiology[13] admitted the necessity of serological criteria for group limitation, adopting the definition of Bruce White (1929). In accord with this definition is understood:

> An extensive group of gram-negative bacilli, non-sporulating and serologically related. . . They do not ferment lactose nor saccharose, do not coagulate milk nor liquefy gelatine nor produce indol, and attack glucose generally with production of gas, but not always. All the known species are pathogenic for man, for animals, or for both.

Only the species which possess antigens of the group can be considered, therefore, as true salmonellas, and it is a foregone conclusion that the biochemical properties adapt themselves to those admitted by the same. The case of S. cerro illustrates this criterion. This type, which we isolated in 1936,[14] possesses all the biochemical properties of the salmonellas; it is agglutinated, moreover, by some agglutin-serum of the group, of low titration (especially somatic sera carrau), but does not absorb in an appreciable degree the agglutinins of these sera. Although it appears probable that it may really pertain to the group, we keep for it these symbols, XVIIIz[4], and we designate it tentatively "Bact. cerro" in the hope of finding a type which, possessing the same antigens, may also contain others already recognized among the salmonellas.[15]

The biochemical properties of the salmonellas cannot be defined with complete accuracy; however, as was done in 1934, Kristensen and Kauffmann[16] obtained varieties of S. eastbourne which produce indol. In 1937 Kauffmann[17] isolated from the strain of S. anatum varieties which ferment lactose, even though very slowly; and, last of all, we recognize various types of salmonella which liquefy gelatine: S. dar-es-salaam, Jordan,[18] S. abortus bovis, Kauffmann,[19] and S. schleissheim, Kauffmann and Tesdal.[20] Taking these new findings into account, the Salmonella Subcommittee[21] modified its definition as follows:

"They do not ferment saccharose, nor coagulate milk, and rarely ferment lactose, liquefy gelatin or produce indol, but attack glucose, etc."

In reality, in proportion to the increase of our knowledge, it becomes more difficult to define clearly what should be exacted as demarcation of the precise limits of the genus; however, we believe that for the present there is no other way but to fix its boundaries arbitrarily in this manner. Today we know that species exist which, though they possess the antigens of the salmonellas, behave like colon-bacilli in respect to their properties in fermenting carbohydrates, such as those studied by Gard in 1939,[22] which possess antigens 1, 3, and 5 of the non-specific phases. Kauffmann and others, more recently, have also isolated strains of coli which possess not only flagellar, but also somatic antigens, already recognized in the species of the genus Salmonella.

It may be disputed as to whether these species may be considered as salmonellas or colon bacilli, i.e. whether one should emphasize the value of their serological or of their biochemical criteria. In our opinion no definite answer is possible at the present. If we accept the proposition that the inclusion of a species in a genus depends upon its possession of the antigens of the group, we shall arrive at an unsatisfactory conclusion, for we know from what Schutze[23] demonstrated in 1928, that a species which could not be classified by a bacteriologist as a salmonella—P. pseudotuberculosis rodentium—possesses the somatic antigens of the salmonellas of Group B. On the other hand, the strains of colon bacilli already mentioned, which are related to the salmonellas by possessing common antigens, possess other characteristics also. If we consider them as being included in the group, the existence of these new antigens in other coli will oblige us also to include these among the salmonellas, and thus we shall probably arrive, step by step, at enumerating salmonellas, for the greater part if not all, as a colon-bacilliary species.

Taking all this into account, it is possible that the future will prove this mode of procedure to be the most logical. In reality, to understand the genus, it is necessary to note that the pathogenic action is an important matter, and it is possible that these colon bacilli with salmonella antigens, about which we have little or no information, may be either quite as pathogenic as are salmonellas or

perhaps are a phase of transition between these and the saprophytic colon bacilli.

Whatever may be the value attributed to bio-chemical properties for the limitations of the genus Salmonella, its study is fundamental for determination of type, which is, in the last analysis, the highest interest in practice.

Some types, with identical serological formula, are differentiated by certain bio-chemical properties: S. gallinarum and S. pullorum are distinct from each other because among other properties the former does not produce gas or H^2S; S. cholera suis and S. typhi suis are also distinct from each other because the latter ferments arabinose and trehalose. For reasons of conciseness we are not listing the bio-chemical properties in the species of the genus. The last publication we know of in which they have been summarized is that of Tesdal,[24] appearing in 1938.

In the course of our investigations we have proceeded to verify the classification, studying as a first consideration whether the suspicious colonies possess the fundamental group properties. As we said before, we separated the isolated colonies on plates of lactose-litmus-agar, which we picked into tubes of saccharose-litmus-broth. Thus, in twenty-four hours we eliminated by fermentation of this hydrate of carbon everything which did not belong to the group. We inoculated the remainder in the litmus broth (with bell for gas) with lactose, glucose, and manitol, as well as on simple agar slants, Pringsheim medium (for the investigation of indol), and agar of acetate of lead (H^2S). At present we add a tube containing Simmons medium, to which we add urea. The development of the urea with change of the medium to blue permits differentiation of the proteus from the salmonellas, which exert no action on it.

Before proceeding to the serological determination of the type of salmonella, we test all the colonies we have obtained, whatever their biochemical properties may be, in the presence of a polyvalent agglutinating serum containing agglutinins for all the antigens, somatic and flagellar, of the salmonellas. We use the quickest agglutinating process, using as antigen a thick suspension of the bacteria to be tested in physiological saline. We place a thick drop of polyvalent serum on a glass slide and then another of equal volume of the suspension. It can be read in a few moments, and permits us to examine a great number of colonies in a short time. If the agglutination is positive, we proceed to study all the bio-chemical and serological properties of the isolated strains.

Determination of serologic type. At present, the identification of the salmonella types is based on the methods of agglutination, which allows the recognition of the group antigens present, designated by special symbols according to the scheme of Kauffmann and White. The somatic antigens are designated by Roman numbers; the specific flagellates (phase 1 and phase 2) by small letters, and the non-specific flagellates (phase 2) with Arabic lettering.

We reproduce the present plan as approved by the Salmonella Subcommittee in 1939:[25]

THE KAUFFMANN-WHITE SCHEME (1939)

Diagnostic Antigenic Scheme

	Type	*O-Antigen*	*H-Antigen*	
			Phase 1	*Phase 2*
		Group A		
1	*S. paratyphi* A	(I), II	a	—
		Group B		
2	*S. paratyphi* B	(I), IV, (V)	b	1, 2...
3	*S. abony*	I, IV, V	b	e, n, x
4	*S. typhi murium*	(I), IV, (V)	i	1, 2...
5	*S. stanley*	IV, V	d	1, 2...
6	*S. heidelberg*	IV, V	r	1, 2...
7	*S. chester*	IV, (V)	e, h	e, n, x
8	*S. reading*	IV	e, h	1, 5...
9	*S. derby*	(I), IV	f, g...	—
10	*S. essen*	IV	g, m...	—
11	*S. budapest*	IV	g, t...	—
12	*S. brandenburg*	IV	l, v	e, n
13	*S. bispebjerg*	IV	a	e, n, x
14	*S. abortus equi*	IV	—	e, n, x
15	*S. abortus ovis*	IV	c	1, 6...
16	*S. abortus bovis*	I, IV, XXVII	b	e, n, x
17	*S. bredeney*	I, IV, XXVII	l, v	1, 7...
18	*S. schleissheim*	IV, XXVII	b, z_{12}	—

THE KAUFFMANN-WHITE SCHEME (1939—*Continued*)

	Type	*O-Antigen*	*H-Antigen*	
			Phase 1	*Phase 2*
		Group C		
19	*S. paratyphi* C	VI, VII (vi)	c	1, 5 . . .
20	*S. cholerae suis*		(c)	1, 5 . . .
21	*S. typhi suis*		c	1, 5 . . .
22	*S. thompson*		(k)	1, 5 . . .
23	*S. virchow*		r	1, 2 . . .
24	*S. oranienburg*		m, t . . .	—
25	*S. potsdam*		l, v	e, n
26	*S. bareilly*	VI, VII	y	1, 5 . . .
27	*S. mikawashima*		y	e, n
28	*S. montevideo*		g, m, s . . .	—
29	*S. oslo*		a	e, n, x
30	*S. amersfoort*		d	e, n, x
31	*S. braenderup*		e, h	e, n
32	*S. newport*		e, h	1, 2 . . .
33	*S. kottbus*		e, h	1, 5 . . .
34	*S. bovis morbifican*	VI, VIII	r	1, 5 . . .
35	*S. muenchen*		d	1, 2 . . .
36	*S. narashino*		a	e, n, x
37	*S. glostrup*		z_{10}	e, n
		Group D		
38	*S. typhi*	IX, (vi)	d	—
39	*S. enteritidis*		g, m . . .	—
40	*S. dublin*		g, p . . .	—
41	*S. rostock*		g, p, u . . .	—
42	*S. moscow*	IX	g, q . . .	—
43	*S. blegdam*		g, m, q . . .	—
44	*S. berta*		f, g, t . . .	—
45	*S. eastbourne*		e, h	1, 5 . . .
46	*S. sendai*	(I), IX	a	1, 5 . . .
47	*S. dar es salaam*	I, IX	l, w	e, n
48	*S. panama*	I, IX	l, v	1, 5 . . .
49	*S. gallinarum*	IX	—	—

THE KAUFFMANN-WHITE SCHEME (1939—*Continued*)

	Type	*O-Antigen*	*H-Antigen*	
			Phase 1	*Phase 2*
		Group E		
50	*S. london*		l, v	1, 6 . . .
51	*S. give*		l, v	1, 7 . . .
52	*S. anatum*		e, h	1, 6 . . .
53	*S. muenster*	III, X, XXVI	e, h	1, 5 . . .
54	*S. nyborg*		e, h	1, 7 . . .
55	*S. amager*		y	1, 2 . . .
56	*S. zanzibar*		k	1, 5 . . .
57	*S. shangani*		d	1, 5 . . .
58	*S. newington*		e, h	1, 6 . . .
59	*S. selandia*	III, XV	e, h	1, 7 . . .
60	*S. new brunswick*		l, v	1, 7 . . .
61	*S. senftenberg*	I, III, XIX	g, s, t . . .	—
62	*S. niloese*		d	z6 . . .
		Further Groups		
63	*S. aberdeen*	XI	i	1, 2 . . .
64	*S. poona*	XIII, XXII	z . . .	1, 6 . . .
65	*S. worthington*	I, XIII, XXIII	l, w	z . . .
66	*S. wichita*	I, XIII, XXIII	d . . .	—
67	*S. carrau*	VI, XIV, XXIV	y	1, 7 . . .
68	*S. onderstepoort*	[I], VI, XIV, XXV	e, h	1, 5 . . .
69	*S. hvittingfoss*	XVI	b	e, n, x
70	*S. gaminara*	XVI	d	1, 7 . . .
71	*S. kirkee*	XVII	b	1, 2 . . .
72	*S. kentucky*	[VIII], XX	i	z6 . . .
73	*S. minnesota*	XXI, XXVI	b	e, n, x
74	*S. tel-aviv*	XXVIII	y	e, n

( ) = These antigens may fail. The only H-antigens to be specified in this way, are the antigens c and k whose absence results in the two non-specific variants most important in practice, viz. *Salmonella cholerae-suis* var. *kunzendorf* and *S. thompson* var. *berlin*.

[ ] = Only part of the O-antigen present.

. . . = Especially strongly abbreviated formulae.

As this Scheme is mainly intended for practical use in the determination of serological types, only antigens of diagnostic importance have been included in the abbreviated formulae.

The most practical method of proceeding with the identification of the serological type of salmonellas consists of using rapid agglutination. In preparing the agglutinating sera, deprived of part of their agglutinins by saturation to the extent that they agglutinate one sole factor, it is easy to recognize in a few minutes which of the somatic and flagellar antigens the strain under study possesses, and comparing the result obtained with the scheme of Kauffmann and White, one can make identification with sufficient accuracy for all practical purposes. It must be recorded that the plan cited does not include the complete antigen of the salmonellas, but rather the simplified form, the lesser antigens being omitted; and for this reason it is necessary to acquire certain facility in the interpretation of the partial reactions, due to the presence of these antigens.

(*a*) Somatic sera. For the preparation of somatic sera we inject the rabbit intravenously with a culture of twenty-four hour preparation in broth, heated to 100° C for two hours, to destroy the flagellar antigens. If one employs the "O" strain, this heating is not necessary. One can employ one or another of the types included in the scheme, but it is indispensible to be assured previously that the strain used is of an "S" form. For this we use the reaction from rapid agglutination in the presence of a solution of trypaflavine, 1:500 in distilled water; the "R" forms give an instantaneous agglutination in thick clumps. The form "Vi" of S. typhi and some non-specific phases of salmonella in form "S" sometimes agglutinate; but in this case the clumps are fine, which permits differentiation from the "R" forms.

To obtain pure somatic factors, one saturates the sera, adding them to a heavy bacterial suspension of the type desired. We use forty-eight hour cultures in agar, and collect the bacilar mass by scraping it off with a wide spatula. This method is preferred because the dilution of the serum is avoided, inasmuch as when the bacteria are collected, suspending them in saline solution makes dilution inevitable. Phenol is added to the mixture of serum and bacilli up to a final dilution of 5 percent and incubated for six hours at 37° C., and eighteen hours at room temperature or in the refrigerator. The serum is separated by centrifugation, and by slow agglutination we avoid the disappearance of the desired agglutinins and, as a preservative, we add glycerine to the amount of 40 percent. Although when thus treated the sera remain stabile at room temperature for a long time, it is wise to keep them in a refrigerator so that the strength of the agglutination shall not vary.[26]

It is important to obtain complete absorption without traces being left, and for this one must obtain a superabundance of bacilli. As the necessary quantity consists of a great mass of bacilli, when the volume of serum to be saturated is considerable, we employ as receptacles for the culture aluminum crystallizing units 30 centimeters in diameter, covered with gauze, cotton and paper.

We sow them, collecting the suspended bacteria in 30 c.c. of distilled water. After forty-eight hours the water evaporates completely and the scraping produces a pasty mass of bacilli. The standardization of a good somatic crude serum should not be lower than 1/1,280, measured by slow reaction. After saturation, it is often possible to dilute them for use in rapid agglutination but, in general, it is advisable to use dilutions in which agglutination is almost instantaneous.

The necessary factor may be obtained by following this plan:

Factor	*Serum*	*Saturation with "S"*
I	Paratyphi A (I, II, XII)	Paratyphi A, var. Durazzo (II, XII)
II	Paratyphi A, var. Durazzo	Enteritidis (IX, XII)
III, XIX	Senftenberg (I, III, XIX)	Paratyphi A
IV	Reading (IV, XII)	Enteritidis
V	Typhi murium (IV, V, XII)	Derby (IV, XII)
VI_1	Cholera suis (VI_1, VII)	Cholera suis (VI_2, VII)
VI_2	Cholera suis (VI_2, VII)	Cholera suis (VI_1, VII)
VII	Oranienberg (VI, VII)	Muenchen (VI, VIII)
VIII	Newport (VI, VIII)	Paratyphi C (VI, VII)
IX	Gallinarum (IX, XII)	Typhi murium
IX, XII	Gallinarum (IX, XII)	.....
Vi	Typhi (IX, Vi)	Gallinarum + Typhi ("R")
X	Anatum (III, X, XXVI)	Newington (III, XV) + Minnesota (XXI, XXVI)
XI	Aberdeen (XI)	.....
XIII, XXII	Poona (XIII, XXII)	.....
XIV, XXIV	Carrau (VI, XIV, XXIV)	Paratyphi C (VI, VII)
XV	Newington	Anatum
XVI	Gaminara (XVI)	.....
XVII	Kirkee (XVII)	.....
XVIII	Cerro (XVIII)	+++ .....
XIX	Senftenberg	Paratyphi A + Newington
XX	Kentucky (VIII, XX)	Newport (VI, VIII)
XXI	Minnesota	Anatum
XXI, XXVI	Minnesota	.....
XXII	Poona	Worthington (I, XIII, XXIII)
XXIII	Worthington	Paratyphi A + Poona
XXIV	Carrau	Onderstepoort (I, VI, XIV, XXV)
XXV	Onderstepoort	Paratyphi A + Carrau
XXVII	Bredeney (I, IV, XXVII)	Derby (I, IV, XII)
XXVIII	Tel-aviv	.....

(*b*) Flagellar sera. Flagellar sera are obtained from rabbits by repeated intravenous injections of twenty-four hour cultures (in broth or saline) with 5 percent formol. Usually it is sufficient to make three injections of 0.5, 1, and 2 c.c. at intervals of three or four days. The standardization of a good flagellar serum should not be less than 1:20,000, calculated by slow agglutination and readings after four to six hours submersion in a water bath at 50° C.

It is important to use actively motile strains, acquiring these by means of frequent microscopic examinations of the hanging drop, thus assuring ourselves of an abundancy of flagellar antigens. Always, when possible, select monophasic types, but when the antigen, the sera of which is to be prepared, does not exist except in biphasic types, it is desirable to chose a strain which has a tendency to retain the phase sought for. For this, one must make the isolation of the colonies on plates of agar and pick from 10 to 15 of them on agar slants after twenty-four hours incubation. Then perform the rapid agglutination with the agglutinating sera for both phases, and select one which agglutinates only with the phase sought for. Repeat this process several times until it is certain that at least 90 percent of the colonies obtained remain in this phase, and inoculate them in tubes of broth to be used for rabbit injections. Even under such conditions, and when the chosen strain agglutinates only with the serum of a determined phase, its injection generally produces agglutinins for both phases. Therefore, it is advisable to saturate these sera, depriving them of the somatic agglutinins. To do this, it is preferable to use strains which have the same somatic and flagellar composition in one of the phases, which permits the usage of a lesser quantity of saturating bacteria. If one, for example, saturates an anti-paratyphi B serum in phase 1 (agent b), with S. typhi murium in phase 2, the somatic and flagellar agglutinins of phase 2 are absorbed at the same time.

The monophasic strains which possess only one flagellar antigen, as for example, S. typhi, do not offer difficulties for classification; but when one has to do with monophasic types of a complex flagellar composition, it is desirable to arrange the pure "sera agents," prepared by saturation in the same manner as are the somatic. The distinction, for example, between S. diblin (gp) and S. rostock (gpu) is easy, when regulated by serum "u." Some biphasic types are easily classified, which demonstrates that a definite antigen exists in one of the phases. The flagellar sera "6" and "7" permit the differentiation between the types S. london and the type S. give.

For these reasons it is preferable to work with pure factors notwithstanding that the usage of total flagellar sera has the advantage that they contain residual factors not included in the plan, which may be very useful as means of recognition of the relationship existing between a new type and a known salmonella.

We have employed the following sera:

Agent	*Serum*	*Saturated with*
a	Paratyphi A	Paratyphi A, heated to 100° C
b	Paratyphi B, var. Java	Typhi murium
c	Paratyphi C (phase 1)	Cholera suis, var. kunzendorf
d	Typhi	Eastbourne
eh	Newport (phase 1)	Muenchen (phase 2)
en	Mikawasima	Bareilly (phase 1)
enx	Amersfoort	Gaminara (phase 1) + S. oranienburg
f	Derby	Essen + Budapest
fg	Derby	Brandenburg
fgt	Berta	Eastbourne
gm	Enteritidis	Eastbourne
gms	Montevideo	Bareilly
gp	Dublin	Eastbourne
gpu	Rostock	Eastbourne
gq	Moscow	Eastbourne
gst	Senftenberg	Niolese
h	Eastbourne	Dar-es-salaam (phase 2)
i	Typhi murium (phase 1)	Paratyphi B (phase 2)
k	Thompson (phase 1)	Cholera suis, var. kunzendorf
lv	London (phase 1)	Anatum (phase 2)
m	Oranienburg	Senftenberg + Berta + Bareilly
mt	Oranienberg	Bareilly
n	Dar-es-salaam (phase 2)	Eastbourne (phase 1)
p	Dublin	Enteritidis
q	Moscow	Enteritidis
r	Heidelberg (phase 1)	Paratyphi B (phase 2)
s	Montevideo	Oranienburg + enteritidis
t	Oranienburg	Montevideo + enteritidis
u	Rostock	Dublin
v	Panama (phase 1)	Dar-es-salaam (phase 1)
w	Dar-es-salaam (phase 1)	Panama (phase 1)
x	Amersfoort (phase 2)	Mikawasima (phase 2)
y	Bareilly (phase 1)	Thompson (phase 2)
z	Poona (phase 1)	London (phase 2) + Poona heated to 100° C
z_4	Cerro	Cerro, heated to 100° C
z_6	Kentucky (phase 2)	Typhi murium (phase 1) + Thompson (phase 2) + Kentucky heated to 100° C
z_{10}	Glostrup (phase 1)	Mikawasima (phase 2) + Muenchen
z_{12}	Schleissheim	Abortus bovis (phase 1)
1, 2..	Paratyphi B (phase 2)	Abony (phase 1)
1, 3..	Newport (phase 2)	Chester (phase 1) + Glostrup
1, 5..	Cholera suis, var. Kunzendorf	Montevideo
1, 6..	Anatum (phase 2)	Chester (phase 1) + Anatum, heated to 100° C
1..7.	Gaminara (phase 2)	Amersfoort (phase 1) + Hvittingfoss

In order to obtain agglutinating factors "2," "3," "5," "6" and "7" in a pure state, it is necessary to saturate respective sera with mixtures of the remaining phases "2," for relationships not mentioned in the plan exist between them.

(*c*) Technique of serological classification. To prove rapid agglutination, we employ a glass plate of 25 x 50 centimeters, divided by lines 1 to 2 m. in width, in squares of 2 x 2 centimeters.

We begin by determining the somatic group to which the strain to be classified belongs, which we already know from a previous test agglutinates with a polyvalent serum. To do this, we deposit a thick drop of each of the somatic agents along one row of squares, writing with a pencil for glass writing in the square immediately above the symbol corresponding to the agent used. We add from 3 to 4 c.c. of physiological saline to the culture on the agar slant, which provides a sufficiently thick suspension for a development which is good. We then deposit a drop of suspension on each drop of serum and mix them with a platinum needle, gently shake the glass plate, and make the reading within a few minutes. In this manner one can determine the somatic group to which the strain belongs, to a sufficient degree to determine the existing flagellar antigens in the salmonellas belonging to the same group, thus avoiding the test of the strain in the presence of all the flagellar sera. Once one of the colonies obtained from the patient is classified, we can examine the rest of the colonies—generally from 15 to 20—testing them only in the presence of those sera which have given positive agglutinations. The process needs to be repeated only when more than one type of salmonella is encountered, or when a new type is found.

3. INFANTILE SALMONELLAS

Resumé of our experiments.

Here we are referring to the cases which we studied during the period between June 1, 1936 and May 31, 1940.

During these four years we studied 2,141 children who came, for the most part, from the hospitals of Montevideo and who ranged in age up to twelve years. Without previous selection we examined the feces of the hospitalized children, whatever might have been the illness for which they were hospitalized, and whether they had intestinal symptoms or not. In those cases in which we found salmonella in the feces, and from the year 1939 on, we practiced a systematic series of cultures of blood, urine, pharyngeal exudate, pus from the ear (when present), cerebral-spinal fluid (when meningeal symptoms were present), and also agglutination reaction with the serum of the patient in the presence of the salmonella or salmonellas met with. The total number of examinations was 5,798.

In 266 children (or 12.14% of the cases investigated) we isolated one or more species of salmonella. In all, 287 families, among which the following types were distributed:

S. typhi murium, 117; S. newport, 67; S. montevideo, 24; S. anatum, 17; S. derby, 16; S. oranienburg, 10; S. paratyphi B (of animal origin), 8; S. bredeney, 6; S. muenchen, 5; S. cholera suis (var. kunzendorf), 3; S. panama, 2; S. muenster, 2; S. minnesota, 2; S. senftenberg, 2; S. chester, 2; S. amersfoort, 3; S. newington, 1; S. carrau, 1; S. gaminara, 1. We found also a strain of a new type, still being studied, which we are calling S. arechavaleta, the abbreviated formula of which is IV, (V.), a–1.7. . . . .

Together with salmonellas we found in 10 cases dysentery bacilli (8 Flexner, 1 Sonne and 1 "Boyd 88"). Five times they were found to coexist in the feces. The rest were distributed as follows: Salmonella in the feces and dysentery bacillus in the urine, 1; salmonella in the pharyngeal exudate and dysentery bacillus in the blood culture, 1; salmonella in blood culture and dysentery bacillus in vaginal exudate, 1; salmonella in the feces and dysentery bacillus in pharyngeal exudate, 1. In a later case we isolated (during the life of the patient) salmonella in the feces, in the pus from the ear, the urine, and the blood; at autopsy, salmonella in the spleen and blood and dysentery bacillus from the lung.

In 15 cases we isolated more than one type of salmonella from the same patient; 12 cases had two types; 1 had three; 1, four; 1, five; we have recently observed more details of these cases, showing association of various salmonellas.

The materials in which we found the salmonellas mentioned were divided thus: feces, 269; urine, 19; blood, 15; pharyngeal exudate, 12; pus from the ear, 10; cerebrospinal fluid, 2; peritoneal pus, 1. It must be observed that the total of enumerated strains (328) is higher than the total of the types met with, which we have just mentioned (287). This apparent contradiction depends on whether, when we isolated from the patient the same type of salmonella in different materials, we considered it as the same species or not.

In relation to the months of the year, our cases were distributed as follows: In January, 49; February, 28; March, 49; April, 34; May, 8; June, 11; July, 6; August, 8; September, 5; October, 8; November, 20; December, 40.

examinations proved negative for both bacteria, we classified the case in the group of "unknown causes."

The relative importance of the etiologic factors mentioned as a cause of infantile mortality by enteritis is shown in the following table:

Mortality from Infantile Enteritis

	Salmonella			*Shigella*			*Causes Unknown*		
Ages	*Cases*	*Deaths*	%	*Cases*	*Deaths*	%	*Cases*	*Deaths*	%
0–1	46	14	30.43	70	32	45.71	157	47	29.29
1–2	21	3	14.27	54	14	25.92	43	10	23.25
2–3	11	0	0	20	1	5.00	10	3	30
3–12	11	0	0	19	0	0	8	1	12.50

Anginas and Otitis.

In numerous cases of infantile salmonelliasis we have observed the presence of a red angina, generally without exudate. We made a bacteriological examination of the pharyngeal secretion in 74 cases in which we had previously detected a salmonella in the feces, urine or other materials. Twelve times we found salmonellas in the pharyngeal secretion: S. typhi murium, 7; S. montevideo, 2; S. cholera suis, var. kunzendorf, 2; S. amersfoort, 1.

According to the intestinal involvements we classify 6 of these cases as enteritis, 5 as simple diarrheas, and the rest as septicemias.

The suppurating otitis medias as complications of infantile salmonelliasis are quite frequent. We examined the collected pus from the external auditory conduit, after puncturing the typanum in 30 cases, isolating salmonellas in 10 of them. In 2 cases the pharyngeal secretion was also positive on examination; in the remainder, negative. The types of salmonella were: S. typhi murium, 8; S. montevideo, 1; S. newport, 1. As to the pictures of intestinal clinical symptoms, they were: four enteritis; five simple diarrheas; one septicemia.

Meningitis. In two cases of meningitis, both fatal, we isolated S. typhi murium from the cerebrospinal fluid. In one of them the meningeal localization appeared as a complication of septicemia. In the other case, we discovered pulmonary tuberculosis at autopsy.

Septicemias. We practised hematological examination in 89 cases, isolating salmonellas in 16. In 5 cases the clinical picture was that of septicemia; 3, pure forms, without localization; one accompanied by enteritis, and the other, by simple diarrhea. In the remaining cases the development followed an irregular course, and in some there were signs of travelling bacteraemias accompanying different localizations; 3 presented enteritis and 7, simple diarrheas. In another case, however, we found no localization which could explain the finding of salmonellas in the blood.

Urine Examination. We made a bacteriological examination of the urine in 71 cases and isolated salmonellas in 19. The types were: S. typhi murium, 10; S. montevideo, 3; S. newport, 2; S. cholera suis, var. kunzendorf, 2; S. derby, 1. In one case we isolated S. typhi murium and S. montevideo together. The clinical pictures were: enteritis, 13; simple diarrhea, 2; septicemia, 3. In another case only the urinalysis showed positive result, apparently without any localization of the salmonella.

Other Localizations. In a recent publication, Del Campo and Aleppo[27] mention clinical observation of a case in which a suppurating peritonitis set in as a sequel to an apendectomy. From the peritoneal pus, S. typhi murium and a strain of colon bacillus were isolated. The agglutination reactions were positive for S. typhi. murium (H, 1/640; O-) and negative for the strain of colon bacillus, which led us to suppose that the causal agent was the salmonella. Following drainage, the child recovered.

CONCLUSIONS

We have concluded that infantile infections produced by salmonellas are very frequent in Uruguay, for we have encountered them in 12.14 percent (266 cases) of the 2,141 children up to the age of twelve years which we have studied.

At present, human salmonella infections of animal origin are practically synonymous with "food poisoning." However, in the case of children, we do not consider the expression appropriate, for such infections differ in many respects from the infections of adults.

The School of Kiel has the merit of having observed and tenaciously defended the epidemiological and pathological differences in human infections by salmonella, according as to whether they were of human or of animal origin. Since 1902, and during the following years, Fischer, Müller, Bitter, and Wagner, of the Institute of Hygiene of the University of Kiel, developed a series of works on their observations, classified in concepts called "The Kiel Doctrine," which is at present accepted (though not always by this name) by all authors who interest themselves in this subject.[28]

The salmonellas of animal origin, of which at that time S. typhi murium (or B. enteritidis breslaviensis, as authors called it) could only be recognized with reasonable accuracy, and the S. enteritidis (B. enteritidis Gärtner) possessed slight virulence for human beings. For this reason, the diseases originated by these organisms seemed more like an intoxication than a real infection, and so they were given the name of "alimentary intoxications" (*Nahrmittelvergiftung*—food poisoning).

The symptomatology of the clinical picture is very characteristic: it begins abruptly after 6 to 12 hours incubation (rarely longer), with frequent vomiting, numerous repeated stool movements, up to 40 in twenty-four hours; colic, tenesmus, a weak and rapid pulse, cyanosis, and an absence of red spots. The illness is located in the digestive tract. There is frequent, gastric catarrh, chiefly in the pure toxic type, and sometimes with purulent gastritis, intense inflammation, and small hemorrhages in the intestines, especially in the large intestine. The mesenteric ganglions and the spleen generally, appear of normal size, though sometimes slightly enlarged. The process generally continues for four or five days, occasionally up to ten, and convalescence is rapid. Complications are rare, especially septicemia, which generally appears in fatal cases a few hours before death. The mortality is low, accepting generally the percentage which oscillates between 1 and 3, and according to Gärtner, death, when it occurs, always takes place within six days of the onset.

The salmonella infections of human origin (the originators of the Kiel Doctrine allude especially to S. paratyphi B) are very different to the above, and the clinical picture is often confused with that of typhoid. The period of incubation is from four to six days, and the general onset is slow and progressive. In some cases, to whose pattern is attributed the presence of a great number of bacteria found in contaminated food, the clinical picture begins abruptly as in alimentary intoxication, and with profuse diarrheas. However, this is the exception, and when it occurs the evolutionary process becomes typhoidal after the first few days, occasionally after a period of normal temperature and abated or vanished intestinal symptoms. Generally neither vomiting nor diarrhea are observed, but on the other hand, there are, as in typhoid, red spots and splenomegaly; also, as in typhoid, the localization in Peyer's patches is the rule, and complications (abscesses, etc.) are by no means rare. There is also, usually, bacillemia, and the mean duration of the disease is about three weeks. Mortality is variable, according to different authors,

but according to unanimous opinion it is greater than in food poisoning and, lastly, according to Gärtner, death usually intervenes between the second and fourth weeks.

The epidemiology of the two forms—gastrointesinal and typhoidal—is, according to the Kiel School of thought, as different as its pathology. In the first, the cases are of alimentary origin and due to the contamination of different food products, especially meat, with salmonella of animal origin. The cases appear, consequently, as outbreaks, attacking a variable number of people who have eaten the same food, and who are stricken simultaneously on the same or the following day. Interhuman contagion is practically nonexistent, and persons who are in contact with, or who nurse patients, remain unaffected. Human "carriers," if they exist, are very rare. According to Gärtner, no case has been absolutely proved, and Savage[29] shares this opinion, because all demonstrations go to show that convalescents do not eliminate bacilli in the dejecta for a longer time than from three to four weeks. On the other hand, accidental carriers are probably frequent enough among subjects who, after having disposed of the infected food, show no symptoms. Typhoid cases do not generally occur simultaneously, but succeed each other one after another, through very rapid human contact from the sick to the sound. Permanent carriers are frequent.

From the bacteriological point of view, lastly, the School of Kiel insisted on characteristics which permitted differentiation between the agents of the two syndromes. It has been demonstrated that the administration of live bacteria *per os* to the rat produces a fatal septicemia in a few days, if the salmonella given is S. typhi murium or S. enteriditis, but, on the other hand, S. paratyphi B. is harmless thus administered. And, reversely, the first two bacilli produce in man the symptoms of alimentary intoxication, but not the typhoid syndrome; while S. paratyphi B. when administered to man produces a typhoid syndrome of so great a virulence that it demonstrates the general invasion of the organism.

The other remaining methods employed by the originators of the Kiel School of thought for the identification of the three types, especially the production of the "mucous barrier" by the S. paratyphi B., today have lost a great part of their significance, because the analysis of the antigenic receptors, based on agglutination, is much more easy and accurate, and has permitted the recognition of the existence of numerous types of salmonellas whose identification was formerly impossible.

There is no doubt that, as the Doctrine of Kiel now maintains, the human adult is highly resistant to salmonellas of animal origin, not only to S. enteriditis and S. typhi murium—to which it specifically refers—but also to the numerous types which have been identified lately in alimentary intoxications. In the human species the illnesses produced by the salmonellas are only accidental, comparable to what occurs when one ingests by accident some toxic substance, and there is no doubt that it is necessary to ingest a considerable quantity of salmonellas to produce symptoms. We know of several cases in which contaminated food was taken without producing any illness. Also, it has been recently observed that several people have partaken of the same meal, and only those who consumed large quantities of the infected material presented symptoms of intoxication. We have demonstrated in a personal experience[30] published in 1936 that the ingestion of S. typhi murium in doses of 2,000,000 to 4,000,000 of live bacteria produced in 4 out of 5 persons only a light fever without diarrhea, or diarrhea without fever; only one of our subjects had a high fever and bowel movements of 15 to 20 daily, for two days. The same doses of heat-killed bacteria occasioned no symptoms at all in another five persons.

Our observations on infantile salmonelliasis are not in accord with the Doctrine of Kiel because, in children, especially in those under one year of age, it is not possible to distinguish syndromes of typhoid from gastrointestinal disorders by clinical or epidemiological investigations only. The infantile salmonelliasis appear in one form or another, and the lower the age, the less marked the differentiation becomes. We believe then that the Doctrine of Kiel, exact enough in general terms as far as adults are concerned, ought to be completed and corrected when it refers to children, and take into account the etiological concepts which, because they originated in our city, are coming to be known as the Doctrine of Montevideo.

Let us consider, in the first place, the pathology of the infantile salmonelliasis. The beginning is frequently slow and progressive, as in typhoid forms, and although it sometimes begins as in alimentary intoxications with vomitings and diarrheas, there are generally premonitory symptoms. We have seen cases that began with sore throat, caused by salmonella infection, or with a fever of apparently inexplicable origin, and only after a few days did the attacks of diarrhea set in. Once this sets in, one notices that the localization corresponds to the lesions in the colon, as occurs in bacillary dysentery, and not to the marked syndromes with the liquid diarrheas of alimentary intoxication.

The duration of the illness is habitually longer than in adults, for it lasts three or four weeks, so that it seems, as one may expect, there exist long, drawn-out forms. On the other hand, the lesions are not exclusively enteric, but there may exist extra-intestinal localizations as already mentioned. Also, in the infant, enteritis is not always a necessary symptom; cases are seen with sore throats, otitis, septicemias, etc., produced by salmonellas, without diarrheas. Last of all, mortality is much greater than in alimentary intoxications, because it rises to 30 percent in cases of enteritis during the first year of life.

As to the epidemiology of infantile salmonelliasis, it is also different from that of alimentary intoxication. These cases do not come in epidemics, but sporadically, and can appear in a family, an isolated group of children, especially in nurselings, when their parents and the elder children remain healthy.

To summarize: In children the salmonelliasis present all the characteristics of true infection, many of them having much in common with typhoid forms, especially the facility with which they are contracted. We must reaffirm the fact which we have emphasized, and that is that the child, especially in the first year of life, is very susceptible to salmonellas of animal origin, much more so than the adult. The susceptibility of young individuals to the salmonellas is recognized in many species of animals. For example, the susceptibility of chickens recently hatched to white diarrhea caused by S. pullorum, in comparison with the immunity of the hen; and the epidemic influenza of pneumoenteritis character in calves, caused by S. enteritidis, whereas adult cattle remain immune.

Owing to this susceptibility of children to salmonellas it is certain that the infection may be produced as a consequence of the ingestion of a reduced number of living bacteria, and from this, the epidemiological course of the disease. What, at present, is impossible to determine, is the origin of the infection.

Let us say, in closing, that contrary to the findings in adults we believe the fact to be demonstrated that salmonelliasis in infants is an infectious, contagious disease, and presents all the symptoms and signs of a true infection, with progressive course, different localizations and a high mortality.