

# IMMUNOLOGICAL INVESTIGATIONS ON TROPICAL SPRUE IN PORTO RICO

## VI. A STUDY OF THE AEROBIC FLORA OF THE STOOLS OF CASES OF TROPICAL SPRUE

## VII. AGGLUTININS IN THE BLOOD IN TROPICAL SPRUE FOR ORGANISMS OF THE COLON-TYPHOID-DYSENTERY GROUP

By O. COSTA MANDRY, M. D., C. T. M.

From the School of Tropical Medicine of the University of Porto Rico under  
the auspices of Columbia University

The present communication forms part of a series of studies on tropical sprue in Porto Rico carried out at the School of Tropical Medicine with the object of establishing if possible the factor or factors concerned in the production of the disease.<sup>1</sup>

The prominence of intestinal disturbances in the common picture of the disease, early suggested the possibility of a bacterial infection localized somewhere in the alimentary tract, and various investigators have made bacteriologic studies of the stools of sprue patients in the hope of discovering the etiologic agent.

Thin,<sup>2</sup> isolated a number of micro-organism from sprue stools and remarked on the apparent scarcity of *B. coli* among them. Castellani described a "Flexner-like bacillus" which he isolated from the feces of three cases presenting sprue symptoms and whose sera agglutinated the organism. Musgrave isolated *B. coli* in pure culture from the heart's blood, but it is quite possible that the organism may have been merely a secondary invader. Faber in an autopsy performed in Copenhagen isolated a diplococcus from the intestinal mucus, the peritoneal exudate, and the heart's blood but the organism proved to be nonpathogenic for guinea pigs. Justi found in the stools a Gram-positive bacillus which was cultivated later by Beneke and Ungermann, but no data regarding the pathogenicity of the organism for animals was ever published.

Bahr found a scarcity of *B. coli* in the stools of nine sprue cases. In one instance he isolated in pure culture a dysentery-like Gram-negative, non-motile bacillus culturally similar to the dysentery bacillus but not agglutinated by the patient's serum or by polyvalent anti-dysentery serum. A Gram-positive diplococcus was isolated in four of the cases. This proved to be either *Streptococcus fecalis* or

<sup>1</sup>The remaining papers of this series already published or in press are listed in the appended bibliography.

<sup>2</sup>Quoted from Bahr.



*Streptococcus brevis*. Bahr also noticed an undue preponderance of Gram-positive bacilli in sprue stools.

In 1914 Distaso reported a scarcity of *B. coli* in sprue stools but many organisms of the lactis aerogenes and Friedlander groups. Schuermann and Fellmer in 1917 isolated from the feces of a case of sprue a diptheroid bacillus which agglutination tests showed to be closely allied to the xerosis bacillus. In 1926 Gore in a bacteriologic study of 200 feces from persons in Ceylon, including 47 cases of clinical sprue, reached the conclusion that the aerobic bacillary flora of sprue stools resembles that of dysentery stools.<sup>3</sup> In view of these inconclusive reports it seemed to us worth while to investigate the problem further; and to this end a study of the aerobic flora of the feces in sprue has been made. We have at the same time tested the blood of sprue patients for immune agglutinins for organisms of the dysentery and related groups.

#### VI. A STUDY OF THE AEROBIC FLORA OF THE STOOLS OF SPRUE CASES

##### *Procedure:*

The stool was planted as soon after passage as possible. A definite amount (approximately 0.5 gm.) both for soft and liquid stools was taken, and this was emulsified in 5 c.c. of normal saline and allowed to stand for 15 minutes. The supernatant fluid was then plated on each of two plated of Endo, McConkey, Levine eosine-methylene blue, and blood agar plates (pH 7.6). If mucus was present in the stool, shreds were picked, washed three times in saline, and seeded on one half of the plates, the remaining half being seeded with the supernatant fluid of the original emulsion. The plates were incubated 24 hours. The colonies were then studied and 20-30 were picked in each case and seeded on tubes of Russell's double sugar. The organisms obtained were then studied as to morphology, motility, Gram stain, hemolysis or coloration on blood agar media and their behavior on various carbohydrates and other differential media. All organisms suspected of belonging to the Salmonella or Eberthella groups were tested with the corresponding sera. No attempt was made to cultivate yeast budding fungi, but when these developed on the media used (pH 7.4 to 7.6), they were studied and identified.

##### *Findings:*

Table I shows the findings in the feces of 20 sprue patients with a detailed account of all organisms isolated from each stool, Table II shows the finding in stool of normal control cases.

<sup>3</sup> Quoted from Fairley, Mackie *et al.*







**Discussion:**

The results obtained from the bacteriologic examination of the stool of sprue patients were compared with those previously obtained by the author from a study of normal feces in connection with a bacteriological study of dysentery in Porto Rico. No marked differences were observed. The findings of *Lactobacillus acidophilus* in two cases of sprue can be explained by the fact that these patients were on a strict milk diet and had taken acidophilus milk. Our experience in regard to the presence of *B.coli* in the feces of sprue cases does not coincide with that of others. We were able in every case to isolate with relative ease organisms belonging to the Coli (*Escherichia*) group. In 55 per cent of the stools aerobic spore bearing Gram-positive motile bacilli were present.

Organisms belonging to the Eberthella group and resembling culturally and morphologically dysentery bacilli but differing serologically were encountered in some instances. However, similar organisms were also found in the control cases. No true dysentery bacilli were found in any of the cases examined. Staphylococci, streptococci and various other organisms were encountered in some of the stools.

**VII. AGGLUTININS IN THE BLOOD OF SPRUE PATIENTS TO ORGANISMS OF THE COLON-TYPHOID-DYSENTERY GROUP**

Thirteen antigens were prepared as listed below, using the technique of Dryer. Eleven of the antigens were tested against homologous sera and all against normal human and rabbit sera in different dilutions. The following is a list of the organisms and the number of strains used in each case in preparing the antigen.

Antigens	No. of Strains
Eberthella dysenteriae Shiga .....	4
Eberthella paradysenteriae Flexner .....	7
Eberthella paradysenteriae Hiss R.....	3
Eberthella paradysenteriae Mt. Desert.....	1
Eberthella paradysenteriae Sonne A.....	2
Eberthella paradysenteriae Sonne B.....	1
Eberthella typhi.....	2
Salmonella schottmulleri .....	3
Salmonella paratyphi .....	1
Salmonella suipestifer.....	2
Salmonella enteritidis.....	2
Escherichia coli .....	4
Bacillus proteus.....	4



*Technique:*

The microscopic slide method was routinely used, being checked in all positive or suspicious cases by the test tube macroscopic test.

The dilutions of serum used for the microscopic test were 1:15, 1:30 and 1:60 and the results were read after one hour at 37°C. With the test tube method the dilutions used were 1:10, 1:30, 1:60 and 1:120. The tubes were placed in a water bath at 37°C. for two hours and then placed overnight on the ice box. The next morning results were read and recorded. Specimens of blood from 25 cases of sprue and of 33 normal individuals picked at random were tested. The 25 cases of sprue were divided into very severe, moderately severe, mild, and recovered cases.

*Findings:*

All final results are recorded in Table III. The results obtained from the microscopic test and the microscopic test tube methods were practically the same.

TABLE III  
FINAL RESULTS OF AGGLUTINATION TESTS

	Sprue (25 cases) Per cent positive	Control (33 cases) Per cent positive
Eberthella dysenteriae Shiga . . . . .	0	0
Eberthella paradysenteriae Flexner . . . . .	0	0
Eberthella paradysenteriae Hiss-R . . . . .	0	0
Eberthella paradysenteriae Sonne A . . . . .	0	0
Eberthella paradysenteriae Sonne B . . . . .	0	0
Eberthella paradysenteriae Mt. Desert . . . . .	0	0
Salmonella enteritidis . . . . .	8	3
Salmonella suipestifer . . . . .	8	0
Salmonella paratyphi . . . . .	12	5
Salmonella schottmulleri . . . . .	4	6
Eberthella typhi . . . . .	8	3
Escherichia coli . . . . .	36	30.7
Bacillus proteus . . . . .	0	3

*Discussion:*

Comparing the results obtained in the sprue cases with those of the control, one finds very slight differences. In both series there were cases in which agglutinins for typhoid or paratyphoid bacilli were present; but this can be explained on the basis of previous attacks of the disease and prophylactic vaccination. Specimens of sprue blood showed in two instances agglutinins for *B. enteritis* and



*B. suispestifer* but at the same time the blood agglutinated paratyphoid bacilli in even higher dilutions. It is therefore reasonable to assume that the agglutinins for *B. enteritidis* and *B. suispestifer* were only group agglutinins. No absorption tests were made. In no cases did we find agglutinins against any of the dysentery bacilli.

*Summary and Conclusions:*

The results obtained from a bacteriologic study of the aerobic flora of the stools of 20 sprue cases and from a study of agglutinins in the blood of 25 sprue cases for organisms of the colon-typhoid-dysentery group are presented. No attempt has been made to investigate the intestinal fungi.

The findings in sprue cases do not differ markedly from those of normal controls. The aerobic bacterial flora in stools of sprue cases is varied and consists of organisms of the *Escherichia (coli)* and *Aerobacter (aerogenes)* groups, spore bearing aerobic bacilli, and at times organisms of the *Eberthella*, *Alcaligenes* and other groups. In no case did we find agglutinins for dysentery bacilli in the blood of sprue patients.

I wish to express my thanks and appreciation to Dr. Charles Weiss, for his valuable aid and advice, to Dr. B. K. Ashford, for identifying the fungi encountered during the work, and to Miss G. Monge for technical assistance.

REFERENCES

- BAHR, P. H. A Report on Researches on Sprue in Ceylon, 1912-1914 London, 1915. Cambridge University Press.
- CASTELLANI, A. J. Trop. Med. & Hyg., 1912, pp. 337. (Quoted from Bahr.)
- COSTA MANDRY, O. Observaciones Preliminares sobre Estudios de Dienteria en Puerto Rico. Bull. P. R. Med. Assoc., 1928, xxi, No. 160, 11-21.
- DISTASO, A. Sur l'Étiologie de la Sprue. Bul. Soc. Path. Exot., 1914, vii, 268-270.
- FABER, K. Tijdsaeftde of Chronisch Tropicdiarrhoë met Anatomisch Undersogelse of Tordojelseskanalen Hospitalstidende, 1904, xlvii, 313 (Quoted from Bahr.)
- FAIRLEY, H. N.; MACKIE, F. P. *et al.* A Progress Report on Researches in Sprue, 1924-25. Indian J. Med. Research, 1926, xlv, 105-123.
- JUSTI, K. Beitrage zur Kenntniss der Sprue. Arch. f. Schiff's u. Tropen-Hyg., 1913 xvii, Beiheft 10, 5-53. (Quoted from Bahr.)
- MUSGRAVE. Sprue in Manila. Am. Med., 1902, iii, 389, 429. (Quoted from Bahr.)
- SCHUERMANN, W.; FELLNER, T. A contribution to our knowledge

of Sprue. Zeitschr. f. Hyg. u. Infektionskr 1916. lxxxi, 432-443.  
(Abst. Trop. Dis. Bull., 1917, x, 299.)

WEISS, C.; COSTA MANDRY, O. Immunological Investigations in  
Tropical Sprue In Porto Rico.

V. Allergic Skin Reactions to Various Food Preparations. J.  
Immunol., 1928, (In Press).

WEISS, C.; LANDRON F. Immunological Investigation in Tropical  
Sprue in Porto Rico.

I. The Monilicidal Activity of Blood.

II. Skin Reactions with Toxins of Various Monilia.

III. Attempts at Transmission of Sprue to Monkeys and Human  
Volunteers.

Amer. Jour. Trop. Med. 1928, viii, (In press.)

WEISS, C.; LANDRON, F. Immunological Investigations in Tropical  
Sprue in Porto Rico.

IV. Studies on the Biology of Monilia psilosis in Relation to Sprue.  
J. Infect. Dis., 1928, xliii, (In press.)