

## STUDIES OF DIARRHEAL DISEASE IN CENTRAL AMERICA\*

### VII. TREATMENT OF PRESCHOOL CHILDREN WITH PAROMOMYCIN AND SULFAMETHOXYPYRIDAZINE UNDER FIELD CONDITIONS IN A GUATEMALAN HIGHLAND VILLAGE

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Acute diarrheal disease maintains its importance in less developed regions largely because of its serious effect on infants and young children of the early preschool years.<sup>1</sup> This in turn is attributable mainly to prevailing malnutrition which interacts synergistically with infection to produce a disease of exaggerated severity, protracted course and high fatality.<sup>2</sup> The accompanying deficiencies in environmental sanitation<sup>3, 4</sup> result in an incidence of such extent that most young children in developing regions experience at least one episode each year. In some localities, the average is as great as 3 or 4 and some children have 10 or more. Rather universally, the disease attains its greatest significance in the first and second years of life, related in many instances to the risks attendant on the weaning process after an initial breast feeding.<sup>5</sup>

Because of the high prevalence of both infection and malnutrition in the central highlands of Guatemala, the Institute of Nutrition of Central America and Panama (INCAP) conducted continuing field studies of diarrheal disease in predominantly Mayan Indian villages from 1959 to 1964. The studies were directed primarily to the important age group of preschool children, those less than 5 years of age.

An organized program of medical care in the village of Santa María Cauqué, population 1025, included such public health measures as control of water supply, building of privies, immunization against childhood diseases, and a clinic for medical treatment of illnesses. It did not

include distribution of water to individual houses or nutritional measures. The village of Santa Cruz Balanyá, population 1459, in the same general area had only the extremely limited facilities for public health and medical care of the usual Guatemalan highland village.

From October 1960 to December 1961, 535 children with acute diarrheal disease under 5 years of age were treated in Santa María Cauqué with paromomycin, sulfamethoxypyridazine, or a combination of the two, along with measures for rehydration and nursing care through home visits by a qualified nurse. During the same period, 266 patients of the same age group were observed in Santa Cruz Balanyá. This report compares the results obtained in the two situations.

#### MATERIAL AND METHODS

Santa María Cauqué had 206 families. They were divided randomly into three equal groups, with one or the other of the two drugs or their combination used for a group. All cases of diarrheal disease in a family received the same drug treatment until 15 days had passed with no member having had the disease. The family then passed into the next treatment group in the order: paromomycin, sulfamethoxypyridazine, and their combination. During the study period, all families at one time or another received each of the three types of treatment.

For the purposes of this study, diarrheal disease was defined as an illness with at least five liquid or semiliquid stools within 24 hours in children under one year of age, and three or more for children over one year. Cases with blood or mucus in the stools were classed as severe. After a case was established as diarrheal disease, a history was taken, a physical examination performed, and a record made of weight, temperature, skin-fold thickness, and clinical nutritional status.

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After February 1961 rectal swabs were obtained from all patients in Santa María Cauqué and rectal swabs or samples of feces from as many contacts as possible. Fecal specimens were examined for enteric bacterial pathogens by the methods described in previous studies.<sup>6</sup> Laboratory examinations were not made in Santa Cruz Balanyá.

Treatment in Santa María Cauqué was through a general medical clinic attended daily by a physician and a nurse. Patients came directly to the clinic or, if the severity of illness so demanded, were visited at home. Case finding in both villages was through continued home visits by two field workers, supplemented by a committee of village women who undertook the responsibility of reporting all known cases in their particular neighborhood. In addition, and irrespective of known presence or absence of diarrheal disease, each home was visited every two weeks.

The primary emphasis in medical management was on rehydration. Mothers were given specific advice by the physician or nurse on means to accomplish this with materials available in the home. The assurance of a satisfactory result was a special concern at home visits by field workers and professional staff. When dehydration remained uncontrolled, oral or intravenous rehydration was instituted at the clinic. General supportive measures and nursing care were a second consideration, especially the need to continue feeding during the attack in place of the dietary restrictions so universally practiced in the village culture. Drug treatment was additional.

Dosage for paromomycin was 25 mg/kg of body weight per day in two doses for five full days, and longer if necessary, up to 10 days. For sulfamethoxypyridazine, amounts were 40 mg/kg of body weight per day in two doses for the first day, 20 mg/kg the next 4 days and, if necessary, continued up to 10 days. Dosages were the same when the two drugs were used together. Each drug treatment was administered at the clinic by the nurse to assure that the drug was actually taken. If the patient did not return as scheduled, the nurse made a home visit. Only under rare circumstances was a patient given the drug to be taken at home in the absence of direct supervision.

Bacterial cultures isolated from feces were first

confirmed for purity, identity, and serotype.<sup>7</sup> *In vitro* tests for drug sensitivity were made with the growth in trypticase soy broth, incubated 18 hours at 37°C. The number of bacteria used, as estimated by plate count dilution, was approximately  $2.5 \times 10^5$  bacilli. Paromomycin (5, 10, 30  $\mu$ g), and sulfamethoxypyridazine (50, 150, 300  $\mu$ g) were tested for sensitivity by the single layer disk technique in the amounts noted. The medium used was Mueller-Hinton agar with added 10% human citrated blood. A zone of inhibition of 14-mm diameter or more after incubation for 18 hours at 37°C indicated sensitivity. With sulfamethoxypyridazine, the presence of a weak overgrowth in the zone of inhibition was not interpreted as indicative of resistance.

#### RESULTS

Of 535 cases of acute diarrheal disease treated with the two drugs or their combination, 17.2% were clinically severe, as were 20.5% of the untreated cases of Santa Cruz Balanyá. A recognized bacterial pathogen was demonstrated in 18.2% of the 417 cases examined in Santa María Cauqué. *Shigella* was the most common (16.3%) with enteropathogenic *Escherichia coli* far less frequent (1.9%). Enteroviruses were equally frequent (about 40%) in children of Santa María Cauqué with and without diarrhea. While patients in Santa Cruz Balanyá were not examined microbiologically, the stated frequencies of bacterial pathogens for Santa María Cauqué have been confirmed in studies of numerous other villages in this general region.<sup>3</sup>

Treatment was initiated in 69% of cases within the first three days of illness, in 24% during the remainder of the first week, in 6% during the second week, and in 1.5% after 15 days. Cases were demonstrated as representatively distributed according to age, severity, time of onset, by whether or not an infectious agent was isolated or no culture made, as well as by drug treatment used.

Neither drug nor the combination of the two, when used in the treatment of acute diarrheal disease under the stated conditions, resulted in significant differences in total duration of diarrhea, which is to say the sum of days before and after treatment, compared with one another or in relation to the series as a whole. For all cases in Santa María Cauqué, 13.5% lasted 3 days or

TABLE 1

*Total duration of acute diarrheal disease, in days, Santa María Cauqué and Santa Cruz Balanyá, Guatemala, October 1960–December 1961*

| Duration (days)    | Santa María Cauqué (treated) |      | Santa Cruz Balanyá (untreated) |      |
|--------------------|------------------------------|------|--------------------------------|------|
|                    | No.                          | %    | No.                            | %    |
| 0–3                | 72                           | 13.5 | 23                             | 8.6  |
| 4–7                | 257                          | 48.0 | 202                            | 75.9 |
| 8–14               | 142                          | 26.5 | 38                             | 14.4 |
| 15+                | 64                           | 12.0 | 3                              | 1.1  |
| Mean days duration | 535                          | 8.5  | 266                            | 5.9  |

less, 48.0% for 4 to 7 days, 26.5% for 8 to 14 days, and 12.0% for 15 days or more (Table 1), an average of 8.5 days. The duration was greater ( $P < 0.01$ ) than for untreated cases in Santa Cruz Balanyá. As Table 1 shows, diarrheal disease in Santa Cruz Balanyá lasted 3 days or less in 8.6% of cases, 4 to 7 days in 75.9%, 8 to 14 days in 14.4%, and 15 days or more in 1.1%, an average of 5.9 days. For treated cases, duration was least in children aged 2 years, 7.4 days, and greatest, 9.1 days, for those one year old. For untreated patients, it was least for those one year old, 5.3 days, and greatest for 2-year olds, 6.9 days. Although incidence was greater in the village where treatment was given, the proportion of severe cases as determined on initial examination was somewhat lower. Death rates in both villages were higher than in preceding experience. The longer duration of illness in the treatment village was evidently not due to a greater initial severity of disease among the treated.

The 417 cases examined bacteriologically in Santa María Cauqué were divided according to demonstrated *Shigella*, enteropathogenic *Esch. coli*, the undifferentiated diarrheas having no demonstrated bacterial agent, and 118 cases not examined. Drug treatment resulted in no difference in subsequent days of duration of diarrhea between cases of shigellosis, those of the cultured but undifferentiated group, and those not cultured. Cases of enteropathogenic *Esch. coli* infection were too few for an opinion. Cases thus are presented in Table 2 according to treatment used and for all forms.

Median duration for all three treatments was the fourth day; it was reached first by cases treated with the combination of drugs, next by those receiving the antibiotic and last by those receiving the sulfonamide, but with no statistical differences. The proportion of cases continuing in excess of one week followed the same order. For confirmed shigellosis, the median duration was also four days, and again slightly better, but not significantly so, for the combination and antibiotic programs than for the sulfonamide.

Duration of treated severe cases, those having blood or mucus in stools, was compared with that of all other clinical forms, one or other of the three treatments being used; treatments were grouped in view of the results just stated. The median for severe cases was on the fifth day (4.3 days) and for those with the less severe reaction on the fourth day (3.3 days), but the difference was not statistically significant.

The effect of early as compared with later institution of treatment was not striking. Of the 369 cases treated within the first 3 days of illness, 36% had a subsequent persistence of diarrhea for 1 to 3 days, 45% for 4 to 7 days, 15% for 8 to 14 days, and 3% for 15 days or more. The corresponding frequencies for the 126 first treated on the fourth to seventh day were 31%, 41%, 18% and 10%. For 32 cases with late institution of treatment, on the eighth to fourteenth day, diarrhea ended within the following week in 50%, 19% cleared within the second week and for 31% of patients the diarrhea continued for the next 15 days or more. Of the 8 patients first treated after the fifteenth day of active disease, 5 still had diarrhea 15 days later.

Deaths from diarrheal disease during the period of observation were relatively few, 2 in the treated group, and 5 in the untreated. The mortality was 7.7 per 1,000 children aged 0–5 years per year in Santa María Cauqué where treatment was practiced, and 17.3 in Santa Cruz with no treatment. The general death rates, however, were expectedly greater in Santa Cruz, 87.5 per 1,000 children aged 0–4 years per year, compared with 54.3 in Santa María Cauqué, with the result that diarrheal deaths accounted for 20% of all deaths in that village and 14% in Santa María, a difference not mathematically different because of the small numbers. Over the 9 years preceding the study, the ratio of deaths from diarrheal disease to all deaths in Santa María

TABLE 2

*Days duration of diarrhea after antibiotic and chemotherapy, all treated cases, Guatemala, October 1960–December 1961*

| Days duration after treatment | Paromomycin |       | Sulfamethoxypyridazine |       | Combination of antibiotic and sulfonamide |       | All treated cases |       |
|-------------------------------|-------------|-------|------------------------|-------|---|-------|-------------------|-------|
|                               | No.         | %     | No.                    | %     | No.                                       | %     | No.               | %     |
| 1                             | 5           | 2.6   | 5                      | 2.3   | 2   | 1.6   | 12                | 2.2   |
| 2                             | 15          | 7.7   | 15                     | 6.8   | 15  | 12.3  | 45                | 8.4   |
| 3                             | 36          | 18.5  | 53                     | 24.2  | 30  | 24.6  | 119               | 22.2  |
| 4                             | 50          | 25.8  | 35                     | 16.0  | 35  | 28.7  | 120               | 22.5  |
| 5                             | 27          | 13.9  | 30                     | 13.7  | 13  | 10.7  | 70                | 13.1  |
| 6                             | 8           | 4.1   | 15                     | 6.8   | 5   | 4.1   | 28                | 5.2   |
| 7                             | 4           | 2.1   | 10                     | 4.6   | 3   | 2.4   | 17                | 3.2   |
| 8–14                          | 30          | 15.5  | 42                     | 19.2  | 14  | 11.5  | 86                | 16.1  |
| 15+                           | 19          | 9.8   | 14                     | 6.4   | 5   | 4.1   | 38                | 7.1   |
| Total.....                    | 194         | 100.0 | 219                    | 100.0 | 122                                       | 100.0 | 535               | 100.0 |

was 15%. During the study period, it was 14%. The usual greater ratio of diarrhea deaths to all deaths in Santa María than in Santa Cruz was reversed in the year 1961, as noted above, but this had happened three other times in the 9 years. The ratio returned to its usual status during the subsequent two years and markedly so, although drug treatment continued.

For the 9 years before the program of prevention and treatment was introduced into Santa María, the crude death rate averaged 71.3 per 1,000 children aged 0–4 years per year, and for the 5 years of the program it was 48.8. In Santa Cruz the decline for the same two periods was from 114.8 to 97.3, a lesser proportion. The rates for diarrheal deaths in Santa María were 8.6 per 1,000 per year in the pre-treatment period and 11.2 thereafter; in Santa Cruz, for the equivalent years, they also rose from 11.6 to 17.5. In neither instance was the general improvement reflected in the diarrheal death rates.

Paromomycin, as used in the field trials, was strongly active *in vitro* against *Shigella* of all serotypes encountered. The 203 strains examined were about equally divided between those isolated from patients and contacts in the village during the time the drug was used, and another lot isolated from cases of acute diarrheal disease or their contacts in communities where the drugs had never been used. With a single exception, all strains irrespective of source or serotype were sensitive to 5- $\mu$ g disks of paromomycin. The one resistant strain was *Sh. dysenteriae* 1, from the treatment village, a serotype never before en-

countered in diarrheal disease of the Guatemalan highlands.<sup>8</sup> Thirty-four of 36 strains representing 9 serotypes of enteropathogenic *Esch. coli* from cases and carriers of the treatment village were sensitive to paromomycin.

When the same 203 strains of *Shigella* were tested against sulfamethoxypyridazine, 61% were sensitive to the sulfonamide (50- $\mu$ g disks), rather less than the usual proportion for a sulfonamide. Of the 124 sensitive strains, the inhibition zone was clear for 25, while 99 strains showed slight overgrowth, more frequently with older cultures than with those recently isolated. The one strain of *Shigella* resistant to paromomycin was also resistant to the sulfonamide. Enteropathogenic *Esch. coli* from cases and carriers of the treatment village were also more frequently resistant to the sulfonamide than to the antibiotic in 8 of the 36 strains examined.

#### SUMMARY

An antibiotic, paromomycin, and a sulfonamide, sulfamethoxypyridazine, were used as an added therapeutic measure to rehydration and nursing care in treatment of 535 patients with acute diarrheal disease, aged less than 5 years, under field conditions in a less developed region of the Guatemalan highlands. About 18% of 417 cases examined bacteriologically were associated with a recognized bacterial pathogen; about 82% were not. *Shigella* was found in 16% of cases.

Under the conditions of the trial, treated cases of one village had a longer clinical course and

duration than did untreated cases observed in a nearby community during the same time period. Patients there received no medical care other than family nursing and the usual folk remedies. Duration of disease in patients with shigellosis could not be distinguished from that of patients with undetermined diarrheal disease. No differences according to the three methods of treatment were discernible.

The general death rate from all causes was lower in the village where general medical care was provided. Deaths from acute diarrheal disease were relatively few in both situations. The observed differences in annual mortality from diarrheal disease in the two villages, 7.7 per 1,000 children aged 0-4 years in the treated, and 17.3 in the untreated, community, represented 14% of all deaths in the treated village and 20% in the untreated. The more favorable rate for treated cases is believed to have been due mainly to general supportive measures during the illness, especially rehydration and nursing care. The effect of antibiotic and chemotherapy cannot be entirely discounted.

The antibiotic paromomycin employed in the present trial proved highly effective *in vitro* against *Shigella*, but not in the clinical tests, as judged by duration of diarrhea in days after treatment. The sulfonamide was effective against only 61% of *Shigella* strains *in vitro*, and the somewhat lesser clinical result when that method of treatment was used is in accord.

*Escherichia coli* strains were sensitive to both drugs, and as with *Shigella*, more so to the antibiotic than to the sulfonamide. The few treated patients precluded evaluation of clinical effect.

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