Newer Procedures in Laboratory Diagnosis and Therapy in the Control of Bacillary Dysentery*

ALBERT V. HARDY, M.D., DR.P.H., SURGEON (R) AND JAMES WATT, M.D., DR.P.H., SURGEON

Division of Infectious Diseases, National Institute of Health, U. S. Public Health Service, Washington, D. C.

There has been recently a renewed concern for the acute diarrheal diseases and, concurrently, a substantial advance in the knowledge of these disorders. It is the purpose of this report to review this newer knowledge as related to the problem of control.

GENERAL OBSERVATIONS

Rational control of disease rests upon reliable diagnoses. To obtain these in the diarrheal diseases, a revision is needed of the prevailing concept of the clinical characteristics which warrant a consideration of Shigella infections. In our clinical series of 555 proven positive cases, the most frequent manifestation was a "simple diarrhea." The classical bloody mucoid stools were notably infrequent; indeed, these scarcely entered into the clinical picture of Sonne, Schmitz, or mild Flexner infections, and were not a prominent feature in severe Flexner cases. Also the onset was marked quite frequently by a prominent fever but only mild enteric symptoms, suggesting "flu" rather than dysentery. The very name bacillary dysentery accounts for many of the missed diagnoses. A designation referable to etiology and freed of its present misleading clinical implications would be preferable. We therefore use and recommend the name "shigellosis" for all infections due to pathogenic Shigellae. Thus shigellosis is to be thought of as a possible, and in many areas as the most probable, diagnosis in the common variety of acute endemic diarrhea and in an uncertain proportion of cases so loosely designated "intestinal flu."

The manifestations of shigellosis vary widely. In the young the illnesses are frequently grave, occasionally fulminating, whereas in older children and adults the disorders are usually mild. Furthermore, in the young, Shigella infection ordinarily results in definite illness, while in older children, adolescents, and adults very mild disorders and subclinical infections are much more common than significant clinical disease. Furthermore, regardless of age of the patient or severity of the disease, infection usually persists beyond clinical recovery. The duration of the convalescent carrier state is commonly three to four times that of the total duration of symptoms. For control, due attention must be given to carriers, convalescent and passive, and...
to cases with disturbances of little clinical significance.

*Shigellae* may be spread in various ways; the relative importance of these must be weighed in formulating control procedures. We found no evidence, in our own experience, which suggested water-borne infection. Milk was notably free of suspicion, since the poor, who suffer most from diarrheal diseases, generally purchase the less expensive canned or dried product. There was some evidence of spread through contaminated food, but this appeared of minor rather than major importance. Where it did occur, usually multiple carriers were found among the food handlers and kitchen helpers. The disease and flies did thrive under similar conditions. Still, cultural evidence suggested that flies rarely harbor viable *Shigellae*. We isolated this organism from flies only once in repeated trials. In contrast we recovered these pathogens with comparative ease from the fingers or from under the finger nails of known cases and carriers. Considering all evidence we are of the opinion that *Shigellae* are transported from group to group through the movement of infected individuals. Within the household and in other groups living together the organisms are most commonly passed from person to person through direct or indirect contact. Among institutional inmates the negative evidence excluding other channels of spread was strikingly clear in several outbreaks. Hence the control of infected individuals (including personal cleanliness) is of much greater importance in the prevention of bacillary dysentery than has been believed.

Explosive epidemics of shigellosis suggesting a common source and single exposure are rare. Ordinarily, an epidemic starts with sporadic infections which gradually become more numerous, the early cases preceding the recognized outbreak by 2 to 4 weeks or more. The total prevalence, which increases gradually, usually reaches a level of between 10 and 33 per cent. Total incidence of primary attacks (clinical and subclinical) in unmodified outbreaks may approach .100 per cent and counting reinfections has gone even beyond this. Clinical cases, which are most numerous early in the outbreak, decline promptly following the peak of the epidemic, but the total prevalence decreases slowly because of persisting convalescent and passive carriers. The duration of an unmodified outbreak is weeks rather than days. Obviously, throughout this prolonged period a rich source for the spread of infection is maintained. The slow evolution of *Shigella* epidemics provides the need and the opportunity for the application of control measures.

**LABORATORY PROCEDURES**

The advances here have resulted in a simplification of bacteriological technics and a marked increase in the reliability of cultural tests. The development and introduction of the highly selective media which grow the enteric pathogens while inhibiting almost all the non-pathogens was of major importance. Earlier we used desoxycholate-citrate agar, now we employ S. S. (Shigella-Salmonella) agar which has grown satisfactorily all strains of *Shigellae* encountered. In the study of known *Shigella* outbreaks this culture medium only is used for primary plating. A heavy fecal inoculation may and should be employed; to obtain this the usual cotton-tipped applicator coated with feces is satisfactory. This makes it convenient and practicable to collect specimens by rectal swabs and to inoculate the plate directly by “painting” the entire surface of the agar with the swab. The device for taking the rectal swab which we recommend is a small rubber tube large enough to contain the cotton-tipped applicator. The
distal end is cut on a bevel and its external surface is lubricated before use. The applicator alone can be used for infants; in adults, also it may be inserted with little discomfort if the cotton tip is moist. The collection of fecal material for culture by this method involves only the skill required in taking rectal temperatures. The whole procedure is so simple that the responsibility for obtaining and plating of cultures can be entrusted (following instruction and demonstration) to a nurse, attendant, or technician.

This procedure opens a new approach to control. It is now practicable to culture large numbers of individuals for the identification of carriers of Shigellae. The plates, prepared and numbered, the sterile swabs, lubricant, and record sheets are taken from the laboratory to the group to be cultured. There the responsibility is divided, one person taking the swabs, a second inoculating plates, a third writing names and culture numbers, and one or more preparing the patients. When the latter are ambulatory, cultures are taken at one place, the patients coming in line, bending over while the swab is being obtained, and leaving by a separate exit. If the work proceeds smoothly, cultures can be taken at about the rate of 200 per hour. It requires more time to handle patients in bed where the materials must be taken from ward to ward. In the presence of an outbreak, the individuals being cultured rarely object, providing the swab is taken by a considerate person of the same sex.

Simplified steps recommended for a prompt and reliable identification of pathogenic Shigellae in an identified outbreak are as follows:

Suspicious colonies are picked to Kligler's iron agar (Russell's double or Krumwiede's triple sugar agar is satisfactory also). Despite the heavy fecal inoculum on the plate a high percentage of pure cultures are obtained if the suspicious colony is touched only at its elevated center without "scooping."

Organisms "positive" for Shigellae on this medium are directly inoculated to three sugars, mannitol, xylose, and rhamnose. A needle is used and dipped in succession into the three tubes. The fermentation reaction after 24 hours' incubation differentiates organisms found in this country as follows:

<table>
<thead>
<tr>
<th>Mannitol</th>
<th>Xylose</th>
<th>Rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexner (including Boyd's 88)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sonne</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Schmitz</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkalescens</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Tube agglutination tests are also set up immediately using growth from the Kligler's slants. Three diagnostic sera are employed, polyvalent Flexner (including Boyd's 88), Sonne, and Schmitz. A supply adequate for several days is diluted to one or at most two dilutions below the titer of the anti-serum. One-half or 1 ml. of this is added to small test tubes. Using a needle, a small amount of growth from the slant is transferred to the tube and is readily brought into a smooth suspension. If the probable variety of the organism is known, only one tube is needed; if not, Flexner and Sonne are both used. Except in outbreaks, Schmitz anti-serum is employed only when indicated by the sugar reactions since this organism is rarely found in endemic cases. Positive agglutination tests may be recorded and reported after 2 to 4 hours in the water bath, but negative readings are delayed till the following morning. For examinations under the conditions stated these simplified tests reliably identify most suspicious organisms isolated. For the few which are neither clearly negative or positive more extensive cultural tests are used. Further procedures are re-
quired when other pathogens, as *Salmonellae*, may be involved.

Enteric bacteriology in the past was difficult, time consuming and often unproductive. That situation no longer holds true. Culturing for *Shigellae* is now one of the simpler laboratory procedures and these tests may be done in substantial numbers when needed, provided the laboratory is not far removed from the individuals to be examined.

**THERAPY**

Attention is limited in this study to the use of sulfonamides in shigellosis.

The following method has been used in evaluating the relative efficacy of different sulfonamides in *Shigella* infections. Cultures were obtained before treatment and at daily intervals after the beginning of medication. All were taken by rectal swabs and S. S. agar plates were inoculated immediately in a uniform manner. The number of clear suspicious colonies per plate was counted (or estimated) and representative types picked for identification. Medication was dispensed in individual boxes or envelopes bearing the patient's name and directions as to dosage. Treatment was continued until at least two consecutive daily cultures were negative. Post-treatment follow-up examinations were obtained. Control cases in adequate numbers were similarly followed. Blood counts and urine examinations were ordered twice weekly on some, not on all, to obtain evidence of any minor toxic reactions. Sulfonamide levels in the blood and in fluid feces (following saline laxative) were determined on series of cases receiving the different sulfonamides. Up to the present over 1,500 individuals known to be infected with *Shigellae* (Flexner, Sonne, or Schmitz) when treatment was started, have been studied. In all, ten sulfonamides were used. The major observations were these:

All sulfonamides had some influence in cutting short the duration of these infections.

Flexner strains responded promptly (with very rare exceptions); Schmitz infection was slightly more resistant; while Sonne cases and carriers cleared least satisfactorily.

The poorly absorbed sulfonamides, though given in larger doses, had no demonstrable superiority in effectiveness in Flexner or Schmitz infections and the response was less satisfactory in one regard. The interval between the beginning of medication and a clinical or bacteriological response was longer with the poorly absorbed response than with the absorbed preparations.

Sonne infections were more resistant to all sulfonamides. The best results were obtained with large doses of sulfasuxidine. All but a small percentage responded in a little longer time to the absorbed sulfonamides; the resistant cases were handled best by changing to sulfasuxidine.

Toxic reactions were rare (not observed with poorly absorbed sulfonamides) as would be expected with treatment usually terminating in 7 days or less.

Cases and carriers responded equally well to chemotherapy.

Concerning the choice of sulfonamides there need be no rigid rules. Sulfanilamide or sulfapyridine is not recommended. Other absorbed sulfonamides (sulfapyrazine, sulfadiazine, sulfamethazine, sulfamerizine, and sulfathiazole) are acceptable. The first appears to have slightly superior properties, the last to be less effective. Among the poorly absorbed compounds, we regard sulfasuxidine as superior to sulfaguanidine and reserve judgment on "sulfathalidine."

We recommend, therefore, that the treatment of shigellosis (cases or carriers) begin with an absorbed sulfonamide. Follow-up cultures should be taken not later than the 4th and again
on the 6th day of treatment. If these or any subsequent two cultures are negative, the individual may be released. If they are positive, and particularly if the organism is "Sonne," a change to sulfasuxidine would be desirable. When cultures cannot be obtained, a treatment of 5 days for Flexner and Schmitz and of 7 days for Sonne is recommended.

A satisfactory dosage for adults for all absorbed sulfonamides is 1 gm.; of sulfaguanidine and sulfasuxidine 5 gm.; and of sulfathaladine 2.5 gm.; all four times daily. Double these amounts may be given as the initial dose. Children (between 25 and 75 lbs.) receive one-half the adult dosage; infants, 0.065 gm. of absorbed sulfonamide per lb. of body weight per day, and proportionately larger amounts of the poorly absorbed compounds. Amounts smaller than the above have been used and found effective in Flexner infections.

**APPLICATIONS**

The reliable diagnosis of communicable diseases on an etiological basis is an accepted objective in public health practice. The diagnosis of the diarrheal diseases is far below standard since the necessary bacteriological tests are infrequently employed. The simplification of the technic of fecal cultures makes it practicable to recommend that more laboratories and particularly the smaller clinical laboratories be encouraged to make this diagnostic procedure readily available. State health department laboratories would need to give leadership, instruction, and supervision. The agglutinating anti-sera also might be provided, and it could be agreed, if deemed advisable, that all suspicious positive cultures would be sent to the state laboratory for full identification.

The prompt institution of sulfonamide therapy in the young is required to prevent deaths and to speed recovery. However, in older children and adults with the milder clinical disease, chemotherapy has it major importance as a control measure, where it is used to prevent and terminate carrier states.

Early diagnosis and specific therapy are of particular importance when the case is the initial infection in a susceptible group. Adequate attention to such cases and to their contacts is considered of major importance in the prevention of institutional outbreaks. Here we advocate the bacteriological diagnosis of all acute diarrheal disorders which continue for more than 24 hours, or which in the first 24 hours are accompanied by fever or other symptoms. If two or more cases occur within a week in a group having common living quarters, all members of that ward or dormitory should be cultured. Such a program is viewed as practicable when the tests can be done in the institution's own laboratory, or in another one nearby which can function as such.

Kuhns has described a control program for an army service command in which, in addition to strict sanitary measures, he advocates the bacteriological diagnosis of all cases of diarrhea, and the treatment of positive cases and recognized carriers with sulfonamides. Presumably procedures may be modified to meet the varying needs of military medicine.

Though advocating methods designed to aid in the prevention of outbreaks, we recognize that assistance in control is usually requested after the outbreak is well established. Ordinarily on the first survey at least 10 per cent of the group are proved infected, and this percentage may reach 35 as was found in a military unit in Puerto Rico. Under this condition the following procedure has been used repeatedly with satisfactory results:

Infected individuals are identified through the immediate examination of all with symptoms and by cultural
surveys. All cases are treated with the onset of symptoms and all carriers on identification. Surveys are repeated usually about twice weekly. If practicable, infected individuals are isolated from the group and are treated with sulfonamides until discharged after two consecutive negative cultures. The group is regarded as free of significant infection when no positive individual is found on two successive surveys.

The results of such a program in a military unit have been described by Cornell, Watt, and Dammin. Further illustrative findings are shown in Table 1 (Group A and B). Cases of dysentery began to occur in late March, 1943, among the inmates of two neighboring cottages in an institution for the mentally defective. Clinical cases only were treated at first, while both groups were followed by cultural surveys. From March 29 to April 13 all in Group A were examined 5 times. The prevalence of the infection was at a high level throughout. Then all individuals positive in any of the last three examinations and all additional positives revealed on subsequent surveys were placed on treatment. The prevalence rapidly fell to a low level as shown. However, the first entirely negative survey was on May 13. Group B was handled similarly. From April 4 to 22, clinical cases only were treated and there were five surveys with the findings shown. During this period the prevalence declined moderately. However, after beginning the treatment of all individuals known to be infected, it dropped promptly to a low level. The last positive was found on the 6th day after this procedure was started. In the subsequent 4 weeks, all individuals were culturally negative in each of four surveys. In these and in other groups the occurrence of clinical disease was promptly prevented by this control measure. Also a high total prevalence was readily reduced to a low one, but it was frequently difficult and in some groups impossible to attain the goal of complete eradication of the infection.

A second approach to the control of established outbreaks is now under investigation and a progress note is offered. Here sulfonamides are used "prophylactically" or as it might better be described for "mass therapy" with minimal doses of the drug. Heavily infected groups, having a combined population of 1,646 have been observed. On this treatment, as with that described above, there was a prompt reduction to almost complete disappearance of clinical cases. The total prevalence of Flexner infections declined with striking rapidity as is illustrated by Groups C and D in Table 1. Sulfadiazine 1 gm. twice daily was used in the former, sulfapyrazine 0.5 gm. twice daily in the latter. The preva-
lence of Sonne infection under small dosage (1 gm. of poorly absorbed and 0.5 gm. of absorbed sulfonamide twice daily) declined more slowly, though the course of infection in the treated groups was modified favorably. Groups E and F treated with sulfadiazine and sulfapyrazine as above are illustrative. Risks are recognized in this "prophylactic" use of sulfonamides. Resistant strains might be developed which would tend to spread in spite of continued medication. Also, the immediate and remote effect of this dosage on the individual must be examined further. Thus the varied aspects of this simple approach to control require continued critical study.

These newer procedures cannot replace the established methods designed to prevent the spread of fecal pollution. We join in emphasizing the importance of sanitation and personal cleanliness. However, these additional supplementary methods of control are now available and are most applicable where diarrheal diseases are most troublesome, i.e., in institutional groups and military units. They can be used most simply and we believe most effectively in the prevention of outbreaks. The handling of widely distributed epidemics is more difficult because of the amount of laboratory work involved. Here the epidemiologist or administrator will need to evaluate the present and potential gravity of the outbreak and adjust control procedures accordingly.

CONCLUSIONS

1. The requirements for more effective control of Shigella infections include reliable diagnoses of mild as well as severe cases, a recognition of the role of carriers and more direct attention to the individuals infected.

2. The slow evolution of Shigella outbreaks provides the need and the opportunity for the application of control procedures.

3. Laboratory technics for the identification of Shigellae have been simplified and increased in reliability.

4. A free use of cultural tests for diagnostic purposes and for the identification of carriers is practicable.

5. Well absorbed sulfonamides, as well as poorly absorbed preparations are effective in Shigella infections, and are recommended.

6. Prompt diagnosis and immediate specific treatment of initial cases and early carriers is advocated for the prevention of outbreaks.

7. In the handling of established outbreaks, cases and carriers may be identified through diagnostic tests and cultural surveys and all discovered infected individuals treated till culturally free of Shigella.

8. The merits of "mass therapy" with minimal doses of sulfonamides are not yet securely established.

9. These newer control procedures have a proper place among the methods now available for the prevention of Shigella infections, particularly among institutional inmates and in military units.

REFERENCES
