greater percentage of them probably would have been able to carry on their activities without this undesirable procedure. At present, we do not have sufficient evidence to conclude that early ileostomy in ulcerative colitis will result in permanent cure of the disease and permit permanent closure of the ileostomy and re-establishment of bowel continuity, thus avoiding permanent ileostomy. It is my impression that the case ileostomized in the early stage without an adequate trial by medical management, with subsequent successful closure of the ileostomy, is the type which would have responded satisfactorily to proper medical management. However, ileostomy at times is definitely life saving and at other times is the only means by which we can make ulcerative colitis compatible with life.

REFERENCES

2. Best, R. Russell: Cod Liver Oil Per Rectum as an Adjunct in the Treatment of Ulcerative Colitis. Am. J. Dig. Dis. 5:426, 1938.

Comments on the Laboratory Diagnosis of Enteric Infections

By

OSCAR FEI.SENFELD, M.D., M.S.,
and
VIOLA MAE YOUNG, M.S.
CHICAGO, ILLINOIS

THE RAPID PROGRESS in bacteriology, parasitology and serology during the last years constantly increases the difficulties faced by the gastroenterologist and his laboratory aid when methods of examination have to be selected which can be carried out in a small laboratory without the use of elaborate apparatus, large series of diagnostic sera and much special experience. While the authors discussed laboratory procedures suitable for large, well-equipped establishments with highly specialized personnel at great length in other papers (1, 2), this article is destined for the use of small laboratories adjacent to the office of the gastroenterologist. The methods described here are, therefore, not necessarily identical with those recommended for large establishments. The efficacy of the procedures described in the present paper is somewhat less than that achieved by more intricate and more extensive methods. The statistical analysis of these procedures, however, shows that their probability of success is higher than .90. Even better results can be achieved by repeated examinations. Thus, in the long run, the final outcome of the series of examinations using simple methods will be the same as if more extensive procedures are applied to a fewer number of specimens.

In the experience of the writers, which is probably shared by most laboratory workers, the crucial points of the examination lie not only in the selection of the proper method but, even to a larger extent, in the way the method is put into practice. The cardinal requirements may be summarized as:

1) Proper method of specimen collection.
2) Immediate preservation or examination, avoidance of drying.
3) Selection of the most suitable portion of the specimen.
4) Properly prepared media, stains and reagents.
5) Satisfactory technic of inoculation and fishing colonies.
6) Correlation of biochemical and serological reactions.

If these points are kept in mind, the methods here described will not fail to give satisfactory results. They will be discussed in detail in the subsequent chapters describing the technics.

For the diagnosis of so-called "enteric" infections, stools, urine and blood are usually examined. Urine examination is important in Salmonella infections, because these organisms are often excreted through the kidneys, chiefly in typhoid and typhoid-like fevers.

STOOL EXAMINATION

For routine examination, we disregard anaerobic organisms, yeasts and molds, because of the difficulties encountered in their identification. The significance of these organisms in the intestine has not been established as yet.
If Streptococci are the object of our search, as they may be in ulcerative colitis, one or two blood agar plates may have to be added to the series of bacteriological media. The differentiation of Streptococci isolated from the intestinal tract should not be attempted in a small laboratory because of the great number of biochemical and serological tests required in this work.

In America, the chief interest of laboratory workers is directed toward Shigellae (dysentery bacilli), Salmonellae (typhoid-paratyphoid-enteric bacilli) and parasites. No stool examination is complete without a search for these bacteria, protozoa and helminths. One of the greatest mistakes made in stool examination, chiefly in chronic intestinal disturbances, is to test the stool either only for bacteria, or only for protozoa. The features of amebiasis (types II and III of Craig) resemble so closely forms of bacillary infections that differential diagnosis is impossible without the aid of the laboratory.

The stool has to be fresh and examined not later than fifteen minutes after collection. If such examination is impossible, preservatives have to be used.

**Stool collection.** The ideal method to collect stools is the procedure described by D'Antoni (3). This method can be carried out only by a gastroenterologist. The patient receives a saline cathartic. A cleansing enema is given. Pre- and post-cathartic stools are collected. The fecal material evacuated after the saline enema is also sent to the laboratory. Finally, sigmoidoscopy is performed. Material is collected with the aid of a suction bulb and with proctologic swabs.

It is not possible to collect material in this way in all cases. We have to be satisfied with diarrheic stools and rectal swabs (preferably collected according to the well-known procedure of Hardy and Watt) in cases of diarrhea. When the lesions sit in the higher parts of the intestine and no diarrhea is present, as, e.g., in shigellosis or amebiasis involving predominantly the ascending colon, post-cathartic stools are more informative. It should be a general rule to examine both post-cathartic and sigmoidoscopic specimens.

A stool specimen evacuated in a toilet near to the laboratory can reach the table of the bacteriologist easily within a few minutes. Sigmoidoscopic swabs, however, will dry out before they are brought to the laboratory. It is recommended, therefore, that one or more such swabs are dumped into peptone broth (for bacteriologic examination) and others used by the gastroenterologist for the preparation of permanent slides (for protozoa). The gastroenterologist is furnished with a Coplin jar filled with Schaudinn's fixative and a few slides. He streaks the fecal material to the slides and places the slides immediately in the fixative. The Coplin jar is brought to the laboratory at a convenient time, together with the peptone broth tubes into which the swabs destined for bacteriologic examination were put.

**Stool preservation.** If stools cannot be examined immediately, they have to be preserved. Two collecting bottles, one ounce each, are used. One of the bottles contains about 20 cc. of 10% formalin, the other about 20 cc. of the fluid of Bangsang and Elliot (see Appendix No. 1). About one gram of the fecal material is thoroughly mixed into each of the bottles. Formalin will preserve parasites indefinitely, while Bangsang's fluid will keep Shigellae and Salmonellae alive for about one week.

**Stool examination.** If possible, a mucous part of the stool is used for examination. Two alternative methods may be used for the bacteriologic testing of stools, both employing plating media which are easy to prepare. It is essential that the plates are sufficiently thick (about 1/4"). Their surface shall be dry. Plates older than four days shall not be used.

Either two S. S. Agar plates (Difco) or two to three E. M. B. (Eosine methylene Blue, Difco) plates are streaked. It is important that a good streak, giving many isolated colonies, e.g., the clock-streak, is used. After 24 hours' incubation, colorless, yellow or brownish colonies are picked to T. S. I. medium (B. B. L.). When S. S. plates are employed, one tube of Tetrathionate broth (Difco) is also inoculated.

The tube is incubated for 24 hours, then streaked to a Bismuth Sulfitite Agar plate (Difco) which is incubated for 48 hours. Black colonies with a halo and metallic sheen are picked from this plate to the T. S. I. medium. Instead of the tetrathionate broth, two brilliant green E. M. B. plates (11) may be streaked. They are picked after 24 and 48 hours incubation.

The growth from the T. S. I. medium is studied according to the reaction observed after 30 to 32 hours incubation.

If there is an alkaline slant, an acid butt and no gas formation, Shigellae and typhoid bacilli are suspected. The growth is inoculated into:

- One tube of semisolid mannitol (see Appendix No. 2).
- One tube of peptone (preferably tryptone or medopeptone) broth.
- One or two agar slants.

The next day motility in the semisolid mannitol is observed. Shigellae are non-motile, while typhoid bacilli are motile. Thus Shigellae will have grown only along the line of stabbing, while typhoid bacilli will spread out into the medium. Typhoid bacilli ferment mannitol, while Shigellae may be mannitol-positive or negative.

The growth in peptone broth is used for the indole test. Typhoid bacilli do not produce indole; neither do Sh. dysenteriae, Sh. sonnei and a number of the Sh. paradytserteriae Flexner-Boyd strains.

The growth from the surface of one agar slant is used for agglutination tests. The slide agglutination method is recommended for small laboratories, using commercial (Lederle) sera. The technic described in the pamphlets enclosed with these sera have to be followed in minute detail. There is no use in testing the suspected strains with other sera than those indicated by the biochemical reactions of the organism. E.g., indole positive Shigellae shall not be tried with Sh. sonnet serum.