containing an excess of iron (Mueller and Miller, J. Immunol., in press). For purposes of attempted purification it has been grown in a heart infusion medium containing glucose, tryptic digest of casein, and an excess of reduced iron. The crude toxins obtained from this medium contain 200,000 to 800,000 mouse M.L.D. per ml (*ca.* 5×10^{-6} mg N_2 per M.L.D.). Several lots of the crude toxin have been purified by precipitation with cadmium chloride according to Eaton and Gronau, by precipitation with 0.5 saturated ammonium sulfate followed by dialysis at 0 to 5 C against 0.9 per cent NaCl, or by precipitation with cadmium chloride and reprecipitation with ammonium sulfate. The best preparation (table 1, toxin no. 4) contained 0.023×10^{-6} mg N_2 per M.L.D. The relatively constant ratio, L_f/M.L.D., indicated that losses during purification did not result from inactivation of toxin; rather, losses resulted largely from incomplete elution from the cadmium precipitate.

Although the N_2/M.L.D. ratio represents a toxin of a hundredfold greater purity than any previously obtained, we have as yet no evidence that a limit of purification has been reached. Further, these data do not yet exclude the possibility that the toxic entity is nonprotein and that it is carried through the purification procedures by adsorption on inactive protein. Indirect evidence, however, argues against this assumption: 80 per cent of the total nitrogen of the purified preparations is precipitated by 5 per cent trichloroacetic acid and 70 per cent by 1 per cent metaphosphoric acid; and the total nitrogen, calculated as protein, constitutes 100 ± 2 per cent of the dry weight in the purified preparations.

TRIPLE-SUGAR IRON AGAR MEDIUM FOR THE IDENTIFICATION OF THE INTESTINAL GROUP OF BACTERIA

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Received for publication March 2, 1945

Kligler's iron agar medium has replaced Russell's and Krumwiede's medium in many laboratories as a primary differential medium for enteric pathogens. The advantage of Kligler's medium over Russell's and Krumwiede's lies in the reaction for H_2S. Since practically all members of the *Eberthella* and *Salmonella* genera produce H_2S whereas those of the genus *Shigella* do not, this reaction is of great practical importance in combination with the characteristic differential reactions of acid and gas production first suggested by Russell, and by means of which enteric pathogens can be distinguished from coliform and other nonpathogenic bacteria. The value of Kligler's medium has been limited by the fact that H_2S is not produced by all strains of *Eberthella typhosa* and by certain members of the genus *Salmonella*. Production of H_2S is sometimes so slow that reactions may be negative at the important 18 to 24-hour incubation period and the reactions are not always so clear-cut as is desirable.

A new triple-sugar iron agar (TSI) medium has been developed which gives

more satisfactory reactions, that is, reactions which are more clear-cut for acid and gas, and more sensitive for H_2S. This is important since selection of carbohydrate test media to be used for preliminary identification of members of the *Eberthella*, *Salmonella*, or *Shigella* genera should be based on the TSI reactions. The formula for the TSI medium is given below.

To each liter of distilled water, add

Agar (dry)	13.0 g
B.B.L. nutripeptone*	20.0 g
Sodium chloride	5.0 g

Dissolve in the Arnold sterilizer or in flowing steam in the autoclave. When melted, adjust to pH 7.5.

Admix

Lactose	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Sodium thiosulfate	0.2 g
Ferrous ammonium sulfate	0.2 g

Check the pH, which should then be 7.4.

Lastly, add

Phenol red (1% aqueous solution)	2.5 ml.

Dispense approximately 5 ml. in 15 x 100 mm tubes. Autoclave at 12 pounds pressure for 15 to 17 minutes.

* Bacto-peptone 15 g plus bacto-proteose peptone 5 g gives equally good results.

Sodium sulfite and meat extract have not been found to increase materially the production of H_2S. The role of sucrose in the medium is to eliminate certain sucrose-fermenting, non-lactose-fermenting bacteria of the *Proteus* and paracolon groups.

All of the *Salmonella* cultures listed in Circular 54 of the University of Kentucky, plus 26 types recently furnished by Dr. P. R. Edwards, were used in this investigation. Very few *Salmonella* organisms failed to produce H_2S, notably *S. paratyphi* A, *S. abortus-equi*, *S. typhi-suis*, *S. berta*, *S. sendai*, and *S. papuana*. *S. cholerae-suis*, hitherto distinguishable from *S. cholerae-suis* var. *kunzendorf* by its failure to produce H_2S, produced it in the medium described here, though in lesser quantity than did the Kunzendorf variety. *Eberthella typhosa* and *S. gallinarum* are indistinguishable in this medium.