(This work is a part of the investigation of food poisoning, conducted under the direction of Dr. M. J. Rosenau, professor of preventive medicine and hygiene, Harvard Medical School. The investigations are done under the auspices of the Advisory Committee of the National Research Council on the Toxicity of Preserved Foods, and under a grant to Harvard University from the National Canners' Association.)

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On methods of isolation and identification of the members of the colon-typhoid group of bacteria. Further studies on C. R. indicator.

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Some time ago we described an indicator for the direct measurement of the hydrogen ion concentration in growing bacterial cultures.[1] This indicator consists of the mixture of China blue and rosolic acid, and covers the range of changes in the hydrogen ion concentration between $C_H = 1 \times 10^{-9}$ and $C_H = 5 \times 10^{-5}$. The choice of the dyes was made on the basis of their possessing suitable turning points coupled with the fact that their respective phases of highest color lie on the opposite sides of the point of neutrality. China blue is colorless at the concentration of hydrogen ions below 1×10^{-7}, and gives graded intensity of blue with the increase in the hydrogen ion concentration up to the point of about $C_H = 5 \times 10^{-5}$, when it reaches its maximum color. Rosolic acid, on the other hand, gives graded intensities of pink beginning with the hydrogen ion concentration close to 1×10^{-7}, and increasing gradually with the increase of the hydroxyl ions concentration reaching its maximum color at the $C_{OH} = 1 \times 10^{-5}$ (or $C_H = 1 \times 10^{-9}$). With the increase in concentration of hydrogen ions above 1×10^{-7} the rosolic acid has a pale yellow (straw) color, which is masked by the color of ordinary culture media.

[1] J. Bronfenbrenner, *Jour. Med. Res.*, XXXIX, I, 25, 1918.

The combination of the two dyes thus offers an indicator which has a faint gray tinge at the neutral point with pure blue and pink on the acid and alkaline sides respectively. Due to the high tinctorial power of the dyes composing it, this indicator is incorporated into the media in very minute quantities. The actual concentrations of China blue and rosolic acid in the medium are respectively 0.0025 per cent. and 0.005 per cent. by weight.

This indicator, however, can be used only for the study of the Gram negative organisms, because the rosolic acid it contains exerts selective bactericidal action against the Gram positive organisms.[1] While this property of the rosolic acid is useful in certain circumstances (as for instance, for the purpose of suppressing Gram positive bacteria while isolating the Gram negative from the mixtures of both)[2], it limits the usefulness of the mixture as an indicator. In order to permit the use of our indicator in the study of Gram positive as well as Gram negative bacteria, we suggest the substitution of corallin (Harmer) for the rosolic acid (Merk) in the above mixture. The turning point of corallin is the same as that of rosolic acid, and its tinctorial power, as well as the color, are much the same: Corallin (Harmer), however, has no bactericidal action upon Gram positive organisms.

As the supply of either China blue, rosolic acid or corallin of foreign manufacture is very low at present, and as the domestic dyes sold under the same names are manifestly different from the foreign dyes used by us, it was necessary to try a number of related dyes before a suitable choice could be made. While this work is still in progress, we have already found a few preparations which seem to answer the requirements.

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[1] J. Bronfenbrenner, M. J. Schlesinger and D. Soletsky, *Jour. Bact.* (in press).

[2] J. Bronfenbrenner, M. J. Schlesinger and D. Soletsky, *Jour. Med. Res.* (in press).