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NOTES

Modification of Schaudinn Fixative

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In a search for a less toxic fixative, a Schaudinn solution substituting a divalent metallic cation for mercuric chloride proved to be satisfactory.

Schaudinn solution, widely used to fix protozoa and helminths in human fecal specimens (2), contains mercuric chloride ($HgCl_2$) which is a potential hazard to patients and laboratory workers. If the solution is disposed of through drains, the $HgCl_2$ will contribute to environmental hazards. It would be desirable to replace the $HgCl_2$ in the fixative with a less toxic compound.

The mercuric ion of $HgCl_2$ bonds unsaturated fatty acids in the fecal specimens (1). One should look, therefore, for a similar, less toxic, divalent cation that would bond fatty acids, and in place of $HgCl_2$, add it to an otherwise unaltered Schaudinn solution.

I prepared six 1-liter aliquots of distilled water which were heated to 100°C. To the first solution we added 20 g of cupric sulfate ($CuSO_4 \cdot 5H_2O$); to the second, 19 g of cobalt chloride ($CoCl_2 \cdot 6H_2O$); to the third, 13 g of cobalt nitrate ($Co[NO_3]_2 \cdot 6H_2O$); and to the fourth, 9.2 g of ferric chloride ($FeCl_3 \cdot 6H_2O$). The fifth solution, a Schaudinn solution without the addition of a divalent metallic cation, was the negative control, and the sixth was the conventional Schaudinn solution, used as positive control. These solutions were then prepared as fixatives by combining 2 parts test solution with 1 part 95% ethanol and glacial acetic acid at 5% (vol/vol) and glycerol at 1.5% (vol/vol).

Two dozen smears of unpreserved fecal specimens containing *Entamoeba coli*, *E. histolytica*, *E. hartmanni*, *Dientamoeba fragilis*, *Iodamoeba butschlii*, *Endolimax nana*, and or *Giardia lamblia* trophozoites or cysts or both were tested to determine whether any of the solutions would prove to be satisfactory. The fresh fecal specimens were smeared on glass slides (75 by 25 mm), immersed in each of the six solutions for 30 min at room temperature, stained in Gomori trichrome, and mounted according to standard technique (see Table 1). Subsequently, more than 100 fecal specimens were tested to determine the reliability of the $CuSO_4$ fixative in comparison with the standard Schaudinn fixative.

To see whether polyvinyl alcohol powder (PVA) would dissolve in the satisfactory test fixatives, 50 g of PVA was added to three freshly prepared solutions—one of copper sulfate, one of the negative control, and one of the conventional Schaudinn fixative—while air was bubbling through to mix and dissolve the PVA at 65°C. Each of the three solutions was allowed to cool after the PVA had dissolved. Fresh fecal specimens, prepared as described above, were then immersed in the three solutions.

TABLE 1. *Experiments with modified Schaudinn solutions*

Soln	Results
$CuSO_4 \cdot 5H_2O$	Satisfactory
$CoCl_2 \cdot 6H_2O$	Unsatisfactory
$Co(NO_3)_2 \cdot 6H_2O$	Unsatisfactory
$FeCl_3 \cdot 6H_2O$	Unsatisfactory
Negative control	Satisfactory
Schaudinn solution (positive control)	Satisfactory

Two of the modified fixatives for conventional Schaudinn solution were satisfactory (see Table 1), the one containing cupric sulfate and the negative control. PVA dissolved more readily in cupric sulfate solution than in conventional Schaudinn solution.

Although the negative control produced satisfactory results when used alone, PVA would not dissolve in it; hence, it was unsatisfactory for use in the field, where "kits" containing PVA are provided to patients.

On the slides fixed with the cupric sulfate solution, nuclear and cytoplasmic morphological characteristics of protozoa were comparable in appearance to protozoa fixed with the negative and positive controls (Fig. 1 through 3).

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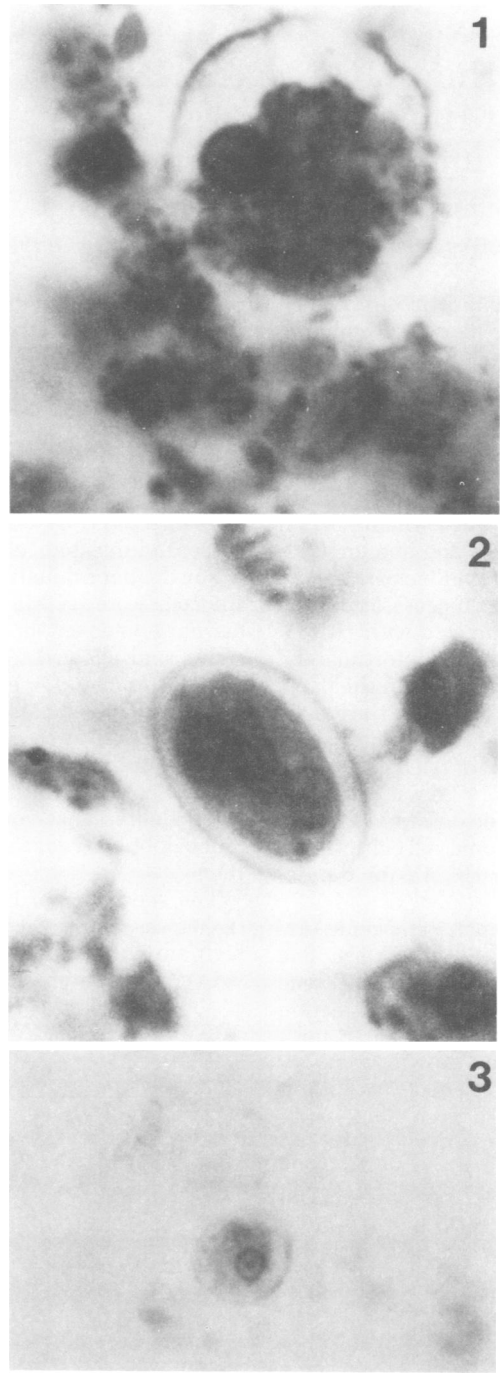

FIGS. 1–3. *Photomicrographs of protozoa fixed in the $CuSO_4$-ethanol-acetic acid solution and stained by the trichrome method.*

FIG. 1. *E. histolytica trophozoite.* × *1,000.*

FIG. 2. *G. lamblia cysts.* × *1,000.*

FIG. 3. *E. hartmanni trophozoite.* × *1,000.*