APPENDIX

KRAJIAN'S "20 MINUTE" RAPID STAINING METHOD OF TREPONEMATACEAE IN FROZEN SECTIONS*

REAGENTS

1. Uranium nitrate ..... 1 gm
   95% Formic acid, C.P. ..... 3 ml
   Glycerin, C.P. ..... 5 ml
   Acetone, C.P. ..... 10 ml
   95% Ethanol ..... 10 ml
2. 1% Silver nitrate solution, made up just before use from a 10% aqueous stock solution. The stock solution keeps for months in a dark bottle. The 1% working solution should be used the same day, not more than 3 times. Solutions with a brownish tinge are discarded.
3. Gum mastic solution.
   Saturate Absolute ethanol..... 35 ml
   with Gum mastic..... 25 ml
   Let stand for 3 to 5 days in dark bottle at room temperature, shaking occasionally. Use the supernate.
4. Developer.
   Mix 40% Formaldehyde ..... 2.5 ml
   Acetone C.P. ..... 2.5 ml

* Krajian, A.A.: *Am.J.Syph.*, *23*:617, 1939.
Krajan, A.A., and Gradwohl, R.B.H.: *Histopathological Technic*, 2nd ed. C.V. Mosby Co., St. Louis, Mo., 1942.
Frankel, S., and Reitman, S. (Editors): *Gradwohl's Clinical Laboratory Methods and Diagnosis*, 6th ed. C.V. Mosby Co., St. Louis, Mo., 1963.
The above is a revision by Dr. Addine G. Erskine.

Dissolve in this mixture
first Hydroquinone..... 0.31 Gm
then Sodium sulfite ..... 0.1 Gm
and Pyridine ..... 2.5 ml
Add to above solution #3 ..... 2.5 ml
then Distilled water ..... 15 ml

The developer should not be used more than 3 times. The solution will keep for 1 or 2 weeks in a dark bottle at room temperature. It should be replaced if a sediment is formed.

5. Thin celloidin solution. (Krajian formula).

PROCEDURE

a. Tissues fixed in 10% formalin at 67° C for 10 minutes are preferred. They are then frozen and sections, 7 to 10 μ thick, are washed in distilled water.

b. Prewarm solutions #1 and#4 in small Stender dishes in a paraffin oven at 60° C.

c. Prepare 3 Stender dishes with distilled water, one with about 5 to 10 ml 95% ethanol, another with 5 ml 95% ethanol to which a few drops of solution #3 have been added with a small glass lifter. (*Note*: metal instruments and tap water should not be used in this procedure).

d. Put the freshly made solution #2 in a 250 ml Pyrex or Kimex beaker.

e. Place the section in solution #1 at 56° to 60° C in the paraffin oven for 5 minutes.

f. With a glass lifter, carry it to a Stender dish with distilled water. The section should open and float. Then pass 3 times quickly but gently through the Stender dish with 95% ethanol and mastic (see under c.).

g. Rinse in distilled water in a Stender dish. The section should spread out.

h. Transfer the section to the beaker with solution #2. Heat the contents of the beaker to 70° to 73° C for 2 minutes, while exposing it to the light of a 60 W electric bulb from a distance of 4 ft.

i. Transfer the section on the glass lifter to the warm solution #4, lifting it in and out of the solution, exposing every part to

the electric light and taking care that the section opens up every time it is put back into the solution. Repeat this 6 to 8 times. The tissue should appear brown.

j. Transfer to 95% ethanol to remove excess gum mastic.

k. Transfer to distilled water. If the tissue does not spread out, repeat step j for a few seconds and return section to distilled water.

l. Place the section again into the now cool solution #2 and expose to the electric light for another 10 to 30 seconds. This step is very important. Too cold or too warm silver nitrate solutions may cause improper results.

m. Transfer to a large dish with distilled water, then to a glass slide.

n. Dehydrate with isopropanol dropping it gently onto the surface of the section and leaving for about 30 seconds, blowing first gently, then harder to remove the water.

o. Blot gently with several thicknesses of Whatman No. 1 filter paper, then apply isopropanol anew to the slide for 1 minute. Drain and blot again.

p. Dip once in #5 solution. Wipe the bottom of the slide dry. Blow gently at the surface until the celloidin is dry.

v. Dehydrate in a staining dish with isopropanol for 1 minute.

r. Carry through two changes of xylene for several minutes each.

s. Clean excess celloidin around the tissue and mount in gum damar.

Treponemataceae, including borreliae, appear black against a yellow or brownish background.

This method is applicable to paraffin sections but the period of staining has to be doubled.