Injection of ecgonin-hydrochloride and benzoyl-ecgonin produced no effect on the behavior of the rats even when administered in doses much larger than that of cocain. Injections of sodium benzoate solution produced no effects. Neither was there any depressant or stimulating effect noted after injections of small doses of methyl alcohol solution (1 per cent.).

Various mixtures of ecgonin hydrochloride, benzoyl-ecgonin, sodium benzoate and methyl alcohol in different proportions were found to produce very little effect on the behavior of rats. As a result of the various experiments it is therefore concluded firstly, that cocain, as such, exerts a depressant action on the intelligent behavior of albino rats, secondly, that in no cases even after minute doses of the alkaloid was there a primary stimulation noted and thirdly, that the various components into which the cocain molecule can be split up, when injected individually or as a simple mixture of each other, do not cause the same action as their chemical combination in the form of cocain. The complete data of this investigation will appear in the *Archives Internationales de Pharmacodynamie et de Therapie.*

38 (1620)

Improved methods for staining spirochaeta pallida in tissue.[1]

By ALDRED S. WARTHIN and ALLEN C. STARRY.

[*Ann Arbor, Michigan.*]

The two methods outlined here present the first satisfactory ones devised for the staining of spirochetes in single sections of tissue mounted upon cover-glasses. We consider them of great value for the following reasons: They are more certain than the Levaditi method of staining in bulk; the time required is shortened to hours, instead of the days required by that method; they have a much greater applicability to practical diagnostic work in that they can be used for single sections, thus permitting a closer control of histological findings.

[1] Researches conducted under a grant from the Interdepartmental Social Hygiene Board, Washington.

1. WARTHIN AND STARRY'S COVER-GLASS METHOD.

1. Fix tissues in 4 per cent. formol.
2. Wash thoroughly in distilled water.
3. Imbed in paraffin (alcohol, xylol, paraffin).
4. Cut; mount sections on cover-glasses with albumin fixative.
5. Remove paraffin from section (xylol, alcohol, water).
6. Place cover-glass in a saturated solution of ferric alum, or a 4 per cent. solution of ferrous ammonium sulphate, in incubator for 1 to 2 hours.
7. Wash in distilled water.
8. Rinse cover-glass with section in a 2 per cent. silver nitrate solution. Cover section with another perfectly clean cover-glass which has also been rinsed in the silver solution, so that the cover-glasses are held together by capillary attraction. Then place them carefully on the bottom of a wide-mouthed dark bottle covered with black paper, and cover them with the silver nitrate solution. Cork tightly, and put into incubator for 3 to 24 hours.
9. After impregnation pour off the silver nitrate solution and rinse in distilled water without removing cover-glasses from bottle, by pouring the water into the bottle, shaking gently, and then pouring off.
10. Pour reducing fluid (pyrogallic acid, 4 grams; 40 per cent. formol, 5 cc.; distilled water, 100 cc.) into the bottle. See that fluid passes between cover-glasses by pressing upon them with a glass rod, or by shaking. Reduction is almost instantaneous; it should occur evenly over the section or brown lines will result. After 2 to 3 minutes remove cover-glasses, separate, wipe off with a cloth any precipitate on the albumin fixative about the section, taking care not to touch the latter.
11. Wash in distilled water.
12. Dehydrate in absolute alcohol, clear in xylol, then balsam.

The reduced section should have a faint, dull brownish-yellow color. If the color is bright yellow the organisms will be poorly stained. The spirochetes should have a deep reddish brown color contrasting sufficiently well with the background, if the procedure has been successful.

2. WARTHIN AND STARRY'S SILVER-AGAR COVER-GLASS METHOD.

1. Fix tissue in 4 per cent. neutral formol.

2. Imbed in paraffin (absolute alcohol, xylol, paraffin).

3. Cut, mount sections on cover-glasses with albumin fixative.

4. Remove paraffin (xylol, alcohol, water).

5. Rinse cover-glass with section in 2 per cent. silver nitrate; cover wet section with another perfectly clean cover-glass, so that they are held together by capillary attraction; then place them carefully in a bottle of 2 per cent. silver nitrate, and place in incubator for 30 to 60 minutes; then remove the silver nitrate and separate cover-glasses. They must not separate while in the solution.

6. Place cover-glass with section in the following reducing mixture:

2 per cent. silver nitrate solution	3 cc.
Warm glycerin	5 cc.
Warm 10 per cent. aqueous gelatin	5 cc.
Warm 1½ per cent. agar suspension	5 cc.
5 per cent. aqueous hydroquinone solution	2 cc.

7. Reduce until section is a light reddish brown (several seconds); remove and rinse in 5 per cent. sodium hyposulphite solution.

8. Rinse in distilled water. Clean off with a cloth any precipitate on the cover-glass.

9. Absolute alcohol-xylol-balsam.

The mounted section should have a light reddish brown color; if too deep brown or black the spirochetes will not be sufficiently contrasted. They should appear dark reddish brown to jet black against a very light brown background.

In some cases of poor fixation of the tissue with poor staining of the spirochetes we have found that if the sections before being put into the silver solution are treated for several minutes in a 1 per cent. solution of uranium or copper nitrate, or a 0.5 per cent. solution of ferric alum, the tissue-reaction is changed in some way so that good staining results. These solutions do not act uniformly in all cases. After their use the section must be thoroughly washed in distilled water before it is put into the silver nitrate solution.