EM PREPARATION OF TISSUES THAT CONTAIN ELASTIC FIBERS

(3 stopping points are listed = places that you can leave the tissue overnight)

1. Dissect tissue into 0.5 - 1.0 mm^3 pieces

2. FIX: 3% Glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 4 hrs at 4˚C

For 10 mls:	25% glutaraldehyde	1.2 ml
	0.2 M buffer	5.0 ml
	dH_20	3.8 ml

3. WASH: 4 x 10 min in 0.1 M cacodylate buffer
STOPPING POINT #1 in fresh cacodylate buffer at 4˚C **OR** continue to stopping point #2

5. **POST-FIX:** 1% osmium tetroxide in 0.1 M cacodylate buffer for 30 min - 1 hr at 4˚C
(use OsO_4 in fume hood)

6. **WASH:** 4 x 10 min in 0.1M cacodylate buffer
STOPPING POINT #2 in fresh cacodylate buffer at 4˚C **OR** continue to stopping point #3

7. **EN BLOC STAIN:**
(a) 2% tannic acid in 0.1M cacodylate buffer for 1 hr at 4˚C
wash: 4 x 10 min dH_2O
(b) 2% uranyl acetate in dH_2O for 1 hr at 4˚C
wash: 1 x 5 min in dH_2O

8. **DEHYDRATE:** 8 min each in 10, 25, 40, 55, 70, 85, 90, 95, 100, 100% methanol
(STOPPING POINT#3 is in 70% MeOH at 4˚C)

9. 2 x 5 min propylene oxide (very toxic - fume hood)

10. **INFILTRATION:** propylene oxide:Epon mixtures (on rotator - caps on)
3:1 for 1 hr (or 2 hrs if you have more time)
1:1 for 2 hrs (or 4 hrs if you have more time)
1:3 overnight

11. Next day - Pure Epon for 4 - 6 hrs (in hood - caps off)

12. Embed tissue in fresh Epon and put in oven at 65˚C or approx. 48 hrs

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0.2 M SODIUM CACODYLATE BUFFER: 4.28 g sodium cacodylate in 80 mls dH_2O
pH to 7.4 and make up to 100 mls
(contains arsenic - wear gloves)

EPON: 23.0 gm Epon 812
14.0 gm NMA
13.0 gm DDSA
1.0 ml DMP-30 (keep in fume hood)
stir for at least 3 min in fume hood (wear gloves - Epon is carcinogenic)

NOTE:
- precipitates are formed by: 1) glutaraldehyde in contact with osmium tetroxide; or 2) cacodylate buffer in contact with uranyl acetate - WASH WELL.

CAUTION:
- osmium tetroxide is very toxic - use fume hood
- sodium cacodylate contains arsenic - avoid breathing fumes, wear gloves

FOR COUNTERSTAING SECTIONS:

- Immerse the grid (sections up) in a large drop (or better - a small dish because it will evaporate - I use a rubber embedding mold from one of the EM companies - it has 6 round wells in it) of 7% uranyl acetate in absolute methanol (filtered) for 2 minutes

- Wash by dipping 20 - 30 times in 3 different changes of absolute methanol

- Put it down on filter paper (sections up) to let dry (1 minute)

- Incubate 2 minutes on drops of Reynolds lead citrate (float grid on the top of the drop)

- Wash by dipping 50 times in 3 different changes of dH20

- Let dry