Final grade A

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Report of Project

MATERIAL:

I was assigned Pheasant wattle, Dove embryos, Pigeon and Dove testis, duodenum, and "something else", with Bouins, Formalin and Hellys fixitives recommended.

Actual Tissues and Fixitives I used:

Pheasant Wattle: (1) from young male pheasant after one month of testosterone injection. Fixed Sept. 15, 1948 in 10% ($MgCO_3$) formalin.
(2) from natural cock moderately developed. Fixed in neutral formalin.

Dove Embryos:
I obtained embryos from the Genetics Dove Colony and fixed them in Bouins and in Picro-Sulphuric 'fixer' (as recommended by Guyer).

Male Pigeon G307B of blood type "CD" was available since it had avian tuberculosis and would have been discarded. Parts (or all) of the testis, pancreas, duodenum, liver, and kidney were fixed in bouins and the same in formalin.

Dove: G333-, a synthetic male (i.e. a hybrid backcross whose blood type was indistinguishable from ring neck, Streptopelia risoria.) Parts (or all) of the testis, duodenum, pancreas, liver and kidney were fixed in bouins and the same in Helly's. Also, the heart was fixed in formalin.

DISCUSSION OF MATERIAL AND PROCEDURE:

I tried more than I could readily handle (as might be surmized from the above list.) I was able to finish and process all the dove tissues in Hellys, the heart in formalin, and all the pigeon tissues in both bouins and formalin.

Formalin fixed material, it seems to me, gives inferior results to bouins. The nuclei werenot as distinct as in bouins in practically all cases. A partial blurring of background may have been responsible for this effect. Bouins fixitive gave good results; and Hellys fixitive gave excellent results. I would recommend Hellys and Bouins (but not formalin) for Pigeons and Doves. Bouins and Picro-Sulfuric fixitives for embryosboth worked equally well though I grew toprefer the latter. I fixed 15 embryos ranging up to 96 hours in age. I was able to find time to finish only seven--3 in whole mounts(poor results) and four sectioned, 2 frontal sections (poor results) and two transversely. Embryos must be cut fairly thick (10 mcrrons did not work).

I used Rogers silver method on the Pheasant wattle to distinguish nerve cells (refer to Dr. Collias). Thismethod is somewhat complicated but the only trouble I had was that the gold chloride didn't take off enough excess silver. Thismay have been due to the excessive amount of egg albumen which hadto be used. Even so, a few mounts came off here which was the only trouble of thiskind I had all during the course.

A general trouble I encountered was that part of the tissues (any) would fail to spread and would leave overlapped areas.

Report of Project (con't)

STAINING:

Iron hemotoxylin nuclear stain either leaves a cleaner background when washed or else it stains the nuclei better than Harris h. leaving the nuclei more distinct and clear to view. Change in procedure (washing time etc.) did not alter this for me.

I got best results when I counterstained in Picric acid. I believe this is due to technique, since toward the end of the semester I was able to get good results in FeAlum by removing the slides a few minutes before they looked ready (i.e. I had previously been overdestaining).

I think students should be warned that when coming up the alcohols from tripple staining, $\frac{1}{2}$ minute is the maximum time allowable for (using the same protocol otherwise), since it destains very rapidly in the alcohols with water in them.

Eosin counterstain was the hardest to learn well (for me). Fast green is the only good counterstain for the silver method (Rogers) on Pheasant wattle since feather follicles in the wattle have a natural yellow or orange color. The green contrasts well with the black and orange.

Whole mount embryos were stained in dilute Harris H. Tripple stain works well on embryos though both iron and Harris H. seem usable.