Annual Laboratory Reports of the Biology Society

February 1940 to and through January 1941.

Histology Section

Joshua Lederberg, Leader.

January 8, 1941.

These reports are abstracted from the running notes, too voluminous to copy.

(N.B. — This is almost a thesis! J.L.)
4500 words.

4 pages

To:
Mr. Schur

Fall 1945 - Lebenberg

The major accomplishments and experiments of the Histology Group are exemplified in the slides that it has prepared. Since the preparation of these slides follows, generally, a basic preparatory process, it would be well to indicate this process. In the preparation of tissues for histological study, the tissue must be killed and hardened in the condition it was when alive, and preserved against future decay and deterioration. This is accomplished by the use of a fixative solution, and the process is called fixation. The tissue must then be made ready for cutting very thin slices; in order to accomplish this, the fixed tissue, after having had its fixative thoroughly washed out, is dehydrated, the water being replaced by paraffin, through an intermediate stage of dioxane and a solution of ~~[illegible]~~ paraffin in dioxane. The tissue is now said to have been embedded. The embedded tissue can now be sectioned; this is done by a microtome, and which delivers sections of about 10 to 12 μ (1μ = .0001 cm. = $\frac{1}{25,000}$ inch) as indicated. These sections are now mounted and attached to a slide, and the slide, holding a thin section of tissue embedded in paraffin, is ready to be stained. To do this, the paraffin is removed by xylol, the xylol is removed with dioxane and that with water. After the proper immersion in the desired staining fluid or fluids, the slide is washed thoroughly, put through dioxane and xylol and is finally covered with a drop of Canada Balsam dissolved in xylol, and a thin cover-glass. This is for mechanical protection and to make the section transparent. The tissue-section has had to be stained in order to bring out and differentiate the various elements and cells in the tissue.

Notes respecting the work of the ~~for~~ large part of the first term of this last year were lost ~~[illegible]~~ during revision, so that a good deal of chronological information is lacking; However, all information relative to the preparation of the tissues that is necessary for the interpretation of the results. Dates, unless otherwise stated, refer to the date of primary staining.

Late in February 1940, a fat body was freshly dissected out of a male frog, a small fragment, 2 x 5 mm. placed in 10% Neutral formal in water, for 2 or three days, washed one hour in 35% methyl alcohol, then dehydrated overnight in dioxane, infiltered 5 hours in dioxane-paraffin, 3 hours in molten paraffin, and embedded. The block was then sectioned at 18 μ, stained in Delafield's hematoxylin and in 1/2% eosin in 50% dioxane, and mounted under balsam. In June, 1940, the slide was borrowed by the

Weinberger, and thence disappeared in some recess of the Biology Department.

From the same frog, a quite liver was dissected out and subjected to a similar process, with the sections at 12 u, stained in Delafield's haematoxylin. Unfortunately, this section is somewhat crumbled. This was not sectioned or stained until April 16, 1940.

From the same frog, and fixation, etc., the cartilage and muscle of the epistemum. Stained after 12 u section in Delafield's. 4/16/40.

From the same frog, and fixation, etc., the spinal cord of the frog in the cervical region, and, also, the lower brain. Sectioned at 10 u, stained for 3-4 seconds in Delafield's, washed, then restained in 1% Methylene Blue, differentiated in 99% dioxane, and finally mounted under balsam. A very poor photomicrograph from this slide, effective magnification about 450 X, ~~no~~ enlarged 2.5 X. The picture, which is the worst of a series, was taken by M. Finch. It shows the ciliated ependymal epithelium surrounding the central canal. A series of these sections were stained at about this time.

April 27, 1940, a smear of human blood was stained about one minute in Wright's stain.

(Part of a more detailed notation follows:-)

(Mon., April 1 - "No work was allowed today in 521."

April 2, 1940 - dissection of a rabbit in 419: "The maxillary turbinals are curious, unfamiliar structures. I am not sure whether the paired set of nerves found just subcutaneously above the rear maxillary teeth are the superior maxillary or one of the facial - facial, trigeminal - nerves. They pass just under the orbit and enter the brain near the optic nerve. Also, there is a small white patch, medially at the roof of the mouth, between the soft and hard palate. What is it? Without even a temporary use of the incubator (which was at the time full of bacteriological tubes) it seems almost hopeless to consider most types of histological work in the near future. Oh, for a freezing microtome! No dehydration. No difficult and messy infiltrations, no rehydrations to unravel a section from the slide. The block of the frog's central nervous system is well embedded. Everything is so disorganized." Quotation from my journal, 4/2/40.

4/3/40. "A cross-section of the frog spinal cord was stained. Because of examinations on April 11 and 12, there will be no work this week. Slide was apparently not clear. Am stained, differentiated, restained. More experience in technique required.

4/4/40. Remained afternoon and evening till 6:50 P.M. with Teachers-in-Service Course. Spent one hour staining nerve slides. Both xylene and aniline are unsatisfactory in arresting destaining in acid alcohol. Stain was by trial and error until right depth of stain was revealed. Eosin counter stain only confuses the field."

4/15/40. "Till about 2 o'clock, aid Mr. Towne in cleaning Petri dishes. Thence, some further microtomy of block of frog nervous system."

4/16/40., same as. In lecture before biology, technique used in histology was demonstrated.

4/17/40. 12 slides, all nervous, were stained with methylene blue, in a dioxane modification of a Nissl Stain

4/18/40. "Till 6:45 P.M. at the In Service Course" - see 4/4/40. "All afternoon and evening was spent in assisting and in staining some more of the unstained sections thus far. Today I used an 85% dioxane in water solution to destain, absolute dioxane to arrest differentiation. The chief difficulty lay in obtaining sufficient intra-cellular detail simultaneously with inter-cellular contrast. In earlier afternoon Fixation of a 10 mm chick embryo that had been kept about 4 hours in salt solution for macroscopic examination in Bouin's.

4/19/40. "to 5 21 blue staining of the last nerve slides was completed. One in haematoxylin and one in safranin. The remainder in Nissl-Methylene Blue. The Canada Balsam we have must be quite acid as the haematoxylin stained slide has turned reddish again.

4/22 - 4/23/40. Embryo dehydrated at home. In afternoon infilter. Later, in evening, at Sid Zakaria's microbatar; embed the embryo on 4/24/40.

May 1, 1940. Sectioning of embryo, more anterior region: Cephalic region found not embedded at all. Upper and abdominal sectioned poorly, much wrinkled. Some sections, more posterior, flattened out nicely. About 12 sections were made.

May 8, 1940. Stain many such sections, largely methylene blue; poor wrinkling, staining. (Slides: II/22-25, II/1.

May 9, 1940. "Upon request to Mr. Weinberger, I was able to obtain today a number of frog embryos and larvae (tadpoles) for embryology. All were placed in Bouin's.

May 13, 1940. Stain all embryo slides thus - Slides: II/2, 3, 4, 7,)
Prepare whole mount of hydra; unsuccessful attempt to kill a planaria extended

May 16, 1940. Section blocks of ~~tadpole~~ embryo frog, as begun on May 9, 1940. Yolk causes poor sectioning.
May 20, 1940. Stain two slides from 5/16/40. Hematoxylin.
May 23-24. Biology Exhibit. Stain a number of slides, ~~etc~~: chick embryo, and a block of head of snake embryo secured by John Jaquy. Also stain a section of Cat cerebrum secured by him.
May 23, 1940 - Frog blood smear, stained with methylene blue and eosin.
May 27, 1940. - Some blood smears, stain with Wright's.
May 28, 1940. Stain a few sections of cat cerebellum - Methylene Blue, Wright's.

June 5-6, 1940. Preparation of about 200-250 sections of stomach, lung, heart, intestine and tongue of frog prepared largely for Mr. Weinberg's study course. In month of June, 1940. a series of sections of stomach, etc. as above, were stained, a great deal in assistance to Mr. Weinberg. Also, a block of kidney was embedded

Second Term 1940, September to January 1941.

1 School opened Monday, September 9, 1940.
September 13, 1940 — Blocks A 1-14 were sectioned. Among them, small intestine and kidney of a male frog. (Slides VI/17, 22, VII/21, etc.) These had been prepared in June of the previous term with J. Jaquy, Bouin's fixation. The sections were not mounted, but floated in a pan of water till September 16, when they were mounted. and, on September 17, when the types of slides indicated above were stained at home with hematoxylin and eosin.
September 18, 1940 — the group prepared 105 slides for the Bio Dept consisting of different colored threads variously mounted. Study of stomach and kidney.
September 19, 1940 — Examination of I/8. Note possible granulation or cilia of cells of frog marrow at 430X.
September 20, 1940 — Examination at 970X of many slides, particularly I/8.
September 23, 1940 — A lecture meeting in the Histology Group, dealing with the use of the microscope, demonstrating the oil-immersion microscope, and lecturing on the structure of the elementary structures. Also, preliminary examination of prepared blood smears at 970X. There was also a discussion of Howell's Theory on Blood Coagulation.
September 25, 1940 - A lecture on the general structure of the cell and the theories of histological fixation and staining. Then, the mechanism of the microtome, and general basic histology technique.

continued - 9/25/40. Some sections were taken of tissues the same as previously ~~taken~~. Wright

- 9/27/40. Staining Technique; introductory. Group members stained sections previously sectioned.

- 9/30/40. Wright Stain Technique on blood smears; also examination thereof. General group work.

~~At~~ October 2, 1940. Various stains applied to Blood Smears to determine optimal staining: All were fixed in Absolute Methyl alcohol after the smear dried. Then dried again. Then stained, washed and dried. Stains used were picric acid, methyl violet, picric acid followed by Methylene Blue; light Green, Carbol-Fuchsin; methylene blue and alcoholic gentian violet. Nuclei of leucocytes seen best stained by methyl and gentian violet. These also stain the hemoglobin of the erythrocytes.

- October 9, 1940. Fixation - Theory reviewed; ~~Cooking~~ Entire histological technique demonstrated. All Group work.

October 14, 1940. Blood Histology for entire group. Demonstrate and explain slides made ~~therefore~~ previously thereof. Also group examination on Wright staining technique. Preparations 1, 2, 3, 4, 5, 6, 7, and 02 outlined on attached sheet were performed by the entire group. Slide I/8 on frog marrow was shown to demonstrate 01. Materials for 03 were not available. Results were highly successful, but detailed notes were taken more by the members of the group.

For two weeks we tried ~~to~~ get a rabbit at a usable time, and were unsuccessful until October 30, 1940. Also, Jewish holidays entirely disrupted a plan schedule: 10/3, 4; 10/17, 18; 10/24, 25; also, work was not allowed on 10/21/40

October 23 - Histology Theory review for Group. Also theory on connective tissue.

October 28 - Lecture from 10/23/40 continued; examination of slides showing cartilage, fibrous, areolar and elastic tissue. No rabbit was available.

~~October 30 - Same as October 28. Instructions regarding the work of Sheet Two were discussed~~

October - 30. A rabbit was finally gotten, so that preparations 1, 2 and 3, 4, and 6 on Sheet 2 were attempted, with reasonable success, except in the case of 3; there was no sunlight; dunlap-

ment did not proceed; 11 was available ~~with~~ from external sources. Cartilage was studied indirectly from organ slides – rat embryo, late chick embryo, larynx. In preparation 1 and 2, a peculiar type of striated fibre, resembling algae, was seen, still unidentified. Van Gieson's had to be prepared at the time, but not hastily.
November 1 – Acista . . .

November 4 – Lecture and slides on cartilage, bone and bone development, with 11 of Sheet 2. Slides on rat and chick embryo, long ago prepared, were most helpful. A number of stomach slides were restained with Van Gieson's, Methylene Blue-Acid Fuchsin, and Wright's stain.

November 8, 1940 – Stain sections of kidney with hematoxylin-eosin. Cursory study.
November 13, 1940 – Due to general lack of knowledge of anatomy in group, a rabbit and a frog were rapidly dissected with explanation.
11/14,15 – Midterms.

November 18, 1940 – Lecture on muscle structure (summary due to lack of facilities) and also of the alimentary canal in general.

11/20/40 – Lecture on structure of pharynx, esophagus and stomach, with examination of slides and discussion.
– in afternoon, at home, fix from a far from fresh frog, in 10% formalin, the: liver, spleen, pylorus region of stomach, tongue, lung, kidney, oviduct, sciatic (spinal) nerve, episternum, femoral and cardial regions of stomach. This is to be known as series B. Fixation proceeded to Monday, 11/25/40. Tissues whose sections do not appear in following accounts were discarded because of obvious decay before fixation.
Mond. 11/25 – series B transferred to 50% methyl alcohol for washing. Aid to Mr. Towne.
Tues, 11/26 – B series transferred to dioxane for dehydration overnight.
11/27 – placed in dioxane-paraffin for infiltration; later in afternoon, into molten paraffin over two nights, when, on
11/29 – the series, or a large part of it, was embedded and blocked off.

~~must did not proceed.~~

December 2, 1940. ~~Stain~~ Section blocks embedded 11/29/40. and mount on slides. Fix narcissus root tips in: Carnoy's, 10% formal, Zenker's, Bouin's,

T December 3, 1940. – Some sections were stained. 7% CrO_3, Flemming's achromatic, from blocks sectioned 12/2. The stomach was Zenker's fixatives. found to be somewhat autodigested with a little detail remaining, however (hem-eosin.) Fixed root-tips from yesterday washed in ~~5~~ 70% Methyl Alcohol, with Li_2CO_3 or Iodine where indicated. Stained were – stomach, kidney and oviduct. Cursory examination.

W December 4, 1940 – root-tips in dioxane; overnight, for dehydration. Stain Frog Liver, series B, hem-eosin; Frog spinal cord, series A with Methylene Blue. Section more stomach ~~(B)~~ liver, kidney, and spleen (B.) Oviduct was much ripped and wrinkled.

T December 5, 1940; root-tips in dioxane – paraffin, then paraffin for ~~infilt~~ infiltration; Buy slide labels. Bring classification to date, clean, relabel slides.

F December 6, 1940: Embed root-tips; Help Mr. Torme.

M December 9: Group members stain stomach, liver, kidney cells; + liver technique still imperfect, but fair.

W December 11 – Help Mr. Torme.

T December 12 – Section and stain root-tip (no. 4 – ~~on~~ fixed with Flemming's achromatic – ~~chrom~~ dichromate – chromic acid – acetic acid fixative. Results inconclusive. Also section Spleen B. Close group examination of slides ~~[illegible]~~ on Rat, chick and snake embryo respectively.

F. 12/13. Examine stained, mounted sections of Spleen B – with hem-eosin. "Sinuses and blood vessels are very characteristic, but well-defined. Malpighian bodies are not at first recognized. Capsule transmits few trabeculae. Dense pulp. Striated venous walls. Also, stain smear of blood of turtle. Apparently, type of amphibia. Rearrangement and house-cleaning of group property.

12/16/40. M — Aid Mr. Torme — preparation of glass holders with nichrome wires for bacteriology.

12/17/40. — Duodenum, Ureter, peritoneum, liver, and esophagus-trachea were taken from a rabbit and all fixed in Bouin's. Subsequently washed through to 70% Methyl Alcohol, wherein they now repose.

12/18/40 — Dissection and fixation in Bouin's of skin, peritoneum, liver, appendix, spleen, duodenum, pyloric, stomach, duodenum, pancreas, fat body (subcut), kidney, lung, heart, esophagus and trachea of a male white mouse. Subsequent washing; now in 70% Methyl Alcohol.
Fix in 10% formal, subsequent washing — the brain and high spinal cord.

Th. 12/19/40 — ~~[illegible]~~ "subsequent operations" of yesterday. ↑

F. 12/20/40 — Prepare slide of Paramecia caudatum, hematoxylin stain. Fix in Schaudinn's, wash through iodine alcohol, water. Stain, dehydrate in dioxane, clear in xylol, mount in xylol-balsam. Stain not too precise or clear. Also, stain stomach, pyloric B, in bem-rosin.
Take inventory.

Christmas vacation.

January 2, 1941. Group buys a quantity of chemicals, with personal funds.
1/3/41. Fr. Stain a section of Liver B with Iron-Hematoxylin. Mordant in 4% Ferric Alum, 25 min., wash one minute in tapwater; Stain 5 mins. in 1/2% hematoxylin aq.; differentiate to suit with Ferric alum, wash, dehydrate, etc.

Stain some wet blood smears fixed in absolute MeOH. Procedure as above for iron-hematoxylin.

Stain a number of specimens from a protozoa culture marked Spirostomum. Form resembles Blepharisma. Iron-hematoxylin. (See 12/20/40 for technique.)

1/6/41. Restain Spirostomum from 1/3 to satisfy. Mount in xylol-damar.

1/9/41. — Section, again, a number of blocks in series A, B, C (root-tip) (Date.)