of the chicken's body, hinged to one end (C). A long notch (D), 1¼″ across, cut lengthwise in the board with two circular perforations (E) located on either side and joined to it, provides a place for the legs and sternum when the chicken is in the ventral position, and for the vertebral column and ilium when in the dorsal position. The loop (C), which can be attached at various points (G) depending on the size of the chicken, fits over the body in either the dorsal or ventral position and holds it firmly against the board. It is fastened with the chain and spring (F). Another chain (L) holds the legs when a chicken is fixed in the dorsal position and may be used with the loop or alone for fastening the body to the board. It is particularly useful for restraining a chicken in either the dorsal or lateral position with the wings through the notch (D).

A detachable head holder (Fig. 2) in the form of a fork set at an angle of approximately 45 degrees to the parallel bars of the loop (C) may be easily adjusted by the set screws (M and N). By using this the head may be either raised well above the body or extended horizontally.

The board is especially useful for bleeding chickens from the carotid and wing vein. It can be used for operations, photography and for a variety of other laboratory procedures. It can be made by any worker in sheet metal, is easily sterilized and has few parts to get out of order. A larger size can be used for geese and turkeys.

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FREEZING TECHNIQUE FOR THE HISTOLOGICAL STUDY OF PIGMENTS IN AMPHIBIAN INTEGUMENT

IN a problem which is now in progress on pigmentation in Triturus it became necessary to make histological preparations. It was found that when the usual procedure of fixing, embedding and sectioning of tissue was pursued, the yellow and red pigment disappeared. This pigment, it was found, is very soluble in strong alcohols, cedar oil, aniline oil, xylol and strong formalin solutions. The frozen method of sectioning was then attempted. Trouble was encountered here. The skin is very thin and unless cut fairly thick (30 to 40 microns) would roll and wrinkle to such an extent during the process of washing and staining that the section would be made useless. This was remedied by placing the tissue in a weak solution of formalin for a half an hour to an hour. Too strong a solution of, or too long a time in formalin would cause the pigment to be dissolved. But a half hour in 10 per cent. formalin was sufficient and gave the desired rigidity to the tissue.

Other disturbing factors were that, first, when making sections in warm weather, the blade would melt the frozen block while cutting the tissue; and secondly, it was difficult to get the tissue off the blade before it melted. There was always great danger of damaging the tissue when taking it from the blade in the melted condition. These disturbing factors were remedied by our "ice dam."

The entire procedure for sectioning and staining is given below:

Place the fresh tissue in a 10 per cent. solution of formalin for half an hour to an hour.

Wash the tissue in water for an hour after taking it from formalin. Then place in a concentrated solution of dextrin for three to twelve hours.

Build on the microtome an "ice dam" of paraffin. This ice dam is made by warming some paraffin and shaping it into a long three-sided block. Place the base of the block on the blade and let the block extend almost the length of the blade. Curve the ends of the paraffin block over the edges of the upper portion of the blade in order that the dam may not slip. Make the dam high enough for the water from the melting ice to flow over the back of the knife. A few drops of dextrin solution on the inner edge will prevent water from running under the dam. Place small blocks of ice in the dam. Keep the cutting edge of the knife as dry as possible by continually blotting off the water of condensation.

To freeze the tissue: Place drops of concentrated dextrin solution on the freezing block of the microtome and open the valve very gradually to let the drops freeze slowly. Then place the tissue on the frozen drops and add dextrin slowly until the tissue is finally surrounded by a small block of sugar solution. The very best results are obtained when the valve is opened slightly. Not only is carbon dioxide wasted when the valve is opened so that a heavy blow of gas is obtained, but there is also the possibility of the tissue freezing too hard.

To obtain the maximum efficiency from the carbon dioxide, place the tank with the nozzle end at the level of the microtome and the base of the tank at least a foot higher than the nozzle level.

As soon as the tissue is frozen, begin sectioning. Hold the handle firmly in the right hand and pull the

knife across the block quickly. With a camel's hair brush in the left hand, quickly remove the section from the knife before it melts. If the section melts, one is likely to damage the tissue in brushing it from the blade.

Place the section in a syracuse dish of Ringer's or physiological salt solution. When a sufficient number of sections have been made, they may be stained.

By means of a small glass rod, headed on the end like a balsam dropper, remove the section from the solution. Let the section wrap around the beaded portion; it has been hardened enough in formalin to hold its shape fairly well. The section may be held up slightly in the solution by means of a dissecting needle while the glass rod is being slipped under it. With the section remaining on the glass rod, dip it into the stain for ten seconds (the stain I found best for this tissue was polychrome methylene blue). From the stain place the section into Ringer's or physiological salt solution. It is all right to let the section drift in this solution, for the stain gives it added rigidity so that it may easily be picked up again with the rod.

Now, still by means of the glass rod, transfer the section to a slide on which is a drop of Brun's Glucose Medium. By rolling the rod through the drop of liquid the section can be made readily to come off of it. (Glycerine alone takes the stain from the tissue.) A cover slip may now be placed over the tissue. This must be done carefully, for the tissue is often wrinkled in the placing of the cover slip.

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SPECIAL ARTICLES

THE CULTIVATION OF A SPECIES OF TROGLODYTELLA, A LARGE CILIATE, FROM THE CHIMPANZEE

THE examination of several chimpanzees at this laboratory recently revealed that one was infected with a large ciliate of the genus *Troglodytella*. Reports of such an infection are not numerous and the organism seems to have been very little studied. Although this species has not been definitely determined it has been studied alive and stained and found to answer very closely the description of *T. brassarti* var. *accuminata* Reichenow.[2,3] Attention was at once directed to it in the hope that it might be cultivated and be used for further studies.

At the time of the discovery, *Balantidia* from other individuals of the same group of chimpanzees were being cultivated. In this preliminary work the *Troglodytella* has been cultivated for a short time following the same technic that was used in the cultivation of the *Balantidia*. The medium which has proven to be most satisfactory in the writer's experience for the *Balantidia* has also given the most encouraging results for the *Troglodytella*. This medium is that of Tanabe and Chiba[4] for the cultivation of *E. histolytica*. Pig serum only has been used in these experiments.

Greatest success was obtained in an experiment in which six test tubes containing 10 cc of solution were inoculated with about 1 gram of fecal material containing *Troglodytella* and a few *Balantidia*. These tubes were incubated at 37.5° C. The results were as follows:

Tubes	Serum	24 hrs.	48	72	96	120	144	168	172
1	5 per cent.	+	+	+	+	+	+	+	–
2	5 " "	+	+	+	+	+	+	+	–
3	5 " "	+	+	+	–				
4	10 " "	+	+	+	+	+	+	+	–
5	10 " "	+	+	+	–				
6	10 " "	+	+	+	–				

In 24 hours many very active *Troglodytella* and a few *Balantidia* could be seen swimming in all the tubes. Subcultures were made at this time from tube 4, which seemed to contain the most ciliates. In 48 hours the *Troglodytella* had apparently increased in numbers and a number of dividing individuals were seen at this time. The ciliates were very active and some could be seen boring into the agar slant. The *Balantidia* had also increased in numbers. At the end of 96 hours all ciliates in three tubes had died and in the other three the *Balantidia* had multiplied much more rapidly than the *Troglodytella* and outnumbered them. The latter, however, were still active and both *Troglodytella* and *Balantidia* could be seen

[1] From the department of protozoology, Johns Hopkins School of Hygiene and Public Health. The writer wishes to express his appreciation to Drs. Van Volkenburgh and Long and the Committee on Cold Research, from whose chimpanzee the material for this work was secured.

[2] E. Reichenow, "Den Wiederkäuerinfusorien verwandte Formen aus Gorilla und Schimpanse," *Arch. f. Prot.*, 41: 1–33, 1920.

[3] J. Buisson, "Les infusoires ciliés du tube digestif de l'homme et des mammifères," Trav. Lab. Parasit. Fac. Méd., Paris, 1923.

[4] M. Tanabe and E. Chiba, "A New Culture Medium for *Endamoeba histolytica*," *Acta Med. in Keijo*, 11: 1–4, 1928.