other parts of Alaska. Neither have we an explanation for the behavior of *articum* and/or *corbis,* so far reported only as a crawling nuisance during the summer but biting readily in the Anchorage area in late fall at air temperatures well below the minimum temperature for flight activity recorded earlier in the season. *P. hirtipes* has also shown the same tendency, biting much more readily in late October than in September.

Many of the observations reported here are contradictory, and the importance to be assigned to Alaskan blackflies is still a matter of conjecture. It is quite possible that the differences in the behavior of a species at different times and places reflect racial adaptations to local conditions. The fact that *venustum* readily bites man at Kotzebue though not elsewhere in Alaska is suggestive. Since pre-Columbian times the human population of interior Alaska has consisted of scarcely more than a few hundred people scattered over a vast expanse of land in which wildlife, including several species of the larger mammals, has been relatively abundant. In such an area there would be little opportunity for blackflies to feed on man. By contrast Kotzebue has been the site of a sizeable village for at least 700 years.

If the question is one of host preference, then the importance of blackflies can be expected to increase rapidly as the population of Alaska grows. Such growth is already proceeding at a rapid pace and will doubtless be accelerated by the increasingly great strategic and economic importance of the Territory. With a larger population the wild host animals will in all probability become less numerous, and the blackflies may be expected to turn, from necessity, to feeding on man and his domestic animals.

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PREPARING MOSQUITO EGGS FOR EMBRYOLOGICAL STUDY

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There is, at present, a great deal of interest in the biology of mosquitoes. To understand their development, methods of preparing the eggs for embryological study are needed. DeCoursey and Webster (1952) have described a method for clearing the chorion of *Aedes sollicitans* (Walker) which permits a rough appraisal of the degree of development. McLintock (1951) had done some work on the egg shells of *Culiseta inornata* (Will) using a modification of the Crabb (1949) technique of double imbedding in celloidin and paraffin for sectioning. This method is satisfactory for the very early stages of the *Aedes hexodontus* Dyar eggs used in the present writer's work but does not give good results after the chorion has completely hardened. With Japanese beetle eggs Gese (1952) used a cedar-wood oil process prior to imbedding which softens the chorion and permits sectioning. This, however, is not effective with *Aedes hexodontus* eggs. Most of the workers on *Drosophila* use dechorionated eggs which fix and imbed readily with standard techniques (Demerc, 1950). Mosquito eggs can be dechorionated but only after they are 48 hours old when the transparent embryonic cuticle, which is resistant to sodium hyperchlorite, has formed. Fixatives enter the dechorionated egg rather

readily and it is not difficult to section them. If the eggs are not dechorionated the problem begins with fixation. Since no known fixative will penetrate the eggs in less than two or three hours and most fixatives require days, the eggs must be punctured with a fine needle while in the fixative. Since puncturing must be done with the stages prior to the formation of the cuticle it is convenient to puncture all the material and this means that the chorion has to be dealt with in sectioning. With standard techniques paraffin enters through the puncture and infiltrates the yolk and developing embryo but there is little or no infiltration of the very dense chorion. When sectioned, the hard, brittle chorion breaks apart and falls out of the imbedding block damaging the section. Although a few sections per egg may be usable, the majority are worthless. To improve the infiltration of the chorion, clearing agents such as toluol, benzol, naphtha, chloroform and dioxane as well as xylol were used with no added success. Infiltration under a partial vacuum gave no better results. To hold the chorion in the imbedding block a mixture of beeswax and paraffin, half and half, was tried but it was difficult to work with and showed little improvement over paraffin.

A satisfactory method was finally discovered using Mayer's chlorine technique as described in Lee (Gatenby & Beams, Ed. 1950) which removed the pigment from, and softened the chorion. The eggs were taken from the 70 percent alcohol in which they were stored and placed directly in the bleach. In about one half hour the chorion became light brown and transparent, the results being similar to those shown in the photographs published recently by DeCoursey and Webster (3) using aqua-regia. No harm resulted to the tissue even if left in the bleach for an hour. The eggs were then washed in 70 percent alcohol, dehydrated in absolute alcohol or carbol-xylol and cleared in xylol. In xylol, morphological features could be studied through the light brown chorion. When the bleached eggs were infiltrated with paraffin for from 2 to 12 hours they gave good sections in nearly all of the cases. Since there was a tendency for the sections to wash from the slide during the staining procedure they were attached with celloidin. The tissue stained readily with Feulgen and hematoxylin stains.

Another satisfactory method of sectioning the eggs involved double imbedding in plastic and paraffin. The eggs were dehydrated in absolute alcohol and placed in a solution of n-butyl methacrylate and catalyst, using a gelatin capsule of convenient size as a mold. The plastic used was n-butyl methacrylate obtained from Rohm and Haas Co., Philadelphia. Ten cubic centimetres were mixed with 0.3 gms. or less of catalyst. The catalyst was Luperco CBD obtained from the Lucidol Division, Novadel-Agene Corp., Buffalo, New York. The mixture remained unpolymerised at 4° C. and polymerised to the solid at 50° C. The capsules were covered and placed at 50° C. and in eighty percent of the cases the plastic hardened in from 6 to 8 hours. Those capsules which did not harden were drained and refilled with new plastic. The hardened capsules were placed in water up to the lip until the gelatin softened and could be removed. The plastic remaining was trimmed, dehydrated in absolute alcohol for 2–3 minutes and placed in xylene for the same amount of time. It was then placed in melted paraffin for no more than 2 hours and imbedded in the standard manner. Sections were easily made at 10 microns. It was found convenient and almost necessary to run the sections onto the surface of water in a box attached to the microtome knife as is done in sectioning material for the electron microscope (Beams, *et al.*, 1952). If water was not used the static electricity generated by the plastic made the ribbons most difficult to handle. The ribbons readily spread on warm water and became firmly attached to a clean slide or a slide coated in egg albumen. Both the paraffin and the plastic

were easily removed in xylol in 15 to 20 minutes and if the slide was coated with celloidin the sections remained in place.

In the method where the chorion is bleached the chorion may sometimes separate from the surrounding paraffin and curl. In the plastic and paraffin technique the inner tissue sometimes becomes detached from the more firmly held chorion and may become distorted. Comparing both methods the saving in time and the ease of handling the material makes the bleaching technique the most practical for mosquito eggs. The double imbedding with plastic and paraffin may prove to be of value in other situations where a brittle object is to be sectioned serially.

Since sections with the chorion intact made observation of the peripheral embryonic tissue difficult, efforts were made to find some way to remove the chorion and not injure the enclosed tissue. Sodium hypochlorite would dissolve the chorion but entered the interior of the egg through the puncture and at all concentrations destroyed the tissue. However, a method was developed in which the egg was imbedded in soft plastic (Beams *et al.*, 1952) and then dissected out of it leaving the inside infiltrated but exposing the non-infiltrated chorion. The eggs were then placed in sodium hypochlorite with the object of dissolving away the exposed chorion while the enclosed tissue was protected by the infiltrant. Since it was previously known that the plastic would be removed in xylol, toluol, or acetone, the easily sectioned embryo, left when the chorion dissolved away, could then be imbedded in paraffin. Although the method proved effective in some cases where great care was exercised in dissection, in the majority of cases some disruption of the plastic could not be avoided, especially in the region of the puncture in the egg where continuity existed between the plastic enclosed within the chorion and that surrounding it. This disruption created capillary pathways for the entrance of sodium hypochlorite and subsequent destruction of at least a region of tissue around the disrupted area. However, the amount of damage was usually not great enough to prevent using the egg as a whole mount for morphological studies. The resulting pellet was transparent and could be handled and mounted with ease. If some manner of sealing the capillary pathways by which the sodium hypochlorite enters is found the method could then be used to obtain superior sections.

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