ture, the character Di^a (+) is present in 20.39 percent.

Inheritance of the Di^a (+) factor was studied in four families by Layrisse, Arenas, and Dominguez Sisco in 1955 (*6*), and later, in 1957, Lewis, Kaita, and Chown (*7*) gave a large pedigree formed by 50 Japanese families. The present study deals with the testing with anti-Di^a serum of 30 Indian matings with 62 children. The results are shown in Table 1 (*8*).

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22 January 1959

Experimental Infestation of Peromyscus leucopus with Larvae of Cuterebra angustifrons

Abstract. Four newly hatched larvae of the warble fly, *Cuterebra angustifrons*, were successfully introduced into the mouse host, *Peromyscus leucopus*, by application to the belly skin, the mouth, and the nose. Regardless of the site of entry, each larva migrated to the inguinal region of the host and completed its development normally.

Cuterebrid flies are widely distributed in North and South America. Their larvae are found encapsulated in the subcutaneous tissues of many rodents (chipmunks, deer mice, pack rats, and so on), rabbits, and occasionally dogs, cats, and other mammals. Larvae of two species of the genus *Cuterebra* (*C. angustifrons* and *C. grisea*) have been found infesting the common deer mouse of wood lots in southern Ontario, *Peromyscus leucopus noveboracensis* (*1, 2*). Maturing larvae are usually observed beneath the skin of the inguinal region but have been seen elsewhere on the body of the hosts. After gaining entry to the host, larvae wander beneath the skin for the first 7 to 8 days and are not visible externally during this period. They then make a small hole in the skin, to which they apply their posterior ends for respiration and excretion. At this time they are in the second instar (recognizable by the characteristic stigmal plate pattern) and may remain in this stage for up to 8 days. After molting to the third instar (recognizable by the change in the stigmal plate pattern) they begin the final phase of larval development, which lasts an average of 12 to 13 days but may vary from 8 to 17 days. When mature, the third instar larva drops from its host, burrows into the ground, and pupates. The time for total development of the larva in its host varies from 19 to 44 days, but probably averages between 25 and 30 days (*2*).

Numerous adult flies have been reared over two winters, but attempts to mate flies in the laboratory have been unsuccessful to date (*2, 3*). Adult flies were not seen in four summers of observation in wood lots from which infested deer mice were trapped. No eggs were seen on the hair or skin of some 1000 deer mice, chipmunks, and other small mammals live-trapped during four summers (*3*). Beamer *et al.* (*4*) found eggs of *Cuterebra beameri* affixed to brush in and around the entrances to burrows of the pack-rat host. Presumably, eggs of cuterebrids are not laid on the bodies of their mammalian hosts. It has been demonstrated that larvae of *C. tenebrosa* may enter the host by mouth (*5*) or by penetration through the skin (*6*); those of *C. angustifrons*, by nose (*7*).

We were fortunate in capturing a female *C. angustifrons* in a cabin at Sparrow Lake, Ontario, 5 Sept. 1958. Since obtaining fertile females for experimental infestations is difficult, in that these flies have not as yet been mated in captivity and fertile females are only rarely taken in the field, we think the following observations worth reporting.

The fly began to oviposit within a few minutes on moistened tissue paper in a glass tumbler and laid a total of 86 eggs within 24 hours. She died 24 hours later. The eggs were placed in a humidor above a saturated solution of sodium chloride (relative humidity approximately 83 percent). Several eggs were examined on the sixth and seventh days after oviposition. Fully formed larvae were found in five eggs; three others showed no development and may have been infertile. One of the larvae, when exposed by removal of the operculum, exhibited feeble movements but died without escaping from the egg.

On the 12th day after oviposition, a larva was seen to have hatched. A closer check revealed three more larvae, one of which was observed in the act of pushing off the operculum and escaping from the egg. Two other larvae had hatched but died before they were found. An examination of the remaining eggs showed that many contained fully formed embryos, but all of these appeared to be dead.

Laboratory-raised *Peromyscus* juveniles were used as hosts for available larvae. Mouse No. 441-48 was prepared by clipping its belly hair and lightly anesthetizing it with ether. A larva was transferred on the moistened tip of a dissecting needle and placed on the dry clipped area. It seemed to be unable to navigate and struggled helplessly among the short clipped hairs. Moistening the hair with water seemed to help, but it still made no attempt to penetrate the skin. A slight abrasion was made in the belly skin. The larva was placed close to but not in the wound. However, fluid from the wound bathed it and it began to burrow into the unbroken skin. When about half buried it remained inactive for a minute or more, and during this time the mouse moved out of the field of the dissecting microscope. When it was again brought into view, the larva was no longer seen. It was not certain at this time whether the larva had been dislodged or had gained entry to the host.

A second larva was placed between the nostrils of mouse No. 431-58. It appeared to be quite active on the moist tip of the nose and moved quickly toward one of the nostrils. The mouse moved out of the field of observation, and the larva had disappeared from view when observations were resumed.

The third larva was placed in the mouth against the lip of mouse No. 432-58. It seemed to be stimulated by the moist environment and was observed to move out of sight along the side of the tongue towards the back of the throat.

With the last larva available, a second attempt at nasal transplant was made. The larva was seen to disappear actively down the nasal passage, but the mouse failed to revive from the anesthetic, even though oxygen was applied. About 20 minutes after the attempt, the dead mouse was necropsied, and the larva was found embedded in the tissue of the nasal septum about ¼ in. from the naris. It was motionless and appeared to have a gas bubble in its alimentary tract. After being removed from the nasal tissues, the larva revived somewhat and was transferred to the tongue of mouse No. 435-58. In this case no anesthetic was used, and the mouse appeared to swallow the slowly moving larva.

Larvae from the first three transfers reappeared in the inguinal region 8 days after entry via skin, nostril, and mouth; in the fourth instance, 12 days elapsed before the larva reappeared. In the first three cases, the molt from second to third instar was observed on the 11th day after experimental infestation; in the fourth case, the molt occurred on the 15th day. There was no significant difference in the length of the third instar —13 days for the first three larvae, 14 days for the fourth. Three of the four larvae dropped on the 24th day, the fourth on the 29th day, after infestation. Three of the four successfully pupated,

but all were dead at the time of this writing. The extended period of early development of the fourth larva in contrast to that of the others may have resulted from adverse effects of extra handling, ether anesthesia, application of oxygen, or a combination of these factors.

It is possible that direct application of moisture is necessary for successful hatching of cuterebrid larvae from the egg, as indicated by the erratic hatching observed in this work. In nature, eggs laid on vegetation would be subject to the influence of dew. Further, they may be ingested by mice in connection with lapping of dew or ingestion of vegetation and may hatch in the mouth or esophagus. On the other hand, larvae may be stimulated to hatch by the action of the tongue alone or through application of moisture by the tongue. Finally, Gregson (*8*) has suggested that the mechanical stimulus of a host brushing past the eggs may be a factor in hatching.

It is interesting to note that all four experimentally introduced larvae reappeared and settled beneath the skin of the inguinal region for development, two on the left side, two on the right. In nature, at least 80 percent of the larvae are seen in the inguinal region, equally divided between the right and left sides. Possibly significant physiological and biochemical differences in the environment beneath the skin of various regions of the body may be factors in this striking regional preference, causing some larvae to reappear beneath the skin close to their point of entrance (perhaps as a result of it), others to migrate a short distance, and most to reappear in the groin. Perhaps many larvae, no matter how they gain entrance to the host, are influenced in their early movements beneath the skin by sensory cues derived from the movements of the body muscles of the host and, in this way, are guided to the inguinal region.

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References and Notes

1. Acknowledgement is made to the Ontario Department of Agriculture for support of phases of this project through allocation of funds to Ontario Agricultural College; to Dr. W. E. Heming, head of the entomology and zoology departments of Ontario Agricultural College, for his sincere interest and encouragement; to the Ontario Research Foundation for specific material aid; and to Miss Helen Shutsa and student assistants for technical aid.
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24 February 1959

Fate of Frog Embryos Implanted into Forelimbs of Adults

Abstract. No teratoma formation and very little growth and differentiation followed implantation of late gastrula-stage frog embryos into forelimbs of adults. This is attributed to poor blood supply and rigid walls of muscle and bone surrounding the implants. The fate of embryos in nonamputated limbs is compared with that of regeneration-promoting implants in amputated limbs.

Several investigators reported formation of tumors following implantation of embryonic material into adult amphibians. Allison (*1*) observed uncoordinated masses of growing tissue which developed from embryonic transplants to the coelom or orbit of larvae or adults, with metastases and infiltrating growth in larval hosts but none in adults. Fankhauser and Stonesifer (*2*) produced teratomas by implanting newt embryos under the skin of the lower jaws of adults. It has been reported that embryonic implants into adult limbs are capable of promoting regeneration of these limbs following their amputation (*3*). The present study deals with the fate of embryos implanted into adult limbs which were not subsequently amputated.

Ten adult *Rana pipiens* served as hosts. Late gastrula-stage *R. clamitans* embryos served as donors. The adults were anesthetized with ether, and longitudinal incisions on the dorsal surface of the left forelimbs were made. The radioulnas and associated arteries and nerves were exposed. Muscles and skin were retracted with small hooks, and donor embryos were placed in these artificially created pockets. The embryos had been denuded of their jelly capsules and vitelline membranes prior to amputation. The wounds were closed with silk. Aseptic technique was used throughout. The wounds healed within 2 or 3 days without complications. The stitches were removed as soon as primary healing took place. The animals were killed at various intervals for histological examination, the last one 3 months postoperatively.

No inflammatory reaction was observed around the implants. The operative wounds healed by primary intention with a minimal amount of scarring. The implanted embryos were clearly distinct in all specimens. Embryonic differentiation and resorption were almost entirely absent up to 3 months following implantation. The only attempts at differentiation noted were formation of a cavity lined by cells, resembling ependymal cells, and a few muscle fibers within the implants. No differentiated nervous elements, notochord, digestive tract, or cartilage were present in any sections. The epidermal covering was lost soon after transplantation. The embryos did not increase in size. The implants were composed mainly of numerous strongly basophilic yolk granules and large round cells with vesicular nuclei. There was no clean and obvious line of demarcation around the implants, and it was impossible to ascertain which cells arose from the implants and which from the host. There was no foreign-body reaction in the host. No invasive growth was observed in any of the implants.

The fate of embryo and larval implants was considerably different in amputated limbs (*3*). Implants remained viable for a prolonged length of time, contributed cells to the regenerating limb bud, and finally blended with the host tissues. The blood supply to the regenerating stump was very good. No neoplastic change was observed in any of these implants.

Very little differentiation and no invasive growth or teratoma formation were observed in the embryos implanted into adult frog limbs. This could be explained by the unusual site of implantation. The embryos were surrounded by rigid walls composed of muscle and bone, which gave them little or no opportunity to expand. In contrast to the situation with amputated limbs, the blood supply to the areas of implantation was exceedingly poor. This of itself could account for the retardation in growth and development of the implants. It is suggested that the embryos received enough oxygen and nutrient materials by diffusion to keep them alive but that the amounts were grossly inadequate for further growth and differentiation.

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