Transplantation of Adult Filarial Worms, *Litomosoides carinii,* in Cotton Rats[1]

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The experimental study of filariasis is handicapped by the prolonged period of development of the filarial worm in man and other animals. Among laboratory animals the dog may be considered a suitable subject for experimental purposes, but the location of the parasite in the pulmonary artery limits observation, is too perilous for manipulation, and does not resemble that of the filarial worms inhabiting man. In recent years the finding of a filarial worm commonly occurring in the pleural and pericardial cavities of the cotton rat, *Sigmodon hispidus,* puts at our disposal an infection which resembles in many ways certain forms of human filariasis. With the view of studying some aspects of this infection, we have resorted to an unorthodox approach which consists of the transplanting of the adult worms from one definitive (*i.e.* final) host to another of the homologous species, which, with a metazoan parasite, is a departure beset with implications.

Cotton rats infected with *Litomosoides carinii* were etherized, tied to a board, and shaved over the thorax. The body was swabbed with a solution of phenol and the thoracic area washed over with alcohol. A slit was made along the midanterior line with scissors and continued along the sternum up to the clavicle. The sides were pinned down, exposing the pleural cavities, which, after this procedure, should be free of blood. The worms were withdrawn aseptically with light forceps or, better still, with a hooked rod and placed in sterile saline in several Petri dishes.

The rat into which the worms were to be transplanted was etherized, tied to the board, shaved and swabbed, and punctured with a thin blade in the central area of the right anterior thorax. By means of blunt hooks the opening was kept sufficiently large to insert 5 to 25 worms with the aid of a blunt probe and with a minimum amount of injury to the worms. The wound closed and healed without aid.

The attempt to transplant live adult *Litomosoides carinii* from the pleural cavity of an infected cotton rat into that of a normal cotton rat has so far failed in 10 animals. As early as three days following transplantation, the worms are dead and are found to be accumulated in a single mass which progressively becomes a syncytium of degenerating worms, as determined from later observations with other treated animals. Live microfilariae, however, may be found for a week or longer in the pleural cavity and in the blood. There is no gross exudation of fluid into the cavity, which appears perfectly healthy, with the syncytium of worms cleanly isolated in a serous covering.

In contrast to the negative results in normal rats, successful transplantation of the adult worm was accomplished in splenectomized cotton rats, the reticuloendothelial system of which had subsequently been blockaded with India ink. Live worms in variable numbers were recovered from the pleural cavity in four out of nine treated rats, with indications that in one other case the worms survived sufficiently long to migrate into the left cavity. In the course of these observations it was noted that the male worm often survived assaults of the defense mechanism of the host which destroyed the females, whose exudations appeared to be much more irritating than those of the males. Worms transplanted into one side of the thorax are later found within both cavities in approximately equal numbers, and may be seen to migrate across the mediastinum, through the fatty tissues, or even between the parietal pleura and the diaphragm, emergent worms having been seen partly through these structures.

Successful transplantation has also been achieved in two out of three rats as the result of X-irradiation with 140 kv., 5 M.A. (without filters) at 25 cm., using a large cone (over 7 cm.) and delivering 120 r per minute. The type of cell and tissue most affected by this treatment, as contrasted with that of blockade, offers significant data in considering the resistance manifested by the host under normal and experimental conditions.

Success has also attended transplantation from one to another naturally infected rat. Also, rats injected four to five times in the course of as many weeks with a suspension of *Dirofilaria immitis* contained in a mixture of Falba, mineral oil, and killed tubercle bacilli (*1*) have accommodated transplants of live adult *Litomosoides carinii,* and in one case lymph node invasion has been observed.

Pathological findings will accompany more detailed publication.

Addendum: Since this article was accepted for publication, Scott and Cross (*2*) have described a tumor in the superior mediastinum of old cotton rats, caused by dead adult filarial worms, and have assumed the neutrophil-infiltrated tumor to have been caused by the dead worms. In view of this observation, it has been thought advisable to note here certain general findings on the pathology of filariasis in the cotton rat which have been made in a number of animals natu-

[1] This work was done with the aid of grants from the Marcelle Fleischmann Fund for the Study of Immunologic and Allergic Phenomena in Tropical and Parasitic Diseases and the American Foundation for Tropical Medicine.

rally and experimentally infected with *Litomosoides carinii*.

Scott and Cross's observation occurs not infrequently in our experience as a result of worms dying in transit through the fatty lymphoid tissue in the region of the superior mediastinum near the hilus of the lung. This is accidental. But a characteristic feature of the infection is a generalized or spotty proliferative reaction of the visceral and parietal pleura which, in its final phase, results in papillary nodulations of the superfices. Neither the worms nor the nodules invade the lung proper, though it shows reaction to the infection. The spleen is hypertrophied.

References

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The Effect of Thiouracil Upon Pigmentation in the Tadpole

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In the course of experimental work concerning the effects of thiourea and thiouracil[1] upon the thyroid glands of tadpoles (*Rana sylvatica*), the latter drug was found to cause pigmentary changes which seem worthy of record.

The tadpoles used in this work were obtained from eggs collected in near-by Maryland. The animals were raised in large finger bowls with 20 tadpoles in each bowl and were fed a diet of boiled spinach, Pablum, and boiled egg. Ten of the cultures served as controls, being kept in tap water; in the case of 10 others the culture fluid was a .05-per cent solution of thiourea, and that of another 10 was a .05-per cent solution of thiouracil. These treatments were started on 19 March 1945, when the tadpoles were in the tail-bud stage. The culture fluids were changed every other day. On 4 April, 16 days after the beginning of the experiment, it was noted that the animals in the thiouracil series were considerably lighter in color than were those in the other two groups. This difference became more striking, so that by 14 April (26 days) all tadpoles of the thiouracil series showed very marked blanching with the melanophores contracted in a manner similar to that seen in hypophysectomized specimens. By this time the inhibiting effect of the drugs upon metamorphosis, reported by other authors (*1, 2*), was clearly evidenced, the controls showing rapid growth of the hind limbs, while the experimental animals of both sets had only rudimentary limb buds. Detailed results of the experiment as they relate to the thyroid and metamorphosis will be reported elsewhere. By 4 May (56 days) 80 per cent of the controls had metamorphosed while none of the experimental animals had shown any signs of metamorphosis. At this time, therefore, the treatment was discontinued and the experimental animals transferred to tap water. The thiourea-treated tadpoles did not begin to metamorphose despite discontinuance of the treatment, and on 27 July (129 days) no increase in hind-limb length had occurred in this group. This does not accord with previously reported results (*1*), but the difference may be related to the slightly higher concentration used in the present experiments. The animals of the thiouracil series, on the other hand, showed signs of the initiation of metamorphic changes very quickly after their removal to tap water. By 26 May (67 days) 50 per cent of these specimens exhibited definite elongation of the hind limbs, and by 11 June (83 days) 80 per cent had metamorphosed. Moreover, these specimens resumed the normal dark color quite rapidly, so that within a week after the thiouracil administration ceased, all of the tadpoles showed the same degree of expansion of the melanophores as had been seen in the controls.

It appears that thiouracil has some specific effect upon the melanophores of the tadpole and that such an effect is not produced by a similar concentration of thiourea. Work is now in progress to ascertain whether this action is exerted directly upon the melanophores or indirectly through the intermediation of the pituitary gland.

Juhn (*3*) has reported an effect of thiouracil upon the pigmentation of the feathers of Brown Leghorn capons, but this is apparently to be attributed to the inhibition of thyroid function by the drug, since it is similar to the pigmentary change which follows thyroidectomy in this animal.

[1] The drugs used were supplied through the courtesy of the Lederle Laboratories, Inc.

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