

Laboratory Transmission of Fibromas (Shope) in Cottontail Rabbits by Means of Fleas and Mosquitoes. (20339)

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Transmission of myxoma virus by mosquitoes, demonstrated by Aragao(1) in Brazil and recently in further detail by Fenner, Day and Woodroffe(2) in Australia, suggests that fibroma virus(3), a related agent, might be similarly transmitted. As will be shown *Aedes aegypti* proves to be a good vector of fibroma virus from cottontail to cottontail (*Sylvilagus sp.*). Fleas collected from wild cottontails also transmitted virus, although results were less striking. These findings indicate the probable importance of biting insects as vectors of this disease, the natural transmission of which is imperfectly understood.

Materials and methods. *Virus:* Fibroma virus employed for insect transmission was originally recovered from a wild cottontail captured at the Patuxent Research Refuge, Laurel, Md., and kindly given to us by Dr. Carlton M. Herman. Passages were made by intracutaneous inoculation of cottontail rabbits. Fibromas used for insect transmission were from 2 cottontails inoculated 48 to 63 days previously. The OA strain of Shope fibroma virus was used in serum neutralization tests. *Rabbits:* Cottontail rabbits were obtained from dealers in Kansas and Arkansas. Serum neutralization tests were performed in white domestic rabbits. Rabbits of both types were prepared for experimentation by removal of hair from the sides with clippers. *Neutralization Tests:* Sera from cottontails used in transmission experiments were tested for specific neutralizing antibodies after inactivation by heating for 30 minutes at 56°C. Undiluted serum was mixed with equal volumes of OA fibroma virus, prepared so as to give final dilutions of 10^{-3} and 10^{-4} which represented 100 and 10 infecting doses in these tests. Serum-virus-mixtures were inoculated intracutaneously into white rabbits in amounts of 0.5 ml. Results were read in 5 days by

noting presence or absence of fibromas. *Fleas:* Fleas were obtained from wild cottontails caught in box traps in the vicinity of Rockville, Md.,‡ and transported in muslin bags. No attempt was made to identify fleas during experimentation, but as many as possible were recovered for identification.§ Three species were involved: *Cediopsylla simplex* (Baker); *Odontopsyllus multispinosus* (Baker); and *Hoplopsyllus affinis* (Baker); the first 2 species predominated. *Mosquitoes:* The mosquitoes, *Aedes aegypti* (Linnaeus), were of an old established laboratory strain. The adults were fed on raisins and through cloth on 5% sucrose solution, and were maintained at approximately 28°C and 70% relative humidity. Unless otherwise stated, the mosquitoes were fed on hosts individually. Each mosquito was confined to a small space near the open end of a 20 mm diameter culture tube, and fed through gauze covering. Those allowed to take full blood meals were confined in like manner to 45 mm diameter tubes, in groups of 5.

Results. Transmission by pins. Fibromas were induced in 3 cottontail rabbits by sticking pins to a depth of a few mm into a fibroma on an infected cottontail and then pricking the skin of a normal animal in similar fashion. After this procedure there was conspicuous oozing of blood from the fibromas but not from normal skin. A large triangular pin led to tumors 1 to 2 cm in diameter which had a gross and microscopic appearance characteristic of Shope fibroma, although they developed a crust and were regressing within 30 days. Finer pins of stainless steel induced smaller tumors on 2 additional rabbits. One cottontail died accidentally, but both of the

‡ The animals were loaned to us by the Maryland Inland Fish and Game Commission.

§ Fleas were identified by C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

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others developed specific neutralizing antibodies along with growth of the fibromas. These preliminary experiments gave encouragement that insect transmission might succeed.

Transmission by fleas: Four cottontails were used in experiments in which flea transmission was attempted by 2 different methods. In initial experiments about 8 fleas were placed in gauze covered glass tubes (4 cm in diameter) which were then inverted over a fibroma allowing the fleas to feed through the open end. As gauze seemed to interfere with feeding it was later dispensed with and fleas were recovered with a suction tube to prevent escape at the termination of feeding. After a delay of 3 or 4 hours, fleas previously fed on an infected rabbit were then held over the skin of a normal cottontail. Each feeding episode lasted about 30 minutes, during which time about half of the fleas appeared to feed. None of these experiments in which the fleas were confined to tubes during both feedings was successful. Recipient cottontails developed neither lesions nor immunity. Successful results were obtained when a fourth cottontail was placed in a flea-proof cage together with fleas which had previously fed on a fibroma. A total of 25 fleas was used on successive days and some were recovered, refed on fibromas, and replaced in the cage. Two and a half weeks later numerous scabby thickenings appeared on the skin of the cottontail, especially at bases of both ears, on one shoulder, and on both outer thighs. These lesions regressed in several weeks at which time serum from this animal, which had previously been negative, neutralized over 100 infective doses of OA fibroma virus. Subsequent to bleeding, the rabbit was challenged

with virulent fibroma virus and found to be immune. Although biopsies were not obtained, we have not encountered such lesions on rabbits infested with fleas. As discussed below, it seems possible that flea bites induce a shallower type of lesion than the more penetrating bites of mosquitoes.

Transmission by mosquitoes. In a preliminary experiment, 6 tubes with 10 mosquitoes each were held for a partial feeding on a fibroma (cottontail No. 343) and then immediately afterwards on the skin of a normal cottontail for completion of the feeding. Four other tubes of partially fed mosquitoes were kept 24 hours before being held on the same rabbit. Six fibromas, the first appearing in 8 days, resulted from the immediate transfer and 2 from the 24-hour delayed transfer, after interrupted feedings on the fibroma. These tumors enlarged to a maximum of 2 cm in diameter, remaining undiminished in size after 80 days. Specific neutralizing antibodies against the OA strain of Shope fibroma virus appeared in this rabbit within 4 weeks. Mutual interference in feeding resulted when many mosquitoes were held in a single tube. In later experiments mosquitoes were kept in individual tubes, which facilitated observations on the number of mosquitoes actually feeding on both fibroma and recipient cottontails.

The efficiency of *Aedes aegypti* as a vector of fibroma virus was demonstrated over various time intervals in a second set of controlled feeding experiments (Table I). Each day's experiment was performed on a fresh cottontail and was limited to mosquitoes which had had only one previous blood meal on a fibroma. This initial feeding was interrupted in the case of mosquitoes refed immediately,

TABLE I. Results of Single Feedings of Mosquitoes (*Aedes aegypti*)* on Recipient Cottontails after Initial Feeding on Cottontail Fibroma.

Initial blood meal	Interval before feeding on recipient	No. mosquitoes feeding on recipient	No. fibromas transmitted	Ratio of No. fibromas transmitted to No. mosquitoes feeding
Interrupted	Immediate	15	18	1.20
"	24 hr	17	15	.88
"	4 days	32	18	.56
Full	1 wk	24	16	.67
"	2 "	16	13	.81

* Mosquitoes 5 days of age at time of initial feeding.

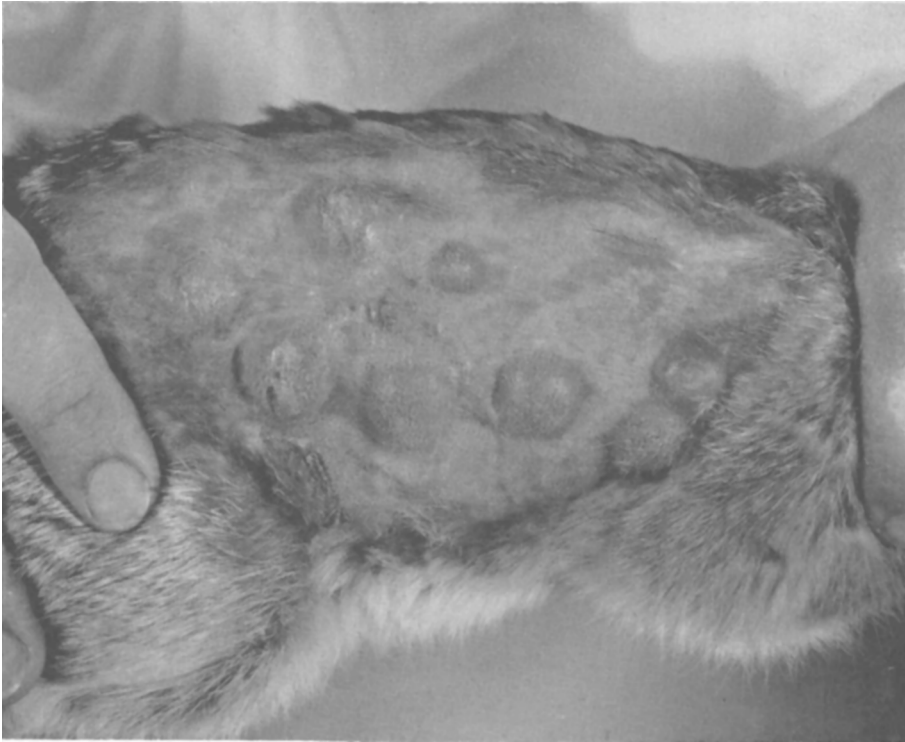


FIG. 1. Fibromas transmitted to cottontails by mosquitoes 14 days after full blood meal on a fibroma. Photo taken 35 days after mosquitoes had bitten.

in 24 hours, and in 4 days. Mosquitoes refed at 7 and 14 days had had a full bloodmeal on fibroma. As shown in Table I best results were obtained when 18 fibromas resulted from immediate interrupted feeding of 15 mosquitoes. As some of these vectors probably probed several times in the course of a single feeding, they may have inoculated fibroma virus in more than one spot. A finding of interest was that mosquitoes transmitted virus approximately as well in 2 weeks (13 fibromas resulted from exposure to 16 mosquitoes) as they did in 24 hours (15 positive out of 17 bites). Transmission of fibromas was somewhat less efficient in intervening periods, with only 18 of 32 bites being positive at 4 days and 16 of 24 being positive at 7 days after the initial feeding. In a control experiment, 5 mosquitoes fed on areas of unaffected skin 3 inches away from fibromas of an afflicted cottontail. No fibromas resulted when these mosquitoes were interrupted in their feeding and refed immediately on a normal cottontail. It should be pointed out that mosquitoes fed

through cloth and on cotton soaked in sugar solution as well as on raisins during intervals between rabbit blood-meals. Gross appearances of fibromas 40 days after mosquito inoculation were not uniform for different time intervals. Those resulting from immediate interrupted feeding remained full and round with minimal scab formation. On the other hand, fibromas developing after the 24 hour, the 4 and 7 day intervals, became flat in 4 to 5 weeks due to formation of a thick dark scab, which had fallen off by 40 days leaving a small scar. Fibromas resulting from mosquitoes partially fed 2 weeks previously were in marked contrast to the preceding, remaining full and round, without scabbing. Many of them were 2 cm in diameter and bore a close resemblance to naturally occurring fibromas.

In a further experiment a normal cottontail with no fur removed was placed in a mosquito-proofed culture chamber. Twenty-four 19-day old mosquitoes were released into this chamber immediately following a partial

TABLE II. Persistence of Capacity to Transmit Fibroma Virus Shown by *Aedes aegypti** in the Course of Repeated Interrupted Feeding.

Intervals between initial feeding and successive bites on recipients		No. mosquitoes biting recipient	No. fibromas transmitted	Ratio of No. of fibromas transmitted to No. of mosquitoes biting
Days				
1	A.M.	13	6	.46
	P.M.	13	6	.46
2		8	2	.25
3	A.M.	8	1	.13
	P.M.	8	2	.25
7		5	None	0

* Mosquitoes 20 days old at time of initial feeding.

blood-meal on a fibroma. All mosquitoes had opportunities to feed on the enclosed rabbit overnight and 10 of them to feed over the following day and night. Two weeks later 2 fibromas had developed, one on the upper eyelid and a second one at the inner canthus of the same eye. No tumors were located elsewhere on the body of this animal. Specific neutralizing antibodies developed in the serum during the disease.

A final experiment was performed to determine how long individual mosquitoes might continue to transmit fibromas in the course of successive interrupted feedings. As shown in Table II, 12 fibromas were induced on a fresh cottontail by 2 separate feedings of 13 mosquitoes within 24 hours of feeding on a fibroma. After 4 previous bites, 8 mosquitoes induced 2 fibromas on the afternoon of the third day, but 5 of these 8, biting a fresh cottontail on the 7th day, failed to transmit fibroma virus. In gross appearance tumors induced in this experiment underwent scab formation and regression within 40 days, being comparable to tumors transmitted in 24 hours, 4 and 7 days in the experiment described above (Table I). The same fibroma-bearing cottontail (No. 337) was used for initial feeding in all experiments indicated in Tables I, II, which were carried on more or less concomitantly.

The lower percentage of transmission attained in the final experiment may be accounted for in part by the fact that the mosquitoes were allowed only to pierce rather than to feed for an extended time. An unusually high mortality in this group of mos-

quitoes, however, complicates interpretation of results obtained. Deaths of the last 5 mosquitoes terminated this experiment.

Discussion. Success of other investigators (1,2) in transmitting myxomatosis by means of mosquitoes suggested that similar results might be obtained with Shope fibroma. There is evidence indicating that myxoma and fibroma are closely related if not variants of the same virus. Thus, one may be transformed into the other(4), there is a high degree of reciprocal cross immunity(5), both are chronic benign diseases of cottontail rabbits (*Sylvilagus*), one in South America (1) and one in North America(3), and finally both can be transmitted by similar vectors. Fenner has recently reviewed classification of these 2 agents(6). As fibroma virus is transmissible by pin pricks, it would seem that mechanical transfer might be possible for a variety of biting insects. Philip(7) found that triatomid bugs would transmit fibroma among domestic rabbits. Domestic rabbits however are not a natural host and react differently to fibroma virus than do cotton-tails. We have therefore confined experiments to the natural host (*Sylvilagus*), hoping that results might give some clue to modes of transmission operative in nature. Fleas, an abundant parasite on most wild cottontails, were used in initial transmission experiments. Positive bites were obtained only when fleas, after feeding on a fibroma, were released on an enclosed cottontail. Resulting lesions were crusted and pox-like, leading to as high a level of circulating antibodies in the course of infection as did typical fibromas induced by

mosquitoes in other cottontails. It is conceivable that flea bites are less penetrating than those of mosquitoes and thus result in a more superficial type of lesion. Similar lesions have been found in wild grey squirrels (*Sciurus carolinensis*) in association with specific neutralizing antibodies and flea infestation(8). These animals may also have typical fibromas(9).

Aedes aegypti is an efficient vector of fibroma, transmitting virus immediately and up to 2 weeks in a high proportion of bites. Transmission is presumably mechanical for the first few days. It is difficult, however, to understand how virus can persist on the proboscis of a mosquito for 2 weeks, during which time the insect has been feeding on raisins or through cloth on sugar water. That virus may get rubbed off is suggested by the final experiment. Here, although numbers were small, 5 successive partial feedings in 3 days led to a reduction in numbers of tumors induced. In general, results obtained in transmitting fibroma parallel those obtained by other investigators in mosquito transmission of myxoma(1,2) and of fowl pox(10-12). This similarity of behavior gives support to the hypothesis suggested by Fenner(6) that these disease agents are related. It would seem to us that further experimentation should be carried out to prove or to disprove whether these viruses proliferate in the transmitting mosquitoes. Fenner and Day(11), however, believe that myxoma and pox viruses are spread by mechanical means, the mosquito serving figuratively as "flying pins".

After mosquitoes carrying fibroma virus were released to feed at will on a recipient cottontail, the animal subsequently developed fibromas close to one eye. Aragao(1), mentions that the only myxoma tumors found on a wild Brazilian cottontail were in this location. Natural fibromas are usually found on the feet and legs of wild cottontails, the reason for which is unknown. One may speculate that either some native mosquitoes are attracted to the shorter fur of these areas or that some different vector is involved, as Shope(13) has suggested. There would seem to be several factors which would favor transmission of fibromas in nature. One factor is the long persistence of virus in fibromas(8).

A second factor of possible importance is that these highly vascular, superficial tumors are readily fed upon by at least 2 blood sucking insects. Thirdly, as we have shown, at least one type of vector can transmit the virus for a considerable period of time after a single feeding. To fully investigate natural modes of transmission one would need to find an area in which cottontail fibromas are epizootic; a situation which, in our experience, has been difficult to locate.

In summarizing present experiments, it would appear that mosquitoes may function as more than "flying pins", especially if one considers the variation of appearance in fibromas 5 to 6 weeks after their inception. Those transmitted immediately and in 2 weeks were larger and persisted with little change after 40 days while those from intervening periods became scabby and regressed early, so that only scars remained after 5 and a half weeks. The latter tumors were comparable to those transmitted by pins whereas the former, especially those transmitted after 2 weeks, bore a close resemblance to naturally occurring fibromas. Possibly virus transmitted in intermediate intervals was less in amount or attenuated by exposure on the mosquito proboscis. A possible explanation of the larger, more characteristic appearance of the other tumors might be that virus was fresh and fully virulent on immediate transmission. If virus growth took place in the mosquito after more than a week, virus transmitted in 2 weeks might again be fully virulent. All mosquitoes used in these particular experiments were of the same lot and had their initial feeding on the same fibroma-bearing cottontail. Although full interpretation of phenomena encountered on a small number of cottontails is difficult, it is not unreasonable to suppose that *Aedes aegypti* may serve initially as a mechanical and subsequently as a biological vector of fibroma virus. Continued experiments are being carried out in an effort to further test this hypothesis.

Summary. 1. Fibroma virus may be transmitted from cottontail to cottontail by means of pin pricks, fleas found on cottontails, and by the mosquito *Aedes aegypti* (Linnaeus). 2. *Aedes aegypti* transmits fibroma virus under favorable circumstances, in as high a propor-

tion of bites at 2 weeks following initial term feeding as at 24 hours. 3. Virus carrying mosquitoes induced fibromas on the eyelid and close to the eye of one cottontail when given the opportunity to feed at will. 4. Possible mechanisms involved in mosquito transmission are discussed.

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Effect of Calcium Salt of Versene upon Metabolism of Plutonium in the Rat.* (20340)

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Because of the potential hazard of the fission products and fissionable materials to individuals who may have been exposed to them, considerable interest has been devoted to the pharmacology of these radioactive substances as well as the development of methods for removing them from the body. Any procedure which results in a greater than normal elimination of these materials from the body has value from two points of view, first it can reduce the ultimate hazard to the contaminated individual, and second it allows a more accurate estimation of the degree of contamination which may be suspected in personnel exposed to such hazards.

One of the compounds which has received attention as a decontaminating agent has been the sodium and calcium salts of ethylene diamine tetra-acetic acid, later referred to as Versene. The pharmacology of this organic compound has been reviewed in detail by the Bersworth Chemical Co.(1). Popovici *et al.* (2) investigated the control of serum calcium levels using the sodium and calcium salts of

Versene. They demonstrated that the sodium salt was many times more toxic than the calcium salt due to the fact that the former compound combined with the serum calcium and produced death by hypocalcemia. Cohn *et al.* (3) has recently investigated the effects of the calcium salt upon the deposition and retention of radio-yttrium using rats as the experimental animals. In their studies the Versene salt and radio-yttrium were administered intraperitoneally, either shortly before or after radio-yttrium was given. The animals were sacrificed at intervals up to 5 days and a very marked diminution of skeletal uptake was observed in the treated animals. The greatest effect was noted in those animals which had been pretreated and the skeletal uptake was approximately 1/10 that of the control groups and the content in the liver was decreased by a factor of approximately 20. Earlier studies at this laboratory have demonstrated that oral administration of the calcium salt of Versene increased the urinary excretion of plutonium(4).

Methods. The dosage of Versene and mode of administration is summarized in each table. Wherever administered, Versene was given as

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