INFECTIOUS FIBROMA OF RABBITS

IV. The Infection with Virus myxomatosum of Rabbits Recovered from Fibroma

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In experiments described in the preceding paper (1) it was shown that serial passage of *Virus myxomatosum* through cottontail rabbits did not modify its pathogenicity for domestic rabbits. Furthermore, cottontail as well as domestic rabbits were found to be resistant to infection with the fibroma virus after infection with *Virus myxomatosum*, and their blood sera were effective in neutralizing both the myxoma and fibroma viruses. So far as these immunological data go, they suggest the identity of the fibroma and myxoma viruses.

However, as disclosed by earlier work (2), the immunological relationship in the opposite direction was quite different. Although domestic rabbits which had recovered from the fibroma exhibited an increased resistance to infection with *Virus myxomatosum*, which is ordinarily fatal, their sera possessed no demonstrable neutralizing properties for this virus. Furthermore, the clinical and pathological pictures of the fibroma and myxoma infections in rabbits were so different that the identity of the two viruses seemed improbable. The experiments to be described in the present paper were conducted in an effort to determine the nature of the resistance to myxoma induced in domestic rabbits by infection with the fibroma virus.

Infection of Fibroma-Recovered Domestic Rabbits with Virus myxomatosum

Fourteen out of 15 fibroma-recovered domestic rabbits were reported earlier (2) to have survived infection with *Virus myxomatosum*. Since then, the number of fibroma-recovered domestic rabbits inoculated

with amounts of *Virus myxomatosum* that would ordinarily be fatal has been increased to 62 and of these 59 have survived the infection. Control animals inoculated each time have regularly succumbed. Of more than 150 normal domestic rabbits infected during this period with the same strain of *Virus myxomatosum* none survived. This uniform fatality of *Virus myxomatosum* for domestic rabbits is in accord with the experience of others investigating the disease. The minimum time for the establishment of a state of resistance to fatal infection with *Virus myxomatosum* is not known. That it is something less than a fortnight is indicated by the fact that 2 rabbits in the series were found to be resistant to fatal myxoma infection 14 days after inoculation with the fibroma virus. Rabbits tested for resistance to myxoma as late as 100 days after their primary fibroma infection proved resistant. In the cases of the 3 fibroma-recovered rabbits above mentioned that died of myxoma following inoculation with *Virus myxomatosum* 24, 35, and 56 days had elapsed between the primary inoculation with fibroma virus and the inoculation with *Virus myxomatosum*.

Repeated injections of fibroma virus failed to enhance the resistance of rabbits to myxoma or to establish antibodies neutralizing *Virus myxomatosum* in their sera. 2 rabbits that had received 2 injections and 1 that had received 4 injections of fibroma virus subcutaneously and intratesticularly were found to be no more resistant to *Virus myxomatosum* than animals receiving but a single injection. Furthermore, the sera of these 3 rabbits possessed no demonstrable neutralizing properties for *Virus myxomatosum*.

The wide time range over which infection with fibroma virus exerts its protective influence against fatal infection with *Virus myxomatosum* (from 14 to 100 days) seems to eliminate the possibility that the increased resistance is of a non-specific nature. This is further indicated by the fact that the mere injection of fibroma virus, even in large dosage, confers no protection; actual fibromatous growth is necessary. For instance, domestic rabbits injected intraperitoneally even with very large amounts of fibroma virus develop no growths and are subsequently still fully susceptible to fatal infection with *Virus myxomatosum*; on the other hand, relatively much smaller amounts of fibroma virus given subcutaneously or intratesticularly regularly result in growths and the establishment of resistance to infectious myxoma.

The Clinical and Pathological Picture of Infectious Myxoma in Fibroma-Recovered Animals.—As was pointed out previously (2), fibroma-recovered rabbits were but rarely completely resistant to *Virus myxomatosum.* Most of the animals developed myxoma in an abortive form, and the lesions, though characteristic of the disease, were limited to the formation of a localized myxomatous growth if the inoculation had been subcutaneous, or to a myxomatous orchitis if the inoculation had been intratesticular. Sometimes these local processes were accompanied by mild conjunctivitis and a purulent type of rhinitis which were transient. Myxomatous swelling of the eyelids, nose, ears, and genito-anal region developed rarely and the picture presented was that of typical acute infectious myxoma, differing from it, however, in that this condition was not fatal. In many instances in which fibroma-recovered rabbits were inoculated both subcutaneously and intratesticularly with *Virus myxomatosum,* myxomatous lesions developed only in the testicle. In respect to the general clinical picture, fibroma-recovered domestic rabbits reacted to infection with *Virus myxomatosum* in much the same manner as did normal cottontail rabbits (1). It appears that preliminary infection with fibroma virus induces in the highly susceptible domestic rabbit a resistance to *Virus myxomatosum* similar in degree to that exhibited naturally by the cottontail rabbit.

The gross and histopathological characters of the local lesions developing in the testicles or subcutaneous tissues at the site of *Virus myxomatosum* inoculation in fibroma-recovered domestic rabbits were similar to those at corresponding sites in fully susceptible domestic rabbits. The healing process seen only in resistant rabbits was characterized by a marked infiltration of the local lesions with round cells. Cytoplasmic acidophilic inclusions were present in epithelial cells of the epididymis of the inoculated testicle and in those of the epidermis overlying local growths in the subcutaneous tissue in resistant rabbits. These were identical in appearance with the inclusions seen in similar cells of fully susceptible rabbits.

Recovery of Virus myxomatosum from the Local Myxomatous Lesions Induced in Fibroma-Recovered Rabbits and Its Passage in Series through Such Animals.—Preliminary experiments showed that when fibroma-recovered domestic rabbits were inoculated subcutaneously or intratesticularly with *Virus myxomatosum* and developed only a local myxomatous lesion, myxoma virus, fully pathogenic for normal rabbits could be recovered from such lesions even as late as 16 days after inoculation. The local lesions by this time were often regressing. This rendered likely the possibility that the virus might prove serially transmissible in such animals.

In order to study the relationship of fibroma to myxoma virus, and because of the possibility of altering the pathogenic properties of

Virus myxomatosum, it seemed advisable to attempt the serial passage of this virus through fibroma-recovered rabbits. Further, the question first raised by Rivers (3) of whether *Virus myxomatosum* is a single virus or composed of more than one virus might be answered. For instance the immunological relationships between the fibroma and the myxoma viruses, outlined earlier in this paper, were in accord with the possibility that *Virus myxomatosum* might be composed of fibroma virus and some other perhaps hitherto unknown virus. If such were the case, it could easily be understood why *Virus myxomatosum* would immunize completely against the fibroma virus, one of its components, while the fibroma virus, being but one part of *Virus myxomatosum*, gives correspondingly only partial immunization.

In the serial passage of *Virus myxomatosum* through fibroma-recovered rabbits at least one normal control rabbit was inoculated at each passage to detect any change in the character of the disease induced by the virus. Male rabbits were used and, in the cases of the resistant rabbits, the preliminary inoculation with fibroma virus had been made subcutaneously and into one testicle. In inoculating such animals subsequently with *Virus myxomatosum* the other testicle and a new subcutaneous site were chosen. Fresh *Virus myxomatosum* from the subcutaneous lesion of a rabbit dead of the disease was used in starting the experiment. At each passage the infected testicle from a resistant rabbit was used in inoculating animals of the succeeding passage. Usually the testicle was removed, under ether anesthesia, on from the 10th to the 12th day following inoculation, thus allowing the animal furnishing the virus to recover and complete its record in the experiment. In the first 2 passages, the animals serving as the source of passage virus were killed. No virus for passage was taken from a resistant rabbit until the normal control animal had died. *Virus myxomatosum* was passed in this manner through fibroma-recovered rabbits for 8 serial passages at which time the experiment was discontinued. An outline of the experiment is presented in Table I.

Consideration of the data given in Table I reveals that *Virus myxomatosum* was readily transmissible in series through the testicles of fibroma-recovered rabbits. Its pathogenic properties, as judged by inoculation into normal domestic rabbits, were unchanged by such passage. None of the fibroma-recovered rabbits used in the experiment died and most showed only a localized testicular myxomatosis.

Rous, McMaster, and Hudack (4) have shown that living cells protect viruses associated with them from the neutralizing effect of immune serum. The possibility, suggested by this work, that living

cells in the inoculum administered at each serial passage served to shield the virus from neutralization in the resistant animals and thus perpetuate it from passage to passage was shown not to be a factor; myxoma virus from infected testicles stored for 2 months in 50 per cent glycerol at refrigerator temperature induced localized myxomatous orchitis when administered intratesticularly to fibroma-recovered rabbits and virus was demonstrable in such local lesions.

The Disappearance of Virus myxomatosum from the Site of Inoculation in Immune Rabbits.—An animal wholly immune to a virus not only yields a specific neutralizing blood serum and is completely refractory to reinfection but in addition, when reinoculated, is capable of rendering injected virus rapidly non-demonstrable. The failure to recover virus from the sites of inoculation in immunized animals has been reported repeatedly.

Kraus, Keller, and Clairmont (5) demonstrated that rabies virus could not be got from the brains of immunized rabbits 5 days following inoculation, and Kraus and Doerr (6) found that while fowl plague virus was still demonstrable in the brains of immunized geese 6 hours following inoculation, this was not the case 18 hours after injection. Levaditi and Nicolau (7) observed that vaccinia virus inoculated into the brain of an immunized rabbit could not be demonstrated 2 hours following injection. Andrewes (8) showed that Virus III inoculated into the testicles of immunized rabbits was not to be recovered 2 hours after injection and Nicolau and Kopciowska (9) made a similar observation regarding herpes virus introduced into the brains of immunized rabbits. Smith (10) noted that while vaccinia virus persisted in the circulation of a susceptible rabbit for as long as 8 days following intravenous infection, it disappeared from the circulation of an immune rabbit within 4 to 6 hours.

The following experiment was performed in an attempt to demonstrate a similar phenomenon in animals immune to *Virus myxomatosum.*

Three male rabbits were made resistant to infectious myxoma by a preliminary subcutaneous infection with fibroma virus. They were next submitted to a subcutaneous inoculation of *Virus myxomatosum.* A local myxomatous lesion developed in the subcutaneous tissue at the site of inoculation. Following regression of the lesion, the sera of these rabbits contained demonstrable neutralizing antibodies for *Virus myxomatosum* and the animals were deemed immune. They were then inoculated subcutaneously and into each testicle with a suspension of *Virus myxomatosum* from glycerolated infected testicles. As controls, a normal and a fibroma-recovered rabbit received the virus in a similar manner. The normal

TABLE I

Passage of Virus myxomatosum Serially through Fibroma-Recovered Rabbits

Passage No. and date	Domestic Rabbit No.	Previous treatment	Infection with *Virus myxomatosum*		Result
			Supernatant of a 5 per cent suspension testicle Rabbit No.	Dosage and route of inoculation	
1 10/31/32	4-72	None (control)	1:50 dilution 5 per cent suspension of subcutaneous myxomatous lesion Rabbit 4-81	1 cc. s.c.* and 0.2 cc. i.t.*	Died, 11th day; typical myxoma
	4-56	Subcutaneous fibroma		1 cc. s.c.	No general symptoms; slight local subcutaneous lesion; recovered
	4-65	Subcutaneous and testicular fibroma		1 cc. s.c. and 0.2 cc. i.t.	No general symptoms; inoculated testicle moderately enlarged; recovered
	4-74	Subcutaneous and testicular fibroma		1 cc. s.c. and 0.2 cc. i.t.	No general symptoms; inoculated testicle moderately enlarged when killed on 11th day
2 11/11/32	5-04	None (control)	4-74	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 8th day; typical myxoma
	4-66	None (control)		0.5 cc. s.c. and 0.1 cc. i.t.	Died, 8th day; typical myxoma
	4-71	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; slight local subcutaneous lesion; inoculated testicle moderately enlarged; recovered
	4-61	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; slight local subcutaneous lesion; inoculated testicle moderately enlarged when killed on 11th day

3 11/22/32	5-13	None (control)	4-61	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 9th day; typical myxoma
	4-87	None (control)		0.5 cc. s.c. and 0.1 cc. i.t.	Died, 9th day; typical myxoma
	4-77	Subcutaneous fibroma		0.5 cc. s.c.	No local lesion or illness
	4-78	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; no subcutaneous lesion; inoculated testicle moderately enlarged when removed on 10th day;† recovered
4 12/ 2/32	4-44	None (control)	4-78	0.5 cc. s.c.	Died, 10th day; typical myxoma
	4-68	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; no subcutaneous lesion; inoculated testicle moderately enlarged when removed on 11th day; recovered
5 12/13/32	5-28	None (control)	4-68	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 8th day; typical myxoma
	5-20	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	Completely negative
	5-21	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; no subcutaneous lesion; inoculated testicle moderately enlarged when removed on 11th day; recovered
6 12/24/32	5-27	None (control)	5-21	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 11th day; typical myxoma
	5-23	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	Clinical picture characteristic of myxoma; recovered
	5-16	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; no subcutaneous lesion; inoculated testicle slightly enlarged and firm when removed on 11th day; recovered

* s.c. = subcutaneously; i.t. = intratesticularly into one testicle.

† All operative procedures were conducted under full ether anesthesia.

TABLE I—*Concluded*

Passage No. and date	Domestic Rabbit No.	Previous treatment	Infection with *Virus myxomatosum*		Result
			Supernatant of a 5 per cent suspension testicle Rabbit No.	Dosage and route of inoculation	
7 1/ 4/33	5-77	None (control)	5-16	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 8th day; typical myxoma
	5-24	Subcutaneous fibroma		0.5 cc. s.c.	No general symptoms; moderate local subcutaneous myxomatous lesion
	5-17	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	Completely negative
	5-50	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; no subcutaneous lesion; inoculated testicle greatly enlarged when removed on 11th day; recovered
8 1/15/33	5-71	None (control)	5-50	0.5 cc. s.c. and 0.1 cc. i.t.	Died, 12th day; typical myxoma
	5-22	Subcutaneous and testicular fibroma		0.5 cc. s.c. and 0.1 cc. i.t.	No general symptoms; scant local subcutaneous lesion; inoculated testicle slightly enlarged and firm when killed on 12th day
9 1/27/33	5-72	None	5-22	0.5 cc. s.c.	Died, 13th day; typical myxoma

control rabbit developed typical myxomatosis and died on the 9th day. The fibroma-recovered control developed localized subcutaneous and testicular myxomata, a transient conjunctivitis, and survived. The 3 myxoma-immune animals remained normal. A testicle was removed, under ether anesthesia, from each of the control rabbits 20 hours following infection, and from the myxoma-immune animals 20, 48, 72, and 96 hours following inoculation. The testicles were ground in a mortar, suspended in physiological saline, and a portion of each suspension thus prepared was inoculated subcutaneously and intratesticularly into rabbits to test for the presence of *Virus myxomatosum*. *Virus myxomatosum*, inducing typical infectious myxoma fatal in 9 and 10 days, respectively, was demonstrable in the testicles removed at the end of 20 hours from the normal and fibroma-recovered control rabbits. The testicles removed 20, 48, 72, and 96 hours following inoculation from the myxoma-immune rabbits were free from *Virus myxomatosum* demonstrable by rabbit inoculation.

The results of this experiment suggest that, as in other virus diseases, virus inoculated into the tissues of rabbits immune to infectious myxoma is promptly destroyed or rendered non-demonstrable.

The Failure of Virus myxomatosum to Invade the Blood Stream of Fibroma-Recovered Rabbits.—In infectious myxoma of rabbits the etiological virus invades the blood stream and is regularly found there throughout the later course of the disease (11 and 12). It seemed of interest to determine whether it was similarly present in the blood stream of fibroma-recovered rabbits after inoculation with *Virus myxomatosum.*

Three fibroma-recovered rabbits that developed a localized myxomatous orchitis after intratesticular inoculation with *Virus myxomatosum* were bled from the ear vein on the 2nd, 4th, 7th, and one on the 11th day after infection. Serum from each of these bleedings failed to produce infectious myxoma in test rabbits to which it was administered subcutaneously in 3 cc. amounts. Similar amounts of serum obtained from non-resistant rabbits, infected with myxoma, from the 7th day post-infection to death regularly produced infectious myxoma in test rabbits to which it was similarly administered.

These experiments indicated that *Virus myxomatosum* did not invade the blood stream in fibroma-recovered rabbits as it did in fully susceptible animals. They did not, however, shed light on the actual mechanism by which *Virus myxomatosum* is restrained to a localized and relatively benign infection in fibroma-recovered rabbits. This will be considered in more detail later.

TABLE II

The Time of Appearance of Myxoma Neutralizing Antibodies in the Blood Serum of Fibroma-Recovered Rabbits Infected with Virus myxomatosum

Serum from Rabbit No.	Drawn, days after inoculation with *Virus myxomatosum*	Effect of subcutaneous injection of mixture of 0.5 cc. 1:25 dilution of *Virus myxomatosum** + serum		
		Amount of serum in mixture	Injected Rabbit No.	Result
		cc.		
8-65	Fibroma-convalescent (before myxoma)	3	8-99	Died, 15 days
	2 days	3	8-91	Died, 17 "
	4 "	3	8-85	Died, 17 "
	7 "	3	8-81	No illness
	10 "	3	9-04	No illness
	15 "	1.5	9-41	No illness
	46 "	1.5	9-39	No illness
8-66	Fibroma-convalescent (before myxoma)	3	9-00	Died, 13 days
	Fibroma-convalescent (before myxoma)	3	9-54	Died, 11 "
	2 days	3	8-83	Died, 17 "
	4 "	3	8-89	Died, 11 "
	7 "	3	8-84	No illness
	10 "	3	9-03	No illness
	15 "	3	9-55	No illness
	46 "	1.5	9-43	No illness
8-70	Fibroma-convalescent (before myxoma)	3	8-98	Died, 12 days
	Fibroma-convalescent (before myxoma)	5	9-52	Died, 12 "
	2 days	3	8-86	Died, 22 "
	2 "	5	9-40	Died, 13 "
	4 "	3	9-05	Died, 18 "
	4 "	5	9-46	Died, 16 "
	7 "	3	8-88	Died, 17 "
	7 "	5	9-42	Died, 13 "
	10 "	3	9-02	Died, 13 "
	10 "	5	9-57	Died, 25 " (probably not of myxoma)
	15 "	5	9-55	No illness
	46 "	5	9-53-A	No illness
10-57	Fibroma-convalescent (before myxoma)	3	11-03	Died, 18 days
	7 days	3	11-00	Died, 15 "
	17 "	3	11-24	No illness

* *Virus myxomatosum* = supernatant of a 5 per cent suspension of glycerolated testicle and subcutaneous lesion from rabbit dead of infectious myxoma.

TABLE II—*Concluded*

Serum from Rabbit No.	Drawn, days after inoculation with *Virus myxomatosum*	Effect of subcutaneous injection of mixture of 0.5 cc. 1:25 dilution of *Virus myxomatosum** + serum		
		Amount of serum in mixture	Injected Rabbit No.	Result
		cc.		
	Fibroma-convalescent (before myxoma)	3	11-05	Died, 15 days
10-58	7 days	3	10-99	No illness
	17 "	3	11-20	No illness
	Fibroma-convalescent (before myxoma)	3	11-01	Died, 16 days
10-69	7 days	3	10-98	No illness
	17 "	3	11-21	No illness
	Fibroma-convalescent (before myxoma)	3	11-22	Died, 17 days
10-70	7 days	3	10-97	Died, 13 "
	17 "	3	11-23	No illness
8-55	Normal	3	8-96	Died, 14 days
14	Normal	5	9-53-B	Died, 10 "
14	Normal	3	10-35	Died, 11 "
10-74	Normal	3	11-07	Died, 16 "
10-74	Normal	3	11-19	Died, 20 "

The Time of Appearance of Myxoma-Neutralizing Antibodies in the Blood Serum of Fibroma-Recovered Rabbits Infected with Virus myxomatosum.—In the preceding section it was noted that *Virus myxomatosum* could not be detected in the serum of myxoma-infected fibroma-recovered rabbits. It seemed possible that the failure of the virus to generalize in these animals might be due to their prompt generation of virus-neutralizing antibodies. Such an occurrence would aid in explaining the benign and localized nature of their myxoma infections. Therefore, the time of appearance of myxoma-neutralizing antibodies in the blood serum of infected fibroma-recovered rabbits was investigated.

A series of rabbits was bled following recovery from infection with the fibroma virus. They were then inoculated either subcutaneously or intratesticularly, or by both routes, with *Virus myxomatosum* in amounts large enough to kill all control animals. Serum was obtained from 3 of the rabbits on the 2nd, 4th, 7th, 10th, 15th, and 46th days and from the remaining 4 on the 7th and 17th days following infection with *Virus myxomatosum*. This serum, together with that obtained prior to their infection, was then tested for its ability to neutralize *Virus myxomatosum*.

The neutralization tests were conducted in the usual fashion. The serum-virus mixtures were set up to contain 0.5 cc. of a 1:25 dilution of the supernatant of a 5 per cent suspension of glycerolated subcutaneous and testicular myxoma lesion (the equivalent of 1 mg. of infectious tissue) mixed with the amount of serum being tested, usually 3 cc. The mixtures were stored overnight (17 hours) in the refrigerator prior to injection subcutaneously into the test rabbits. Rabbits receiving mixtures which were neutral developed no evidence of infectious myxoma and survived. Those receiving mixtures in which the serum did not neutralize the virus came down with the typical disease and died in from 11 to 20 days following inoculation. These rather long survival periods are believed to be the result of the relatively small amounts of virus employed in the mixtures. The amount of virus used was, however, sufficient to kill all control rabbits. The results of the neutralization experiments are outlined in Table II.

The data recorded in Table II reveal that of the serum samples obtained prior to the 7th day following infection with *Virus myxomatosum* none neutralized the virus. Of 7 of the samples obtained on the 7th day following infection, however, 4 neutralized the virus completely. Of the remaining 3 rabbits, the serum of 1 failed to neutralize virus on the 10th day but did on the 15th day, while the other 2 both neutralized on the 17th day post-infection. An attempt to determine a possible relationship between the time of appearance of neutralizing antibodies and the severity of the myxoma infection leads to the impression that the promptness of antibody reaction was determined by the severity of the infection, rather than that the severity of the infection was determined by the promptness with which antibodies were produced. 3 of the 4 rabbits whose serum contained neutralizing antibodies as early as the 7th day post-infection developed either a coryza or a conjunctivitis in addition to myxomatous swellings at sites of inoculation, while 2 of the 3 rabbits in which the appearance of demonstrable antibodies was delayed until later than the 7th day post-infection exhibited no evidence of generalizing infection. The exceptional animal in each group is sufficient to indicate that any

attempt to correlate the speed of production of antibodies with the extent and severity of the disease in so small a group of experimental animals is hazardous. The fact remains, nevertheless, that fibroma-recovered rabbits produce antibodies capable of neutralizing *Virus myxomatosum* during an attack of the modified infectious myxoma that they develop. The sera of fully susceptible rabbits infected with myxoma virus at no time contains neutralizing antibodies and, as was pointed out in the preceding section, is rich in virus from the 7th day post-infection to death. The time of appearance of virus neutralizing antibodies in the sera of resistant rabbits thus approximately coincides with that at which the virus ordinarily generalizes in susceptible animals. It seems possible that this fortunate coincidence of events may be at least partially accountable for the apparent resistance of fibroma-recovered rabbits to *Virus myxomatosum.*

DISCUSSION

The transmissibility of *Virus myxomatosum* in series through fibroma-recovered rabbits without alteration of its disease-producing properties, in contrast with its failure even to survive in the tissues of myxoma-immune rabbits, is of importance as far as reaching a decision concerning the identity of the fibroma virus with *Virus myxomatosum.* The generally accepted criterion for considering two viruses identical is an immunological one. Animals recovered from infection with each virus should not only resist infection with the other virus but their sera should neutralize it. They should, furthermore, be capable of inactivating or destroying the other virus when it is introduced into an ordinarily susceptible tissue. So far as the immunological relationship between the fibroma virus and *Virus myxomatosum* is concerned these criteria of complete cross-immunity are not fulfilled. Even though infection of a rabbit with fibroma virus is known to establish in that animal a state of enhanced resistance to *Virus myxomatosum,* the fibroma-recovered rabbit is usually not completely refractory to myxoma infection as evidenced by the development of a local myxomatous lesion at the site of inoculation. Furthermore injection of *Virus myxomatosum* into a fibroma-recovered rabbit does not result in the destruction of the injected virus. On the contrary, the virus actually multiplies and can be passed indefinitely in series through

such resistant animals. Finally, serum from a fibroma-recovered rabbit, though neutralizing the fibroma virus, is without effect on *Virus myxomatosum*. It is plain that the two viruses are not identical.

The results of cross-protection and cross-neutralization experiments can be best explained on the basis of a partial duplication of the antigenic components comprising the two viruses. It was shown in the preceding paper (1) that rabbits recovered from *Virus myxomatosum* infection were immune to the fibroma virus and their sera capable of neutralizing that virus. This indicated that *Virus myxomatosum* contained antigenic components essential to the production of a complete fibroma virus immunity. On this basis, the incomplete protection of rabbits in the reverse direction might be interpreted as indicating that the fibroma virus is antigenically only a partial replica of *Virus myxomatosum*. The antigenic components comprising fibroma virus and common also to the myxoma virus are sufficient to establish in fibroma-infected rabbits a state of resistance to myxoma, but, because they represent only partially the antigenic composition of *Virus myxomatosum*, this resistance is not the complete immunity conferred reciprocally by two identical viruses.

The experimental data presented are considered to support the view, evident also from the clinical and pathological data, that, while perhaps antigenically and genetically closely related, *Virus myxomatosum* and fibroma virus are different infectious agents.

SUMMARY

The serial passage of *Virus myxomatosum* through fibroma-recovered domestic rabbits did not alter its pathogenic properties. Fully virulent *Virus myxomatosum* persisted in the inoculated testicle of fibroma-recovered rabbits for at least 16 days following inoculation. Virus injected into the testicles of myxoma-immune domestic rabbits, on the other hand, was promptly rendered non-demonstrable. The failure of fibroma-recovered domestic rabbits to destroy injected *Virus myxomatosum* and the absence from their sera of neutralizing antibodies effective against *Virus myxomatosum* are considered to be evidence against the identity of the fibroma and myxoma viruses. The rapidity with which fibroma-recovered rabbits develop neutralizing

antibodies following infection with *Virus myxomatosum* is considered to be a possible factor in their acquired resistance.

It is believed on the basis of all the evidence that infectious fibroma of rabbits is a definite disease entity and not merely a mild and non-fatal form of infectious myxoma.

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