A FILTRABLE VIRUS CAUSING A TUMOR-LIKE CONDITION IN RABBITS AND ITS RELATIONSHIP TO VIRUS MYXOMATOSUM

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In a preceding paper (1) the clinical and pathological characteristics of a tumor-like condition of rabbits were described. The purpose of the present paper is to report experiments indicating the nature of the etiological agent and to point out its possible relationship to the virus of infectious myxoma. As in the preceding paper, the term "tumor" is used in its broadest sense, to indicate a local swelling consisting of a mass of new tissue.

Viability of the Tumor-Inducing Agent in Glycerol

Tumor tissue from domestic rabbits stored in 50 per cent glycerol at refrigerator temperature for as long as 86 days lost very little, if any, of its original potency as judged by the size and character of the tumors it produced in domestic rabbits. Tissue stored for a longer period has not been tested for infectivity.

Etiology

Cultures of tumor tissue on various media, including some suitable for the growth of tubercle bacilli, failed to reveal any significant organism.

The resistance of the etiological factor to glycerol, together with the finding of inclusion-like bodies in the cytoplasm of epithelial cells overlying the original wild rabbit tumor (1), suggested that the causative agent might be a filtrable virus. Attempts were made to transmit the condition to domestic rabbits by means of sterile filtrates of infectious suspensions.

Tumor tissue to be used as a source of infection in the filtration experiments was minced with sterile scissors, ground in a mortar with sterile sand, and suspended in infusion broth (pH 7.3) sufficient to make a 5 per cent suspension. Suspensions thus prepared were cleared of gross particles by centrifugation and the supernatant fluid was filtered through paper. 1 cc. of 24 hour broth cultures of *B. prodigiosus* was added to each 15 to 20 cc. of the paper filtrates and they were then filtered through Berkefeld candles. The resulting filtrates were tested for sterility in amounts of 1.5 to 3 cc. All those recorded were bacteriologically sterile.

The results of the filtration experiments are summarized in Table I.

The testicular tumors produced by filtrates were as large, persistent, and characteristic as those in the control animals which had received unfiltered suspensions. The subcutaneous tumors, on the other hand, were usually smaller, did not last so long, and were less compact than those in control animals. In some of the filtrate-infected animals the subcutaneous reaction consisted in the formation of groups of small, firm, shotty nodules lying in a softer subcutaneous swelling. These regressed very rapidly and without sloughing. Testicular tumors produced by filtrates, however, when used to infect further series of rabbits subcutaneously, caused typical firm, compact tumor formation. The period between the time of inoculation and the first evidence of tumor formation was approximately 2 days longer in animals receiving Berkefeld V and N filtrates than in those receiving the unfiltered suspensions. Only one of the two attempts to pass the tumor-producing agent through Berkefeld W filters was successful, and the incubation period in the rabbit receiving this filtrate was 10 days in contrast to 4 days in the case of its control receiving unfiltered suspension.

Immunity Conferred by Experimental Infection

Domestic rabbits in which either a subcutaneous or testicular tumor had been produced and allowed to regress were completely resistant to reinfection by either route. This immunity became evident before the tumor had completely regressed. Actual tumor formation was necessary for the production of immunity, since an amount of agent that failed to produce a tumor conferred no demonstrable immunity.

The neutralization experiments summarized in Table II show that sera from rabbits whose tumors had regressed were capable of neutral-

TABLE I

Filtration Experiments

Filter		Material filtered (approximately 5% suspension of testicle and subcutaneous tumor Rabbit No.)	Time of filtration	Amount of filtrate	Maximum negative pressure	Rabbit inoculated, No.	Amount of filtrate injected and route		Result, tumor formation at site of inoculation	
Type	No.		*min.*	*cc.*	*cm. Hg*		*cc.*			
Berkefeld V	13	248*	1.5	30	62	268	1	i.t.†	Positive	
" N	8	268 and 269	4	6	62	271	1	"	"	
" "	18	281	12	42	62	286	1	s.c.	"	but slight
							1	i.t.	"	
						287	1	s.c.	"	but slight
							1	i.t.	"	
						289	1	s.c.	"	but slight
							1	i.t.	"	
						290	1	s.c.	"	
							1	i.t.	"	
						292	1	s.c.	"	but slight
							1	i.t.	"	
						293	1	s.c.	"	but slight
							1	i.t.	"	
						294	1	s.c.	"	but slight
							1	i.t.	"	
Berkefeld V	13	300	10	33	62	304	3	s.c.	"	but slight
							1	i.t.	"	
						305	3	s.c.	"	
							1	i.t.	"	
						306	3	s.c.	"	
							1	i.t.	"	
						307	3	s.c.	"	
							1	i.t.	"	
						308	3	s.c.	"	
							1	i.t.	"	
						309	3	s.c.	"	but slight
							1	i.t.	"	
Berkefeld W	9	369 and 409	15	10	62	422	2	s.c.	Negative	
							1	i.t.	"	
Berkefeld W	11		15	25	62	423	2	s.c.	"	
							1	i.t.	Positive	

* The numbers in this and the subsequent tables are taken from laboratory records and bear no relation to the actual number of animals used in these experiments.

† i.t. = intratesticularly. s.c. = subcutaneously.

TABLE II

Neutralization Tests with Serum from Recovered Rabbits

Source of serum	Source of tumor-producing agent	Rabbit inoculated, No.	Amount of mixture of infectious suspension and serum injected and route of injection*		Result, tumor formation at site of inoculation
			cc.		
Recovered Rabbit 234	Supernatant fluid suspension testicular tumors Rabbits 279 and 280	298	2.0	s.c.†	Negative
			0.6	i.t.	Positive; long latent period
Physiological saline		299 (control)	2.0	s.c.	"
			0.6	i.t.	"
Recovered Rabbit 266	Supernatant fluid suspension subcutaneous and testicular tumors Rabbit 307	312	2.0	s.c.	Negative
			0.2	i.t.	Positive but slight
" " 234		313	2.0	s.c.	Doubtful small tumor
			0.2	i.t.	Positive; long latent period
" " 251		314	2.0	s.c.	Negative
			0.2	i.t.	Positive but slight
" " 263		315	2.0	s.c.	Negative
			0.2	i.t.	"
" " 264		316	2.0	s.c.	"
			0.2	i.t.	Positive but slight
Normal " 660		311 (control)	2.0	s.c.	"
			0.2	i.t.	"
Recovered " 263	Supernatant fluid suspension testicular tumor Rabbit 380	387	2.0	s.c.	Negative
			0.4	i.t.	"
Normal " 14		392 (control)	2.0	s.c.	Positive
			0.4	i.t.	"

* The mixtures injected were comprised of equal parts of infectious suspension and convalescent serum.

† s.c. = subcutaneously; i.t. = intratesticularly.

izing the tumor-producing agent completely in tests by subcutaneous inoculation and partially or completely in tests by testicular inoculation.

In this and other neutralization tests described in this paper the mixtures of serum and infectious suspension were incubated at 37°C. for 1 hour and then stored for 2 hours in the refrigerator before use.

TABLE III

Period of Persistence of Tumor-Producing Agent in Tumors

Source of infectious material, Rabbit No.	Length of time after inoculation that material was taken	Material used for inoculation, suspension of	Rabbit inoculated, No.	Route of inoculation and result, tumor formation at site of inoculation
	days			
307	7	Subcutaneous tumor	317	s.c.,* positive
				i.t., "
		Testicular "	318	s.c., "
				i.t., "
305	14	Subcutaneous "	335	s.c., negative
				i.t., positive
		Testicular "	336	s.c., "
				i.t., "
311	21	Subcutaneous "	346	s.c., negative
				i.t., positive
		Testicular "	347	s.c., " but slight
				i.t., "
318	35	Testicular "	359	s.c., negative
				i.t., positive but slight

* s.c. = subcutaneously. i.t. = intratesticularly.

The time necessary for the appearance of antibodies effective against the tumor-producing agent in the blood serum of tumor-bearing domestic rabbits has not been determined. They were, however, present in the serum of 1 animal (Rabbit 305, Table III) 14 days after inoculation. It should be noted that at this time the rabbit had large subcutaneous and testicular tumors that were shown to contain the tumor-producing agent.

Persistence of the Tumor-Producing Agent in Experimental Tumors

Experiments to determine the most favorable time for transmitting the tumor in series have yielded the results recorded in Table III. All rabbits furnishing infectious material in these experiments were inoculated both subcutaneously and intratesticularly and developed typical tumors at both sites.

These results are only sufficient to suggest that the tumor-producing agent disappears or becomes attenuated before the regression of the tumor and that the threshold of natural resistance to infection is higher by the subcutaneous than by the testicular route. The agent persisted in an experimentally produced subcutaneous tumor in a wild cottontail rabbit for at least 77 days after inoculation and material taken from this tumor was still capable of inducing tumor formation when administered either subcutaneously or intratesticularly to domestic rabbits. This relatively long persistence of the tumor-producing agent in a wild rabbit tumor is in marked contrast to its early disappearance from domestic rabbit tumors.

Failure to Demonstrate the Tumor-Producing Agent in the Blood

Blood from infected domestic rabbits 2, 7, and 8 days after inoculation did not induce tumor formation when administered subcutaneously, intracutaneously, or intratesticularly. Animals so tested were found subsequently to be fully susceptible to material known to contain the tumor-producing agent.

Fate of Tumor Grafts

While the implantation of bits of tumor tissue into the testicle or under the skin of domestic rabbits with a large aspirating needle, after the method usually employed in tumor grafting, frequently, although not invariably, gave rise to local tumor formation, histological study of such tumors has been inconclusive in showing that multiplication of cells in the engrafted bit of tumor ever took place. The impression gained from the study of sections of such graft-produced tumors was that they arose as a result of stimulation of surrounding connective tissue by an agent present in the graft. As yet no sufficient experiments have been conducted to establish definitely the fate of grafts. It has been possible, however, to produce tumors by the implantation of grafts from the tumor of a wild cottontail rabbit in domestic rabbits

and, conversely, in a wild cottontail rabbit by the implantation of a graft from the tumor of a domestic rabbit. In view of the generally accepted evidence regarding the impossibility of successfully transferring the tissues of one species to another, the introduction of the tumor-producing agent with the graft seems more probably the cause of ensuing tumor formation than the proliferation of cells introduced with the graft.

Relation of the Tumor-Producing Agent to Virus III[1]

Five male domestic rabbits immunized to Virus III by testicular and cutaneous infection were found, when tested 1 to 2 months later, to be fully susceptible to the tumor-producing agent administered subcutaneously or intratesticularly. This seemed to eliminate the possibility that Virus III, which might have become associated with the tumor-producing agent by rapid serial testicular passage through rabbits, contributed in any way to the tumor-like condition.

Relation of the Tumor-Like Condition to Infectious Myxoma[1]

The strain of myxoma virus used in the experiments to be described here was that used by both Hobbs (2) and Rivers (3) and was originally obtained from Dr. Arthur Moses of the Oswaldo Cruz Institute in Brazil. Since, in recent works, Hobbs (2) and Rivers (3) have very fully described the characteristics of this strain of myxoma virus and the clinical and pathological manifestations of the disease it produces under experimental conditions, only the similarities and differences between infectious myxoma and the tumor-like condition under discussion will be summarized.

Since Moses (5) has found the wild rabbit of Brazil insusceptible to experimental infection with *Virus myxomatosum* except in rare instances, and Hobbs (4) has been unable to infect our native wild cottontail rabbits with the virus, there was no expectation that the condition observed in the original wild rabbit was infectious myxoma. The character of the experimentally produced tumor-like condition in laboratory rabbits spoke strongly against the view that we were dealing with infectious myxoma. The salient characteristics in

[1] The Virus III and *Virus myxomatosum* used in these experiments were kindly supplied by Dr. T. M. Rivers of The Rockefeller Institute for Medical Research.

which the two conditions differed may be briefly indicated here. Infectious myxoma is almost uniformly fatal for rabbits and is characterized not only by a local swelling at the site of inoculation but by edematous swellings about the eyes, ears, nose, mouth, external genitalia, and other parts of the body; while the tumor-like condition is in our experience never fatal, its only evidences of pathology are at the site of inoculation, and it produces no general symptoms. The virus of myxoma is infectious by any route and the resulting disease is highly contagious, whereas the tumor-producing agent is effective only when it can be brought into intimate contact with connective tissue, and the resulting disease is not contagious. The virus of infectious myxoma is found regularly in the blood stream while the tumor-producing agent has not been demonstrated there. The swelling produced at the site of inoculation with myxoma virus is of a diffuse character with an indefinite margin and it has the gross and histological appearance of an edematous inflammatory cellulitis accompanied by connective tissue proliferation. The tumor, on the other hand, arising at the site of inoculation with tumor-producing agent is firm and well circumscribed; it has the gross and histological appearance of a fibroma. One should add that portions of myxoma lesions can be found which bear a closer resemblance to a neoplastic process than they do to an inflammatory one. In the epithelial cells overlying a myxoma swelling, eosinophilic cytoplasmic inclusions can regularly be seen. These are not found in the epithelium overlying the tumor nodules produced in laboratory rabbits although the epidermis may be edematous and vesiculated.

In spite of these differences, very early in the present work two characteristics of the tumor-like condition suggested a possible relationship to infectious myxoma. The first was the spongy consistency of the local swelling in its early stage before it had developed its characteristic firm tumor-like form. The second was the finding of cytoplasmic eosinophilic inclusions very similar to those seen in infectious myxoma in the epithelium overlying the original wild rabbit tumor and in that overlying an experimentally produced tumor in a wild rabbit. The following experiments were undertaken in order to explore this possible relationship.

Resistance to Infectious Myxoma Exhibited by Rabbits Recovering from the Tumor

Domestic rabbits in which actively growing subcutaneous or testicular tumors had been allowed to regress either partially or completely were tested for susceptibility to infectious myxoma. Of these, 8 were inoculated subcutaneously with a very large dose of myxoma virus, while 7 received, in the same way, a somewhat smaller dose. The results are recorded in Table IV.

As shown in this table, only 1 of the 15 rabbits died of infectious myxoma, 2 proved completely resistant, and the others, although they exhibited varying degrees of illness, all recovered. This result, striking in view of the practically uniform fatality of the disease as observed by other investigators, indicated that rabbits, in which tumors had regressed, had been rendered more than normally resistant to infection with the virus of myxoma.

Attempts to Neutralize the Virus of Infectious Myxoma with Serum of Rabbits Recovered from Tumor

In an attempt to obtain information concerning the nature of the increased resistance to infectious myxoma exhibited by rabbits recovered from tumor, the sera of such animals were tested for antibodies effective against the myxoma virus. These sera had previously been shown to contain antibodies effective against the tumor-producing agent. The results of such cross-neutralization tests were completely negative, as shown in Table V.

Neutralization of the Virus of Infectious Myxoma with Serum from Tumor-Recovered Rabbits That Had Been Subsequently Inoculated with Myxoma Virus

While the sera from rabbits recovered from tumor failed to neutralize myxoma virus, these same rabbits when subsequently infected with myxoma underwent a mild and abortive type of disease after which their sera neutralized the virus of infectious myxoma, as shown by Table VI.

TABLE IV

Resistance of Tumor-Immune Rabbits to Infectious Myxoma

Rabbit No.	Site of tumor	Condition of tumor at time of myxoma inoculation	Dosage of myxoma virus subcutaneously	Clinical picture	Result
226	Both testicles	Almost complete regression	1 cc. of the supernatant of a 10% suspension of subcutaneous swelling and testicle of Rabbit 284	Severe illness	Recovered
233	Subcutis	Complete regression		Moderate illness	"
234	"	" "		Mild abortive illness	"
242	Both testicles	Regressing		Abortive very mild illness	"
243	" "	Complete regression		Severe illness	"
265	" "	Regressing		Abortive mild illness	"
267	Subcutis	Complete regression		Moderate illness	"
271	Both testicles	Regressing		Local subcutaneous swelling	No illness
297		Control		Typical myxoma	Died on 7th day
251	Both testicles	Regressing	0.5 cc. of a 1:50 dilution of the supernatant of a 5% suspension of subcutaneous swelling and testicle of Rabbit 310	Completely negative	No illness
263	Subcutis and testicle	Almost complete regression		Local subcutaneous swelling	" "
264	Both testicles	" " "		Typical myxoma	Died on 12th day
266	" "	Regressing		Local subcutaneous swelling	No illness
283	Subcutis	Almost complete regression		" " "	" "
320		Control		Typical myxoma	Died on 9th day
321		"		" "	" " 10th "
416	Both testicles	Beginning regression	1 cc. of a 1:50 dilution of the supernatant of a 5% suspension of subcutaneous swelling and testicle of Rabbit 421	Local subcutaneous swelling	No illness
417	Subcutis and both testicles	" "		Completely negative	" "
426		Control		Typical myxoma	Died on 7th day

TABLE V

Attempts to Neutralize the Virus of Infectious Myxoma with Serum from Rabbits Recovered from the Tumor

Source and concentration of myxoma virus	Rabbit No.	Amount of virus subcutaneously	Serum		Result
			Source	Amount	
		cc.		*cc.*	
Supernatant of a 10% suspension of subcutaneous swelling and testicle of Rabbit 284	296	1	Recovered Rabbit 234	1	Died in 9 days
	297 (control)	1	Normal " 660	1	" " 7 "
Supernatant of a 5% suspension of subcutaneous swelling and testicle of Rabbit 310	295	0.5, 1:50 dilution	Recovered " 263	0.5	" " 11 "
	322	0.5, 1:50 "	" " 264	0.5	" " 7 "
	323	0.5, 1:50 "	" " 234	0.5	" " 11 "
	324	0.5, 1:50 "	" " 251	0.5	" " 12 "
	325	0.5, 1:50 "	" " 266	0.5	" " 10 "
	320 (control)	0.5, 1:50 "	Normal " 660	0.5	" " 9 "
	321 (control)	0.5, 1:50 "	" " 660	0.5	" " 10 "
Supernatant of a 5% suspension of subcutaneous swelling Rabbit 344	367	0.5, 1:25 "	Recovered " 234	1.5	" " 13 "
	368	0.5, 1:25 "	" " 266	1.5	" " 15 "
	365 (control)	0.5, 1:25 "	Normal " 14	1.5	" " 12 "

TABLE VI

Neutralization of the Virus of Infectious Myxoma with Serum from Tumor-Recovered Rabbits Subsequently Inoculated with Myxoma Virus

Source and concentration of myxoma virus	Rabbit No.	Amount of virus subcutaneously	Serum		Result
			Source	Amount	
		cc.		*cc.*	
Supernatant of a 5% suspension of subcutaneous swelling and testicle of Rabbit 310	338	0.5, 1:50 dilution	Rabbit 234 after recovery from myxoma	0.5	Moderate illness; recovered
	337 (control)	0.5, 1:50 "	Normal Rabbit 14	0.5	Died in 12 days
Supernatant of a 5% suspension of subcutaneous swelling of Rabbit 344	364	0.5, 1:25 "	Rabbit 234 after recovery from myxoma	1.5	No illness
	366	0.5, 1:25 "	Rabbit 266 after recovery from myxoma	1.5	" "
	365 (control)	0.5, 1:25 "	Normal Rabbit 14	1.5	Died in 12 days

Inoculation of Wild Cottontail Rabbits with the Virus of Infectious Myxoma and Its Effect on Their Susceptibility to the Tumor-Producing Agent

Hobbs (4) has stated that in his experience the native wild cottontail rabbit is not susceptible to infectious myxoma. Two out of three of our trials to infect wild cottontail rabbits also yielded apparently negative results. In the third attempt the very large dosage of 2 cc. of a 5 per cent suspension of virus-containing tissue administered subcutaneously resulted, after 16 days, in a transitory thickening of the epidermis and subcutaneous tissue in the region of the site of inoculation. No general symptoms or characteristic swellings of the eyelids, nose, and external genitalia were observed. The clinical picture in this one wild rabbit was thus at wide variance with both infectious myxoma as produced in laboratory rabbits and the tumor-like condition produced in wild and laboratory rabbits. These few experiments with wild cottontail rabbits tend to confirm Hobbs' observation that this species does not develop clinically recognizable infectious myxoma as a result of inoculation with myxoma virus.

However, in spite of the apparently negative reaction of 2 of the animals to the virus of infectious myxoma, they, as well as the one that had developed a doubtful myxoma lesion, were subsequently found to be resistant to the tumor-like condition. Blood serum, obtained a month after infection, from the wild rabbit that had shown a doubtfully positive reaction to the virus of infectious myxoma exhibited only slight inhibitory action on both the tumor-producing agent and the myxoma virus in neutralization tests.

Neutralization of the Tumor-Producing Agent by Serum of a Rabbit Convalescent from Infectious Myxoma

While a number of domestic rabbits have been rendered immune to myxoma by preliminary infection with the tumor-producing agent, and while, after subsequent inoculation with myxoma virus, the sera of such animals have proved capable of neutralizing that virus (see Table VI), they were not satisfactory to use in cross-neutralization experiments because of the earlier tumor infection. Consequently experiments were initiated in order to secure a combination of sera

TABLE VII

Neutralization of Tumor-Producing Agent and Myxoma Virus by the Serum of a Rabbit Recovered from Myxoma

Type and concentration of infectious agent	Rabbit No.	Amount of infectious suspension administered and route	Serum		Result
			Source	Amount	
		cc.		*cc.*	
Supernatant of a 5% suspension of myxomatous material from Rabbits 344 and 365	390	0.5, 1:25 dilution, s.c.*	Myxoma-convalescent Rabbit 338	1.5	No illness
	388 (control)	0.5, 1:25 " "	Normal Rabbit 14	1.5	Died in 18 days
Supernatant of a 5% suspension of testicular tumor from Rabbit 380	385	1 "	Myxoma-convalescent Rabbit 338	1	No tumor
		0.2 i.t.	Myxoma-convalescent Rabbit 338	0.2	Scant tumor growth
	392	1 s.c.	Normal Rabbit 14	1	Typical tumor growth
	(control)	0.2 i.t.	" " 14	0.2	" " "
Supernatant of a 5% suspension of testicular tumor from Rabbit 392	396	1 s.c.	Myxoma-convalescent Rabbit 338	1	No tumor
		0.2 i.t.	Myxoma-convalescent Rabbit 338	0.2	Scant tumor growth
	394	1 s.c.	Normal Rabbit 14	1	Typical tumor growth
	(control)	0.2 i.t.	" " 14	0.2	" " "

* s.c. = subcutaneously. i.t. = intratesticularly.

and myxoma virus such that, while preventing a fatal myxoma illness, it would still render the animal immune. Most of the mixtures were so balanced that either there was no evidence of neutralization and the animals died much like their controls, or the neutralization was complete and neither illness nor immunity resulted. One animal (Rabbit 338), however, either because the partially neutralized virus was ideal for immunization, or because of a natural partial resistance to infectious myxoma, developed a non-fatal attack of the disease and recovered completely. Serum from this rabbit was then tested for neutralizing properties against the myxoma virus and the tumor-producing agent. The results of these experiments are recorded in Table VII.

As shown in Table VII, the serum from Rabbit 338 contained antibodies effective against both the myxoma virus and the tumor-producing agent. This animal was subsequently inoculated subcutaneously with tumor-producing agent and found to be resistant to infection.

DISCUSSION

The properties of the tumor-producing agent described in this and the preceding paper (1) are all of the group generally considered characteristic of a filtrable virus. The failure to cultivate any organisms from the tumors or to see them in stained sections, together with the tumor-producing agent's resistance to glycerol, its ready filtrability, the type of immunity it induces, its relative host specificity, its production of cytoplasmic inclusions in the epithelial cells of one of its susceptible hosts, and its apparent tropism for one type of tissue, considered collectively, suffice to place it in the general group of filtrable viruses. Any attempt to classify it in any other way would seem artificial.

The classification of the pathological product of the action of the virus on the host is more difficult. The original tumor seen in the wild rabbit, and, in fact, those produced experimentally in wild rabbits, could, from the pathological picture, especially that revealed by the microscope, be classed as either granulomata or fibromata. However, the gross and histological pictures of the tumor as produced in laboratory rabbits, whether by intratesticular, subcutaneous, or

intramuscular inoculation, resemble those of a fibroblastic neoplasm. The extremely rapid growth of the tumor during the first 10 or 15 days after inoculation, followed later by retrogression, is not usual with transplanted neoplasms. It should be remembered, however, that this is the event in an alien species, and that mouse tumors will grow for a time in rats before retrogressing. Tumors produced in wild rabbits appear to persist unchanged indefinitely, to judge from the few available instances. The production of the tumor by material stored for long periods of time in glycerol distinguishes it from mammalian neoplasms, as also its production by cell-free filtrates. However, these features taken together fail to eliminate the possibility that the tumor is of neoplastic character, for the Rous sarcomas of chickens can be produced by cell-free filtrates of tumor tissue and by glycerolated material. As yet sufficient experiments on the fate of engrafted material have not been conducted to enable one to say whether the introduction of such material involves an actual transplantation of the growth. There can be no doubt that the tumors caused by the injection of filtrates arise as a result of the local action of the virus on the cells of the injected animal. The fibroma-like new growths are the product of this reaction. There is evidence (1) that the effectiveness of the virus in the production of tumors depends upon its being brought into intimate contact with connective tissue cells and that it bears a relationship to connective tissue similar to that borne by neurotropic viruses for the central nervous system or dermatotropic viruses for the skin. From the data at hand the tumor can better be considered as the local hyperplastic connective tissue reaction to the virus than as a true neoplastic process.

When the tumor-like condition and infectious myxoma were compared, utilizing cross-immunity phenomena to determine relationship, surprising results were encountered in view of the marked clinical and pathological differences already discussed. It was found, in fourteen out of fifteen instances, that rabbits in which tumors had regressed or were regressing exhibited marked resistance to infection with the myxoma virus. In only 2 out of 14 animals was this resistance of such a degree as to be classified as complete. In the remaining 12 rabbits myxoma developed in degrees of severity varying from a local lesion at the site of inoculation to a grave illness. Blood serum

from tumor-convalescent rabbits, however, while capable of neutralizing the tumor virus, did not protect against *Virus myxomatosum*. Even the serum from Rabbit 251 (Tables IV and V) was devoid of neutralizing properties against myxoma virus although this animal had proved completely resistant to infectious myxoma on test inoculation. These experiments indicate that the failure of the virus of infectious myxoma to produce a fatal illness in tumor-convalescent rabbits may be explainable on the basis of an acquired resistance, conceivably of fixed tissue type. This view was strengthened by the finding that a rabbit (No. 266), whose serum drawn after recovery from the tumor failed to neutralize myxoma virus (Table V), after subsequent infection with myxoma virus, which resulted in only a local swelling, yielded a serum capable of neutralizing myxoma virus (Table VI). The fact that myxoma virus, which in a tumor-convalescent rabbit produced only a local lesion, was nevertheless capable of establishing demonstrable humoral antiviral bodies, while tumor virus, also causing a local lesion, developed no demonstrable humoral antibodies but did establish a resistant state in the host, seems significant. Two possible interpretations of these observations are apparent. The first and probably least likely is that a fine line of distinction may be drawn between the *resistance* to infectious myxoma generated in a rabbit by its previous infection with tumor virus and the *immunity* it acquires when it is subsequently inoculated with myxoma virus. Under such an interpretation the two viruses could be considered as antigenically distinct from one another. The second and simpler explanation for the immunological differences is that they are quantitative only, not qualitative, and that the tumor virus establishes a lower grade of immunity against *Virus myxomatosum* merely by virtue of being a poorer antigen than *Virus myxomatosum*. Under this second interpretation the two viruses would be thought of as antigenically identical, or closely related to one another. The tumor virus would then be considered merely as a strain of *Virus myxomatosum*, atypical, however, in its ability to regularly infect wild rabbits, in its inability to produce the classically rapidly fatal infectious myxoma in domestic rabbits, and in its failure to generate in domestic rabbits demonstrable virucidal antibodies effective against *Virus myxomatosum*. The tumor virus, considered as a strain of *Virus*

myxomatosum, would further differ from our classical conception of that virus by being incapable of passage from rabbit to rabbit by contact, by not invading the blood stream, by not establishing a generalized infection with widespread pathological alterations, by failing completely to infect domestic rabbits when administered intravenously or intraperitoneally, by having an apparently specific infective affinity for connective tissue, and by producing at the site of inoculation a tumor histologically at wide variance with the local lesion produced by *Virus myxomatosum*.

Wild cottontail rabbits inoculated with *Virus myxomatosum* failed, with one possible exception, to show clinical evidence of myxoma, but in spite of this became subsequently resistant to infection with tumor virus. This acquired resistance too was not accompanied by the appearance in the blood serum of antibodies neutralizing either myxoma or tumor virus.

The cross-immunity developed by a rabbit convalescent from myxoma has also been considered. Hobbs (4) demonstrated that serum from a rabbit convalescent from infectious myxoma would neutralize the myxoma virus and Fisk and Kessel (6) found that rabbits convalescent from infectious myxoma were immune to subsequent infection by the virus. We were desirous of determining whether rabbits convalescent from infectious myxoma would show resistance or immunity to the tumor virus. Only one animal not previously infected with tumor virus has so far survived an attack of myxomatosis. This rabbit was shown thereafter to be resistant to infection with tumor virus and its serum not only neutralized myxoma virus completely but also possessed virucidal properties for the tumor virus.

The relationship of the tumor-producing virus to the myxoma virus is not yet clear. The two diseases appear at present as separate clinical entities produced by viruses that seem immunologically to be related. The recent work of Andrewes (7) on the immunological relationships of fowl tumors with different histological structure and that of Murphy and his coworkers (8, 9) on a tumor-inhibiting substance effective even against tumors of an alien host may point the way to an explanation of the peculiar relationship existing between the two. Another possibility is that their relationship is like that of

vaccine virus and the virus of variola. One might suppose that the two viruses were originally identical or at least had a common ancestor, and that the tumor virus was developed from the myxoma virus by prolonged passage through wild rabbits just as the virus of variola, after passage in series through calves, becomes permanently vaccine virus, or so it is supposed.

Rivers (3), in discussing similarities between the myxoma of rabbits and the Rous sarcoma of chickens, has said, "If, as some believe, the Rous sarcoma appears to be more closely related to true neoplasms than to diseases induced by highly contagious agents, then the myxoma, upon further study, may serve to bridge the gap between the Rous tumor and other virus maladies. . . . ." The tumor-like condition of rabbits discussed in this and the preceding paper probably has a place somewhere between infectious myxoma and the Rous sarcoma in this general arrangement of virus diseases suggested by Rivers, thus bridging still further the gap between the Rous tumor and other virus maladies.

The description by von Dungern and Coca (10) of a transmissible spindle cell fibrosarcoma in wild hares, which could be transplanted to laboratory rabbits, while presenting interesting points of resemblance to the disease under discussion here, was not accompanied by sufficient data on the nature of the inciting agent for a close comparison.

SUMMARY

The properties of the agent causing a tumor-like condition in rabbits have been tested experimentally and the conclusion reached that it is a filtrable virus. While the tumor-like condition and infectious myxoma differ markedly in their clinical and pathological pictures, they have been found to be related immunologically. The relationships of the tumor-producing virus to the virus of infectious myxoma, and of the tumor-like condition to malignant neoplasms have been discussed.

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