INFECTIOUS PAPILLOMATOSIS OF RABBITS

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With a Note on the Histopathology

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Plates 33 to 35

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Our attention was recently called to a disease occurring in wild cottontail rabbits in northwestern Iowa.[1] Rabbits shot there by hunters were said to have numerous horn-like protuberances on the skin over various parts of their bodies. The animals were referred to popularly as "horned" or "warty" rabbits.

Warts from a naturally occurring case of the disease in Iowa were obtained and sent to the laboratory in sterile 50 per cent glycerol. These glycerolated warts furnished us our original material for investigation. A little later, in a shipment of a dozen wild cottontail rabbits from southern Kansas, three were found to be affected with the same wart-like disease. To date, out of 75 wild cottontail rabbits received from Kansas eleven have been found to carry one or more warts. These eleven animals serve as the basis for our description of the naturally occurring disease.

Description of the Naturally Occurring Disease

In wild cottontail rabbits the presence of warts has caused no apparent discomfort in our experience and induced no demonstrable evidence of generalized illness. Most of the animals were sacrificed, shortly after their arrival, for pathological material, but four, kept under observation for 7 weeks or longer, at no time appeared ill and were in good physical condition when finally killed. The number

[1] We are grateful to Mr. T. A. McKichan of Cherokee, Iowa, who first told us of the disease, and to Mr. Clifford Peck of Cherokee and Mr. Earl Johnson of Rago, Kansas, who furnished us with naturally occurring cases of the disease.

of warts on individual animals in our series varied from one to ten in all cases except one. The exceptional animal was almost literally covered with warts and these, when removed at autopsy, were sufficient to fill a 200 cc. flask. The most common sites for the warts were on the inner aspect of the thighs, the abdomen, or about the neck and shoulders. Individual warts varied somewhat in size but the greater number were from 0.5 to 1 cm. in diameter and from 1 to 1.5 cm. in height. They were black or grayish black in color, well keratinized, and the upper surfaces were frequently irregular or fissured. They were roughly oval in shape when viewed from the side (Fig. 1). The bases, in most instances, were narrower than the mid-portion. The lateral surfaces of the warts appeared vertically striated because each individual wart was composed of closely packed and almost homogeneous vertical strands of tissue. On cut section, an average wart had a white or pinkish white fleshy center, and the upper portion and its lateral surfaces were grayish black and keratinized. The vertically striated structure was particularly evident on cut section. Attachment to the skin was loose as evidenced by the ease with which warts were knocked or pulled off when animals were handled. Warts removed in this way left a rather freely bleeding surface which in most instances healed without complication; sometimes a second wart appeared at the same site.

Experimental Transmission

No difficulty has been encountered in transmitting the condition to either domestic or wild cottontail rabbits when materials from naturally occurring cases have been employed. The method used is, in brief, as follows:

Either freshly removed warts or those that have been stored in 50 per cent glycerol at refrigerator temperature are ground to a fine paste with sterile sand and physiological saline in a mortar. More physiological saline is added to make a 3 to 5 per cent final suspension. Such a suspension is then centrifuged and the supernatant fluid, which is only slightly turbid, is removed and used for inoculation. Suspensions prepared in this way remain infectious for at least a month when kept at refrigerator temperature.

Inoculation by scarification was regularly performed in these experiments. Rabbits to be inoculated were shaved on the abdomen or sides and lightly scarified either by needle or by rubbing the shaven skin with a moderately coarse grade of sterilized sandpaper. To obtain discrete warts the former method was employed, while scarification with sandpaper was used when a confluent and massive growth of warts was desired. The scratches were made only deep enough to cause a barely perceptible oozing of blood-tinged fluid. A small amount of the infectious suspension was immediately applied by dropping it from a syringe, and this fluid was rubbed well into the scarifications by means of a spatula or the flat handle of a scalpel. The area thus inoculated was allowed to become almost dry before the animal was released and put into its cage.

Course of the Experimental Infection

The disease produced by experimental infection of either wild cottontail or domestic rabbits followed a typical course when infectious material from naturally occurring cases was employed. The period of time elapsing between inoculation and the first appearance of macroscopically detectable lesions varied from 6 to 12 days with an average of slightly more than 8 days. This variation in the incubation period was probably due more to differences in the potency of the infectious suspensions than to differences in the resistance of individual animals, for when the same suspension was employed in inoculating a series of rabbits the incubation period was the same in the entire series.

The first detectable lesions consisted in minute, barely visible elevations along the lines of scarification. These, on the 1st day, appeared macroscopically to be tiny vesicles, an appearance not supported by histological examination. By the 4th or 5th day the lesions were more definite and numerous and were usually pink in color. They were approximately 1 mm. in diameter and height and had lost their delicate vesicular appearance. From this stage on, growth was constant although its rapidity varied considerably. From the 16th to the 20th day after their first appearance, the lesions were approximately 3 mm. in diameter and height if they were isolated on the shaven areas or, if confluent, they constituted a more or less solid mass of rough wrinkled pinkish keratinized tissue 3 to 4 mm. in thickness. They had, by this time, acquired a definitely warty appearance, their surfaces were keratinizing, and their sides exhibited the peculiar type of perpendicular striations seen in the naturally occurring warts. The warts, whether separate or confluent, continued to increase in size for an indefinite period, and as the lesions became older they became more and more cornified until finally the upper portions were very hard. The lower portions, however, usually remained fleshy to the touch. At 6 weeks, individual warts or confluent masses were from 1.2 to 1.5 cm. in height; the skin on the portion of the body upon which they were developing had become pendulous and was thrown into large stiff folds. Animals sacrificed at this time exhibited an enormously increased blood supply in the subcutaneous tissue underlying the warts. In spite of the great size of many of our experimentally produced wart masses, the animals showed no loss in weight and the entire course of the disease was free from any general clinical evidence of illness. In their gross appearance the experimental warts in both domestic and wild rabbits have been identical with those seen in the naturally occurring disease. Photographs of experimentally produced warts are given in Figs. 2 to 4.

Experimental warts, as well as those occurring naturally, appear to remain stationary when they reach 1 to 1.5 cm. in height. One of our rabbits, however, at present, 6 months after inoculation, is carrying a large wart mass which in places is 3 cm. in height. With two exceptions, we have seen no warts retrogress in animals infected in the usual way. In the exceptional animals, one a wild and the other a domestic rabbit, warts developed slowly after an unusually long incubation period. They reached a maximum height of only 2 to 3 mm. between 30 and 40

days after inoculation and in 60 days had completely disappeared. Both of these animals were inoculated with the same infectious suspension and were the only ones so inoculated. In no animal in which growth of warts took place in the usual fashion and in which the lesions reached a thickness of 1 cm. or more have we seen any evidence of retrogression. To date we have had experimentally infected animals under observation for 6 months only. While there has been no evidence of retrogression of the papillomata except in the cases mentioned, there has also, so far, been no evidence that the lesions of prolonged standing are acquiring malignant properties. Animals are being held under observation to determine what the ultimate fate of the papillomata will be.

HISTOPATHOLOGY

By E. WESTON HURST

Histology of Naturally Occurring Warts

The growths consist of a number of closely adjacent, branching filiform processes of epidermis with very narrow connective tissue cores (Fig. 5). This formation suggests simultaneous growth from many centers, with resulting lateral pressure; at the margins the growth bulges over the neighboring normal skin. Transition from the normal epithelium at the edge is relatively abrupt through a narrow zone showing rapid thickening of the epithelial layers.

In the area of new growth the germinal cells of the Malpighian layer may be taller and narrower than normal, giving a palisade effect; mitoses are always present and often numerous, and are found in layers four or five cells removed from the corium. Melanin pigment is much more abundant than in the surrounding skin. The polygonal cell layer is greatly increased in depth, as is the granular layer; the cells vary greatly in size and include a variable number of monster cells with enormous vesicular nuclei. There is no definite eleidin layer; the granular layer passes more or less abruptly into the horny layer, which is often imperfectly keratinized and shows skeleton cell outlines with pyknotic nuclear remains (Fig. 6).

In many cases the tips of the longer papillae are in a necrotic condition, probably determined by the obviously deficient blood supply; that necrosis occurs suddenly is shown by the detection of remains of mitotic figures in the dead tissue. Acute inflammation may supervene locally. Evidence of mild inflammation in the skin beneath the wart is furnished by the presence, in small numbers, of lymphocytes, plasma cells, and polymorphonuclear leucocytes in the subcutaneous tissues.

No definite cellular inclusions have been recognized. Spherical or ovoid eosinophilic structures exist in the outer layers of the wart; at first sight intracytoplasmic, they can usually on careful examination be seen to lie between the cells or in indentations of the cell membrane. They can be traced into the deeper

layers almost to the germinal layer, and appear to originate from necrosis of individual cells at this level.

Histology of Experimental Warts

As early as the 7th day after inoculation, in a series showing macroscopic lesions on the 10th day, slight thickenings of the epithelium may be detected microscopically in the inoculated area. By the 9th day, quite definite localized thickenings composed largely of rapidly multiplying cells, differing but little from normal epithelial cells, are present. Though to some extent elevated above the general skin surface, the cellular masses project more into the corium beneath, where by lateral extension they come to underlie the normal epithelium (Fig. 7). The granular layer is several times the normal in depth, but as yet there is no excess keratinization. The growth is obviously primarily epithelial.

Sections of warts macroscopically visible for 5 days reveal an almost continuous sheet of thickened epidermis extending over the inoculated area. Mitoses are abundant even some distance from the germinal layer in cells containing many keratohyaline granules. Keratinization of the newly formed cells is beginning and at this stage is more perfectly accomplished than later.

Gradually the warts project as papillae from the surface (Fig. 8). The polygonal cells show greater variation in size, keratinization is less perfect, pigmentation occurs in excess, and all the features of the spontaneous growth are faithfully reproduced (Fig. 9). Necrosis of the tips of the papillae may occur as early as the 20th day when the warts are thickly set on the inoculated area. Growth continues actively for at least 91 days, the most advanced case of the histological series, at which period mitoses are still numerous. No signs of spontaneous regression have been noticed. There is no observable difference in warts induced in wild and in domestic rabbits.

In the wild rabbit, removal of the main mass of the wart may be followed by regeneration of an identical structure. Warts induced in a wild animal already infected show no histological variation from the spontaneous growths.

Throughout the experimental series, a variable degree of inflammatory infiltration with mononuclears and polymorphonuclear leucocytes is evident in the corium; this is never intense.

Viability of the Wart-Inducing Agent in Glycerol

Warts from naturally occurring cases stored in equal parts of glycerol and 0.9 per cent NaCl solution at refrigerator temperature for as long as 106 days remain fully infectious. Warts stored for longer periods have not been tested for infectivity.

Filtrability of the Wart-Inducing Agent

Warts to be used as a source of infection in the filtration experiments were removed from the 50 per cent glycerol in which they had been stored and were washed in three changes of sterile physiological saline. They were then minced with sterile scissors, ground in a mortar with sterile sand, and suspended in sufficient physiological saline to make an approximately 5 per cent suspension. Suspensions thus prepared were cleared by centrifugation. The decanted supernatant fluid was usually almost water-clear with only a faint opalescence, and for this reason was rapidly filtrable. 1 cc. of a broth culture of *B. prodigiosus* was added to each 15 to 20 cc. of fluid just before it was passed through Seitz or Berkefeld filters. The resulting filtrates were tested for sterility in 1.5 cc. amounts. All filtrates recorded were bacteriologically sterile.

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

Warts produced by filtrates, recorded in Table I as positive, were as extensive and characteristic as those in the control animals which had been inoculated with unfiltered suspensions. Furthermore, when domestic rabbits were used as the test animals, filtration, especially through Berkefeld V or N candles, instead of prolonging the incubation period as might be expected because of some possible removal of the filtrable agent by absorption on the filter surface, usually had either no effect or shortened the period. In wild rabbits, from the limited data at hand, it would seem that filtration resulted in a slight prolongation of the incubation period. From the data recorded in Table I it can be concluded that the etiological agent causing warts in rabbits readily passes Berkefeld filters, of V, N, or W porosity but does not regularly pass a Seitz filter when two pads are employed. Filtration through a Seitz filter, using one pad, allowed not only the virus to pass but also *B. prodigiosus*.

No extensive attempts to cultivate visible microbial forms from filtrates of proven infectivity were made. However, during the investigation active filtrates have been cultured repeatedly in plain and blood broth and on plain and blood agar and such cultures have remained sterile both as regards the test organism, *B. prodigiosus*, or any other visible bacterial form. While no special media have been employed in these attempts to demonstrate the bacteriological sterility of active filtrates, the results obtained using the media mentioned above, considered with the fact that sections of actively growing warts or films of

TABLE I

Filtration Experiments

Experiment No.	Filter	Time of filtration	Amount of filtrate	Maximum negative pressure	Rabbit No., inoculated	Result: wart formation on inoculated skin	Incubation period
		min.	*cc.*	*cm. Hg*			*days*
1	Berkefeld V	2	30	62	DR* 621	Positive	6
	Unfiltered suspension				DR 620	"	6
2	Berkefeld V	0.75	30	62	DR 644	"	6
	" N	1	19.5	62	DR 575	"	6
	" "	1	19.5	62	WR† 634	"	9
	" W	2	10.5	62	DR 613	"	8
	" "	2	10.5	62	WR 632	"	9
	Unfiltered suspension				DR 666	"	9
	" "				WR 637	"	7
3	Berkefeld V	0.50	29	62	DR 640	"	7
	Unfiltered suspension				DR 681	"	10
4	Seitz (2 pads)	2	30	Positive pressure	DR 711	Negative	
	" (2 ")	2	30	" "	DR 725	"	
	Berkefeld V	2.5	30	62	DR 729	Positive	12
	" "	2.5	30	62	WR 733	"	12
5	" N	0.5	34	62	DR 790	"	9
	" W	3	27	62	DR 789	"	9
	Unfiltered suspension				DR 791	"	9
6	Seitz (2 pads)	2	34	Positive pressure	DR 793	"	15
	Berkefeld N	1.5	29	62	DR 788	"	7
	" "	1.5	29	62	DR 794	"	7
	" W	3	29	62	DR 784	"	11
	" "	3	29	62	DR 795	"	11
	Unfiltered suspension				DR 792	"	9

* DR = domestic rabbit.

† WR = wild rabbit.

active unfiltered infectious suspensions have failed to reveal the presence of any constant perceptible microbial form, would seem clearly to indicate that no visible organized agent is etiologically essential to the wart production.

Heat Resistance of the Wart-Inducing Agent

The method just described for preparing wart suspensions for filtration was employed in the heating experiments to be outlined. Only the slightly turbid supernatant fluid of centrifuged physiological saline suspensions of glycerolated

TABLE II

Heat Resistance of the Wart-Inducing Agent

Heated for 30 min.	Rabbit No., inoculated	Result: wart formation on inoculated skin	Incubation period
°C.			*days*
45	716, right side	Positive	10
55	715	"	11
60	746, left side	"	7
60	757 " "	"	7
60	713	"	10
60	772	"	8
60	726	"	9
60	785	"	8
65	716, left side	"	10
65	748 " "	"	9
65	759 " "	"	8
65	752 " "	"	8
65	778 " "	"	8
65	738	"	8
65	781, left side	Negative	
67	753 " "	Positive	26 (only 5 warts)
67	779 " "	"	20 (developed well for 10 days, then retrogressed)
67	782, left side	"	20 (developed poorly and retrogressed)
70	753, right side	Negative	
70	778 " "	"	
70	781 " "	"	
73	779 " "	"	
73	782 " "	"	
73	783	"	
75	746, right side	"	
75	757 " "	"	
75	752 " "	"	
75	777	"	
75	780	"	
85	748, right side	"	
85	759 " "	"	

warts was used. The fluid to be heated was sealed in sterile glass ampoules and completely submerged in the water bath for the 30 minute period during which it was exposed to a given temperature.

The results of the heating experiments are given in Table II.

The data recorded in Table II indicate that the activity of the wart-inducing agent is unaffected by temperatures of 65°C. or below for ½ hour but is completely destroyed by temperatures of 70°C. or higher. Heating to 67°C. for 30 minutes, while not completely inactivating the wart-producing agent, did exert a deleterious influence on it. This was evidenced by a marked prolongation of the incubation period in rabbits infected with material heated to this temperature and by scant takes and early retrogression of the resulting warts. It was of interest to note in this respect that the heating of suspensions at temperatures from 45–65°C., instead of lengthening the incubation period in inoculated animals, often shortened it as compared with that shown in animals receiving the unheated control suspensions.

In some of the heating experiments opposite sides of a single rabbit were used to test two suspensions, with adequate care that material from one side did not contaminate the other side. In earlier carefully controlled experiments in which both sides of an animal were shaved and scarified but only one side inoculated with an infectious suspension, it was shown that warts developed only on the inoculated side. In the experiments in Table II, in most instances, inoculations were arranged in such a way that only one side of the animal developed warts.

The thermometer used in these experiments was compared with a standard thermometer and was found to give readings 0.2°C. below those of the standard instrument. This small correction has not been made in the data recorded in Table II.

Routes of Infection

Only the method of inoculation by scarification has yielded constant results in our hands. Inoculation intravenously with infectious Berkefeld filtrates, after first abrading an area of the skin of the abdomen with a sterile needle, led to infection of the abraded areas in two out of four cases. Of the two positive animals, one, a wild rabbit, developed only a single wart; while the other, a domestic rabbit, de-

veloped four warts on the abraded area and two on the back of the neck. The incubation period in both of these cases was over three times as long as that of the control animals infected by scarification. At autopsy, all four intravenously inoculated animals were free from visceral pathology ascribable to the wart-inducing agent. Inoculations of either wild or domestic rabbits intraperitoneally, subcutaneously, intratesticularly, or intracerebrally, with filtrates of proven infectivity on scarification, have yielded entirely negative clinical and pathological results. About 50 per cent of the intradermal inoculations resulted in wart formation although in these instances the warts appeared not at the point where the inoculum had been deposited but at the point where the needle had pierced the epidermis and where some of the inoculum had leaked from the needle tract. The incubation period of warts produced in this way was always longer than when infection had been accomplished by scarification.

Resistance of Infected Rabbits to Reinfection

In a series of 123 wild and domestic rabbits inoculated with suspensions of known infectivity, we have encountered no animal that was naturally immune.

One of the two rabbits in which warts underwent complete retrogression was tested and found to be resistant to reinfection. The serum of this animal, however, when mixed in equal parts with an infectious suspension, failed to neutralize the wart-inducing agent. It did prolong the incubation period considerably.

Ten domestic rabbits carrying warts of various ages have been tested for immunity to reinfection. Five resisted reinfection successfully, while the remaining five, after unusually long incubation periods, finally developed warts at the sites of their new inoculations. These warts were much less numerous and slower in growth than those in the control animals. The time elapsing between the primary infection and the attempt at reinfection was apparently of little importance, for two animals were found to be susceptible to reinfection 76 days after their primary inoculation while one animal was completely resistant to reinfection 31 days after its primary inoculation. Two rabbits tested 14 days after their primary inoculation and 6 days after the first appearance of warts possessed some resistance which was evidenced by the fact that no warts appeared at the sites of their new inoculations for

24 days, whereas the incubation period in the control animals was 8 days.

Three wild rabbits that were carrying warts when received from Kansas were tested for immunity. All three were still susceptible, although the incubation periods were markedly lengthened. One experimentally infected wild rabbit has been found to be immune to reinfection.

Neutralizing Properties of the Sera of Infected Rabbits

Sera from wild rabbits, either naturally or experimentally infected, as well as the sera from experimentally infected domestic rabbits, have been found to contain antibodies effective against the wart-inducing agent. Most of such sera have neutralized it completely, so that warts failed to develop in animals inoculated with mixtures of serum and the infectious agent; in the others, partial neutralization was evidenced by a doubling or trebling of the incubation period.

For these tests the usual virus neutralization technique was employed. Equal parts of serum and infectious suspension were mixed and stored overnight (17 hours) in the refrigerator. The control consisted of equal parts of infectious suspension and normal rabbit serum. Rabbits were inoculated with these mixtures on the freshly scarified skin in the usual way, using one shaven side for the control inoculation and the other for the neutralization test, performing both inoculations on the same rabbit and thus avoiding possible individual variations in resistance.

From the above experiments it is evident that an active wart infection in rabbits not only renders them completely or partially resistant to reinfection but also that it evokes antibodies, demonstrable in their sera, capable of completely or partially neutralizing the wart-inducing agent.

Wart-Producing Agent Not Immunologically Related to the Viruses Causing Infectious Fibroma or Myxoma

In earlier experiments (1), a benign fibroma-like new growth of rabbits caused by a filtrable virus was found capable of establishing a resistance in rabbits to fatal infection with the otherwise uniformly deadly virus of infectious myxoma. To explore the possibility of an immunological relationship between the wart-producing agent and the benign fibroma virus or the fatal myxoma virus, a number of experiments were conducted. It was found that rabbits infected with the

wart-producing agent and carrying large warts at the time of testing were fully susceptible to both the fibroma and the myxoma viruses. No alteration of the normal course of either of these diseases was observed as the result of previous infection with the wart-producing agent. Conversely, rabbits recovered from infection with fibroma virus and demonstrably immune to reinoculation with that virus were still fully susceptible to infection with the wart-producing agent. Rabbits immunized against infectious myxoma by preliminary infection with fibroma virus and subsequent inoculation with *Virus myxomatosum*, and possessing demonstrable virucidal antibodies for *Virus myxomatosum*, were also still fully susceptible to infection with the wart-producing agent. These experiments indicate that the wart-producing agent is not immunologically related to either of these viruses.

It may be noted here that in the original glycerolated wart material obtained from Iowa both the wart-producing agent and the virus of infectious fibroma were present. The latter was easily separated from the former by testicular passage through domestic rabbits. The strain of fibroma virus thus isolated was typical in all major respects of the original strain described earlier (2), and like it was capable of protecting rabbits against fatal infection with *Virus myxomatosum*.

Attempts to Transmit the Wart-Producing Agent in Series through Rabbits

In all, twenty-six domestic and wild rabbits have been inoculated in the usual way with suspensions prepared from experimentally engendered domestic rabbit warts ranging in age from 1 to 116 days. Not only did all such inoculations yield negative results but the animals, when subsequently tested, were found to be still fully susceptible to infection with the wart-producing agent from wild rabbit papillomata. On the other hand, either naturally occurring or experimentally produced warts from wild rabbits proved readily transmissible to either wild or domestic rabbits. Warts from nine naturally occurring cases of the disease in wild rabbits have been tested and all found to be infectious for both wild and domestic rabbits. In like manner, experimentally produced warts from nine wild rabbits have been tested for infectivity. Eight of these proved infectious for either domestic or wild rabbits while the warts from one proved to be non-transmissible.

We have not yet attempted to pass the wart-producing agent through a long series of wild rabbits but in the course of obtaining fresh infectious material it has at present reached its third serial passage. In spite of the fact that the agent cannot be propagated in series through domestic rabbits, it is probable that it can be passed indefinitely in series through wild rabbits and that any of these serial wild rabbit passages can be used in infecting domestic rabbits.

No attempt has so far been made to transmit the domestic rabbit warts by means of tissue grafts, although in a small number of experiments freshly prepared cell-containing suspensions of young actively growing papillomata from domestic rabbits have yielded negative results when inoculated intracutaneously or subcutaneously into domestic rabbits. Instead, it has seemed best to study the rabbit papillomata first as an infectious process caused by a filtrable agent and to determine, if possible, why this agent should be readily transmissible in series when inducing warts in wild rabbits and non-transmissible when inducing similar growths in domestic rabbits.

That the degree of maturity of the warts in domestic rabbits at the time that attempts were made to transmit them in series was not a determining factor is indicated by the fact that warts taken at intervals of 6 to 8 days, from their first appearance until they were 116 days old, yielded no successful infections.

Domestic rabbit warts glycerolated for varying periods of time were repeatedly tested for infectivity to determine whether or not glycerol storage has an activating effect on the agent as it does on herpes virus of low activity (3–5). The results of these experiments were all negative.

In a series of experiments conducted before the presence of neutralizing antibodies in the blood serum of wart-bearing animals had been demonstrated, it was found that when an inactive domestic rabbit wart suspension was mixed with an equal part of a suspension prepared from wild rabbit warts of known infectivity, the resulting mixture was either completely non-infectious or the incubation period was prolonged and the resulting warts few in number as compared with control animals. This suggested the presence in warts from domestic rabbits of an inhibitory substance similar to that found by Sittenfield, Johnson, and Jobling (6) and Murphy, Helmer, Claude, and Sturm (7) in fowl tu-

mors. In the light of subsequent experiments in which the sera of wart-bearing rabbits were found to neutralize partially or completely the wart-producing agent, it seems possible that the inhibitory properties observed in non-infectious domestic rabbit wart suspensions might in reality have been due to contained humoral antibodies. A point of argument against this belief is that, while humoral antibodies were demonstrable in the sera from both infected wild and domestic rabbits, only the domestic rabbit warts possessed demonstrable inhibitory properties. We have as yet made no systematic attempt to render experimental domestic rabbit warts infectious by removal of a hypothetical inhibitory substance. We have tried, however, to infect rabbits with inactive experimental domestic rabbit wart suspensions that had been heated to 60°C. for 30 minutes in the hope that that temperature might inactivate the possible inhibitor without affecting the wart-producing agent, with suspensions prepared from domestic rabbit wart cells that had been washed repeatedly and sufficiently to remove all freely soluble humoral antibody, and with Berkefeld filtrates of inactive wart suspensions. All three of these procedures yielded completely negative results. Both the Iowa and the Kansas strain of the disease were used in these attempts to transmit warts in series through domestic rabbits.

DISCUSSION

The absence of significant visible bacterial forms in highly active wart-producing suspensions together with the ready filtrability of the etiological agent and the inability to cultivate, on lifeless media, any visible microbial form from demonstrably active filtrates; the agent's ability to transmit in series through wild rabbits; its glycerol resistance; its ability to induce in its hosts an immunity which is constant although of variable degree; and its apparent tropism for one type of tissue place this agent in the filtrable virus group.

The non-transmissibility of the agent in series through one of its demonstrably susceptible hosts, the domestic rabbit, is not a characteristic of most of the known virus diseases. An analogy, however, is to be found in the group of filtrable fowl tumors. Des Ligneris (8), working with Rous Sarcoma 1 of chickens, has found that while both turkeys and guinea fowls are susceptible, transmission through these two alien species is limited to two successive serial passages. Similarly,

Andrewes (9) has found that while Rous Sarcoma 1 will produce fatal metastasizing tumors in its first pheasant passage it cannot be transmitted in its characteristic form from pheasant to pheasant.[2] It seems probable that the domestic rabbit (genus *Oryctolagus*) is sufficiently distantly related to the wild cottontail rabbit (genus *Sylvilagus*) to behave towards infection with a filtrable new growth of wild rabbit origin in much the same manner as do turkeys, guinea fowls, and pheasants towards infection with a filtrable chicken tumor.

Another property of the wart-producing agent that is unusual among viruses causing diseases in animals is its resistance to heat. Suspended in 0.9 per cent NaCl solution it proved capable of withstanding a temperature of 65°C. for 30 minutes in sealed ampoules without apparent damage to its wart-producing properties. Virus heated to 67°C. for 30 minutes, while still active, produced, in our limited number of experiments, warts which either developed scantily or retrogressed after a few days' growth. We are aware of no other animal virus which will withstand so high a temperature in the moist state; most are completely inactivated at much lower temperatures. However, among the plant viruses, which are on the whole as susceptible as animal viruses to the effects of heat, there are several which withstand heating to 65°C. or more (10). The virus of tobacco mosaic is an example of a typical plant virus that is relatively heat resistant (11). For this reason it does not seem necessary to consider seriously the possibility that the unusual heat resistance of the wart-producing agent eliminates it from classification as a virus.

The not infrequent shortening of the incubation period in animals inoculated either with virus heated to from 45–65°C. or with virus that had been filtered through Berkefeld V or N candles cannot be explained. Removal of an inhibiting agent by these two procedures is suggested by the data.

In the gross and histologically, the warts of rabbits described in this paper are typical of virus-produced papillomata (12–15) as known in man, cattle, and dogs. It has not been previously observed in studies of mammalian warts of this kind that an epithelial neoplastic process of identical gross and histological appearance can be induced in two ani-

[2] Andrewes has recently reported the successful serial passage of Rous Sarcoma 1 through pheasants (Andrewes, C. H., *J. Path. and Bact.*, 1933, **37**, 17).

mal species, in one of which the condition is not only transmissible in series, but transmissible by cell-free filtrates, and in the other of which it is not transmissible at all. Here then in what is certainly a single clinical entity are examples of the two extremes of neoplastic processes considered from the standpoint of transmissibility. In the wild rabbit the papillomata can be initiated by inoculating the animal with a filtrable agent and they are transmissible in series by inoculation with filtered or unfiltered virus. From an etiological standpoint, then, the wild rabbit warts are analogous to the chicken tumors which by some are not considered as true representatives of neoplastic processes simply because they are transmissible by cell-free filtrates. Thus the wild rabbit papilloma represents the one extreme of a tumor induced by an infectious agent which can be separated from the cells and some of whose properties can be studied.

The other extreme is exemplified by the papillomata induced in domestic rabbits which, while initiated by the same virus, have so far resisted transmission either to domestic or wild rabbits. These are thus analogous to many of the tumors of mammals which cannot be transmitted in series by the usual methods of transplantation. No objection to the eligibility of the domestic rabbit warts for consideration as neoplastic processes could be raised on the grounds that a causative agent distinct from the proliferating cells can be discriminated. A study of this epithelial new growth in domestic rabbits without knowledge of its causation would probably lead an investigator to classify it as one of that large group of so called "spontaneous" mammalian tumors that are non-transmissible. It would not even be suspected that the papillomata had been caused by a filtrable virus of wild rabbit origin.

The question which is naturally brought to mind by the experiments of des Ligneris (8) and Andrewes (9) with fowl tumors and our own with rabbit warts is whether certain "spontaneous" non-transmissible or not readily transmissible tumors may not originally have been caused by viruses which produce transmissible tumors in some other species. A careful study, from this point of view, of the causes underlying the non-transmissibility of these various tumors may bring to light new knowledge of the etiology of neoplastic processes in general, especially in the group of mammalian tumors which are either entirely

non-transmissible or transmissible only by viable cell-containing grafts.

SUMMARY

A papilloma has been observed in wild cottontail rabbits and has been found to be transmissible to both wild and domestic rabbits. The clinical and pathological pictures of the condition have been described. It has been found that the causative agent is readily filtrable through Berkefeld but not regularly through Seitz filters, that it stores well in glycerol, that it is still active after heating to 67°C. for 30 minutes, but not after heating to 70°C., and that it exhibits a marked tropism for cutaneous epithelium. The activities and properties of the papilloma-producing agent warrant its classification as a filtrable virus.

Rabbits carrying experimentally produced papillomata are partially or completely immune to reinfection and, furthermore, their sera partially or completely neutralize the causative virus. The disease is transmissible in series through wild rabbits and virus of wild rabbit origin is readily transmissible to domestic rabbits, producing in this species papillomata identical in appearance with those found in wild rabbits. However, the condition is not transmissible in series through domestic rabbits. The possible significance of this observation has been discussed. The virus of infectious papillomatosis is not related immunologically to either the virus of infectious fibroma or to that of infectious myxoma of rabbits.

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EXPLANATION OF PLATES

PLATE 33

FIG. 1. Spontaneous wart on the thigh of a wild rabbit. The vertically striated appearance of the lateral surfaces can be seen. The upper portion of the growth is hard and well keratinized.

FIG. 2. Experimental warts on the abdomen of a domestic rabbit showing individual discrete wart formation in an animal infected following scarification by needle. These warts are 23 days old.

FIG. 3. Same animal as shown in Fig. 2. The warts are now 52 days old and, considered individually, are accurate reproductions of the spontaneous warts seen in wild rabbits.

FIG. 4. Experimental warts on the abdomen of a domestic rabbit showing massive confluent wart formation in an animal infected following scarification with sandpaper. These warts are 118 days old and are firmly keratinized.

PLATE 34

FIG. 5. Section of a spontaneous wart in a wild rabbit. The long, branching papillae are capped by an enormous amount of keratinized material. Iron alum hematoxylin and eosin. × 10.4.

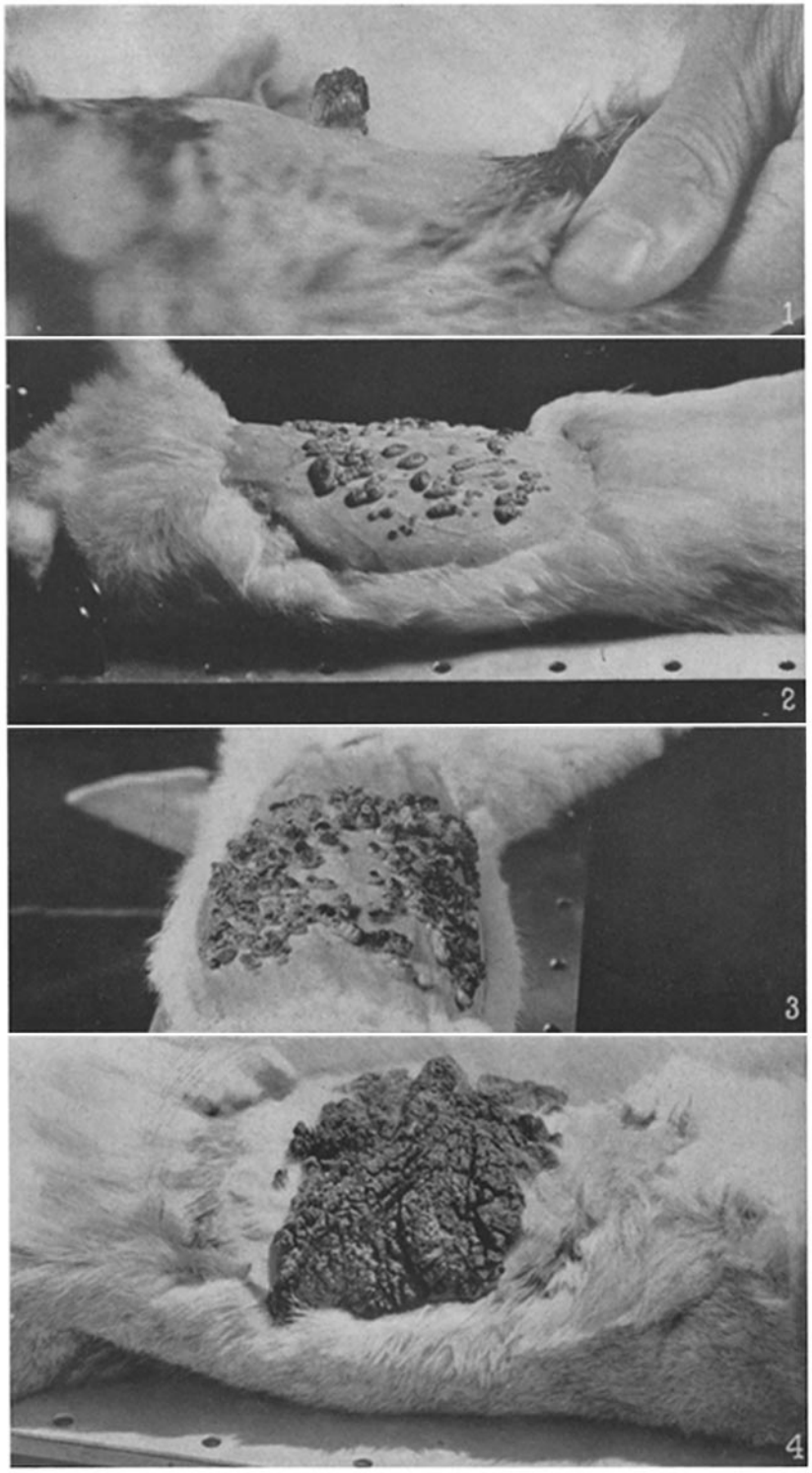
FIG. 6. Higher power of a spontaneous wart showing the tall and narrow germinal cells, the great thickness of the polygonal cell and granular layers, and the imperfectly keratinized surface layer. Iron alum hematoxylin and eosin. × 168.

PLATE 35

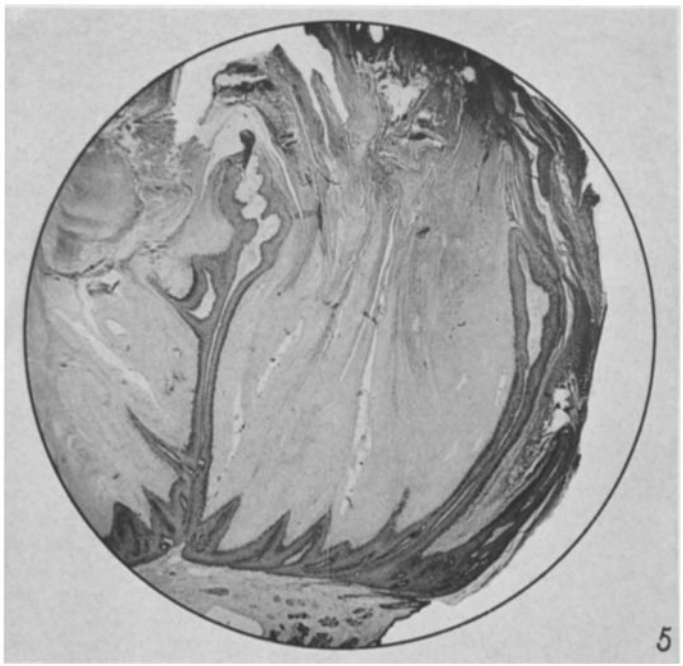
FIG. 7. Section of an experimental wart in a domestic rabbit 3 days after its appearance. The mass of proliferating epithelium lies partly below the level of the normal epithelium, which it underlies at the margins. As yet there is no excess keratinization. Iron alum hematoxylin and eosin. × 33.

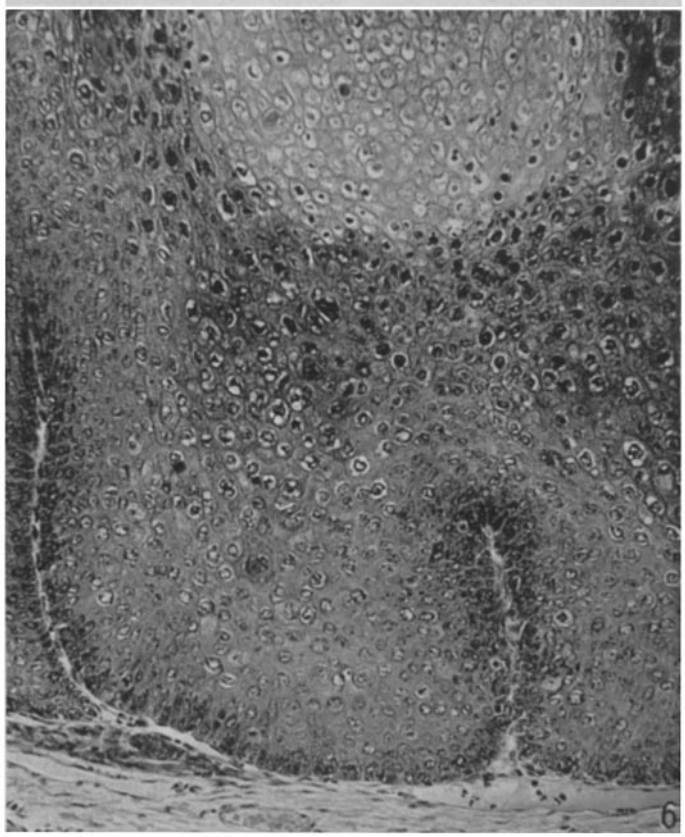
FIG. 8. Section of an experimental wart in a domestic rabbit 18 days after its appearance. The epithelium is greatly thickened and the new growth projects considerably from the surface. Iron alum hematoxylin and eosin. × 77.

FIG. 9. Still later stage of the experimental disease in a domestic rabbit (36 days). The features of the spontaneous growth are by now fairly faithfully reproduced. Iron alum hematoxylin and eosin. × 26.



(Shope: Infectious papillomatosis of rabbits)





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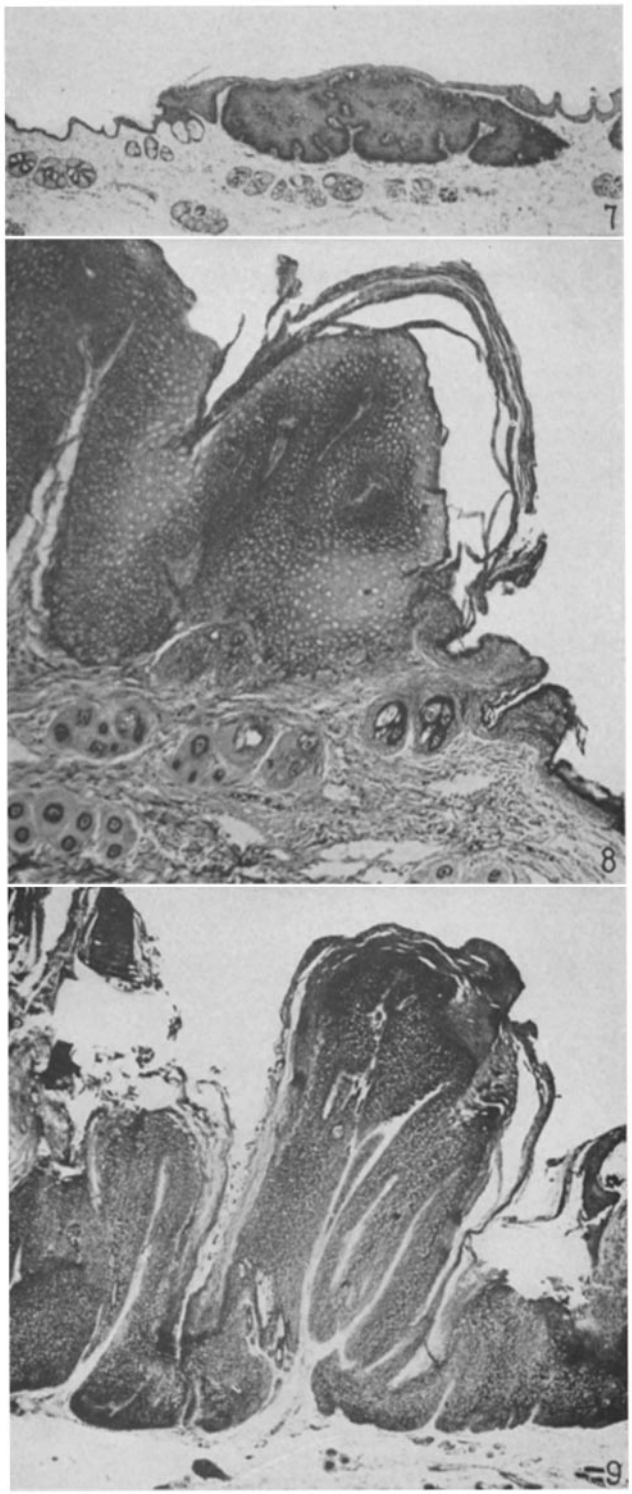

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