SEROLOGICAL REACTIONS WITH A VIRUS CAUSING RABBIT PAPILLOMAS WHICH BECOME CANCEROUS

II. Tests of the Blood of Animals Carrying Various Epithelial Tumors

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Plate 6

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In the preceding paper a method has been outlined for titration of the antiviral power acquired by the blood of rabbits carrying papillomas induced with the Shope virus. Tests of the blood of animals carrying other epithelial growths will be reported in the present communication.

The Sera of Rabbits with Cancers Originating in the Virus-Induced Papillomas

Carcinomas develop from many of the papillomas induced with the virus in domestic rabbits (1). It was manifestly useless to test the blood of the first hosts of the cancers since they had all carried the preliminary papillomas for many weeks, and any neutralizing power encountered could be referred to the influence of these growths. The primary cancers themselves yielded an undesirable transplantation material, since papilloma cells might have persisted amidst the carcinomatous elements, to be introduced with the latter into new hosts. For this reason metastases were employed as transplantation material whenever they were available, though in certain instances autoplastic growths in the leg muscles were utilized. Six attempts at transplantation have been made thus far, all with squamous cell carcinomas. The rabbits employed were of Dutch belted stock but not of wholly pure breed. Detailed histories of the cancers furnishing the materials have been given in a previous paper (1).

The general method was to hash the cancerous tissue, suspend it in a relatively large amount of Tyrode solution, and inject 1 cc. into the upper muscles of each leg. The injection needle was introduced through a slit in the skin, with its lips held wide to exclude all possibility that some epidermal cells might be pushed deep and infected incidentally with virus present in the tumor suspension. On withdrawing the needle some of the inoculum always came in contact with the slit skin, but papillomas never resulted.

1. A suspension of the tissue of a metastasis in an axillary gland from a cancer of rabbit 2-38 was implanted in 3 adult rabbits. None developed growths.

2. The tissue of a leg nodule of 2-39, consequent upon autoimplantation, was injected into the legs of 3 rabbits. None developed growths.

3. The basal portion of a cancer of 2-39 was mixed with material from several large nodules resulting from previous implantation of the same cancer into the leg muscles. The tumor suspension was injected into the legs of 9 rabbits and into the testicles of 3 of these. A nodule developed in a leg of one animal (4-70), and biopsy after 79 days disclosed there a squamous cell carcinoma like the original growth (1). Portions were implanted in the legs of 6 adult and 9 unweaned young rabbits, of which latter only 2 survived 3 months. At the end of this time none of the remaining animals had tumors and the growth in D. R. 4-70 had disappeared.

4. An axillary metastasis from a cancer of 2-53 was utilized, and implantation done into the fore legs of 6 unweaned rabbits, 5 recently weaned, and 6 adults. The 8 young that survived for 126 days were all negative then and were discarded. Of the 5 surviving adults one (5-79) had large growths in both fore legs and another (5-84) a nodule 1 cm. in diameter in one of them which disappeared later. Biopsy of the growths of 5-79 on the 160th day showed them both to be squamous cell carcinomas. Tests were made of the neutralizing power of the sera of these animals (*vide* Experiments 1 and 5). After 210 days, 5-79 was again operated upon and material procured for implantation in 15 half grown animals. To the present (3 months) no tumors have appeared.

5. 10 adult rabbits were implanted in the fore legs with a suspension of an axillary metastasis of a squamous cell cancer of 2-38, and in the hind legs with the tissue from an autoimplant of the same cancer in an upper leg. All were negative when discarded 3½ months later.

6. Tissue from another axillary metastasis of 2-53 was implanted in the legs of 15 rabbits 3 months old and 6 adults. One developed a small nodule, which was incised to hasten its growth, but it retrogressed instead. On incision it had appeared cancerous in the gross.

In only 4 of the 96 rabbits did nodules result from implantation, and in 3 of these retrogression soon ensued. The animals furnishing the cancer materials were not of the same genetic constitution as those implanted; while furthermore some of the tissues employed were

infected with pyogenic bacteria, abscesses developing where they were put.

Rabbit 5-79 developed discrete, smooth surfaced, football-shaped tumors at the sites of implantation (Fig. 1), which were nearly 5 cm. long when cut into after 160 days. Each contained a single large cyst filled with glairy, yellowish fluid, turbid with cell debris. The lining of the cysts was ragged and pink, and their walls nearly 2 cm. thick, tough and close textured, with occasional small yellow dots near the cyst cavity. Slices were taken and the gaps in the cysts' wall were not closed, though the skin was carefully sutured over them. Next day the animal was bled for serum test (Experiment 1). Sections showed the cysts to be lined with the tissue of a rapidly necrosing, anaplastic squamous cell carcinoma, wholly devoid of papillomatous features (Fig. 2), as was the material of original implantation (Fig. 3). The walls consisted in the main of reactive connective tissue into which the carcinoma had extended but a little way, and there it was keratinizing and dying or dead amidst a scattering of lymphocytes.

The gaps left in the cysts healed without extension of the cancers through them, and they enlarged further, with the animal in excellent condition (Fig. 1). After 50 days more (210th day) they measured 6 x 4 x 4 cm., and 5 x 3½ x 3½ cm. respectively. Again they were cut into and the same state of affairs was found as before, both in the gross and microscopically. The animal was bled for another serum test (Experiment 5). 3 days later it succumbed to bacterial infection. The carcinoma proved to be heavily encapsulated everywhere, and there were no metastases.

D. R. 5-84 developed an irregularly rounded nodule in the right fore leg, which reached a diameter of 1.5 cm. within a month after the implantation, remaining stationary for many later weeks. It was perhaps slightly smaller when the animal was bled for serum test on the 161st day, and thereafter it slowly disappeared.

The nature of the nodule present for a time in rabbit 5-84 was never ascertained. The progressively enlarging tumors of 5-79 were of singular character, each consisting of a thin layer of densely encapsulated, carcinomatous tissue, walling a cyst filled with glairy fluid. The original cancer had formed no such cysts and we have seldom encountered similar ones in association with the malignant growths deriving from the papillomas. The great resistance offered by the new host may have had something to do with their development. The living, cancerous lining of the cyst was only 1 to 2 mm. thick and such of the malignant cells as penetrated into the dense encapsulating connective tissue died after but slight proliferation.

Experiment 1 (Table I).—Neutralization tests were carried out in the usual way with serum specimens procured from 5-79 and 5-84, on the 160th day after im-

TABLE I

Neutralization Tests with the Serum of Rabbits Carrying Transplanted Cancers Derived from Virus-Induced Papillomas

Rabbits.	*5 per cent virus*, 0.5 cc. + 0.2 cc. serum + 0.3 cc. Tyrode						*1 per cent virus*, 0.5 cc. + 0.5 cc. serum							*0.1 per cent virus*, 0.5 cc. + 0.5 cc. serum						
	L	S	N 1	N 2	C 1	C 2	L	S	N 1	N 2	C 1	C 2	+ 0.5 cc. Tyrode	L	S	N 1	N 2	C 1	C 2	+ 0.5 cc. Tyrode
Test rabbit																				
16 days 1	0	+−	0	+++	+−	0														
2	0	0	+++	++	+++	+++−														
3	0	+	+++	++++	+++	+++														
4	0	++++	?	++	++++	++														
20 days 1							0	0	++	++	++	+++	+++							
2							0	0	++++	++	++++	+++	0							
3							0	0	+++	+++	++++	++++	++							
4							0	0	+++	+++	+++	++	++							
27 days 1														0	0	+	0	++−	0	0
2														0	0	+	+	++−	++−	+
3														0	0	+	+	++−	++−	+−
4														0	+−	++−	++−	++−	++−	+−
34 days 1	+−	+++	+++−	++++	++++−	+++−	0	+	+++	+++−	+++−	++	++	0	0	++−	+−	++−	+−	0
2	++	++++	++++	++++	++++	++++	+−	++−	++++	++++−	++++	++++	+++−	0	+−	++−	++	+++−	+−	++−
3	+++	++++	++++	++++	++++	++++	+−	++−	++++	++++	++++	++++	+++	0	0	++	++	++	+++−	+
4	++−	++++−	++++	++++	++++	++++−	+−	+−	+++	++++−	++++	++	++	0	+−	++−	++−	++−	++	+

L = with large cancers. S = with 1 small cancer presumably. N = implanted with negative outcome. C = normal control.

0 = negative. +− = 1 or 2 papillomas. + = a few papillomas. ++ = many discrete papillomas. +++ = semiconfluent, discrete papillomas. ++++ = confluent papillomas.

plantation. For comparison specimens were utilized from 2 other animals which had been unsuccessfully implanted with the same material, and from 2 normal rabbits of the same breed and approximate age. All had been kept under identical conditions during the period since transplantation. Mixtures were made of equal parts of the sera with 3 dilutions of a virus fluid prepared by passing a 10 per cent extract of virus material W. R. 1240 through a Berkefeld filter V, and then diluting it to 5, 1, and 0.1 per cent with Tyrode. After 2 hours incubation the materials were rubbed into squares on the skin of 4 normal rabbits. Table I records the consequences. As usual growths appeared first where the most concentrated virus fluid had been inoculated (2). The first notations made when the papillomas were barely or dubiously visible, have been omitted from the table. Dilution of the virus fluid to 0.1 per cent brought it close to the working limit of use, beyond which the influence of chance factors as *e.g.* local variations in skin character, in the depth of scarification, and in reactive inflammation, renders the outcome in terms of papillomatosis unreliable for comparative purposes.

It will be seen from Table I that the serum of the rabbit with large carcinomas had a pronounced neutralizing effect on the virus, while that of the animal with a single small nodule exhibited a slighter yet definite antiviral action. None of the control sera influenced the virus. The results of a second test with the serum of 5-79 procured 50 days later, are given in connection with Experiment 5, to be described further on.

The findings appeared to indicate that the Shope virus was present in the animals that developed growths as result of transplantation of the cancer. But there were at least two other possibilities, namely that the neutralizing power exhibited by the sera lay within the range of the normal, or that it was nonspecific, being called forth by the proliferating epithelium as such. Amongst more than 50 normal rabbits tested at various times only one has yielded a serum with any neutralizing power for the Shope virus; but the effectiveness of this serum (Table V, C 1) fell little if at all short of that from 5-84, the rabbit that had developed a small nodule of problematic character. No normal specimen with a neutralizing power approaching that of the rabbit with large transplantation cancers has been encountered; and as will be seen this power still existed at the time of second test (Table VI).

Tests with the Serum of Rabbits Carrying Tar Papillomas

The papillomas which develop on the tarred skin of rabbits are very like those produced by the Shope virus (3). Tests of the serum of

TABLE II

Neutralization Tests with the Serum of Rabbits Carrying Tar Papillomas

		1 per cent virus, 0.5 cc. + 0.5 cc. serum										*1/5 per cent virus*, 0.5 cc. + 0.5 cc. serum									
Rabbits..		4-58*	6-47	6-48	6-45	6-49	6-60	6-54	6-55	6-63	+ 0.5 cc. Tyrode	4-58*	6-47	6-48	6-45	6-49	6-60	6-54	6-55	6-63	+ 0.5 cc. Tyrode
Tar papillomas..		Large				Medium	None	Very small				Large				Medium	None	Very small			
Test rabbit																					
17	1	0	?	+−	+−	++−	+−	++−	?	+	+−										
days	2	0	++−	++−	+	++−	+	++−	+	++−	++−										
	3	+	++−	+++−	+	++	+	+−	++	+++−	+										
20	1	+	++−	++−	+	++	+	++	++	++−	++−	0	+	++−	0	++−	+	+−	0	0	0
days	2	+	++	++	++−	+++−	++−	+++−	++	++	+++−	0	++	+	+	0	0	+	0	+−	+
	3	++−	+++−	+++	++	++	++−	+	++	+++	++	0	++	++	+	++−	++−	0	0	0	++
27	1	+	++	++−	++−	++	+	+++−	+++−	++−	++−	+−	+	++−	+	+	+	+	+	+	+−
days	2	+	++	+++	++−	+++−	++	+++−	++	++−	+++−	+−	+++−	+	++−	+	+	+	+	+−	+
	3	++−	++++−	++++	++−	++	++−	++−	+++−	++++	++	0	++	++	+	+	++−	+−	+	+	++
40	1											+−	+++−	+++−	+	++	+++	++−	++	++	+
days	2											+−	++++−	+++−	+++−	++	++	+++−	++	+	+++−
	3											0	+++	++++−	++	++	+++−	+	++	+	++++−

* Carrying very large papillomas for more than 3 months.

animals with tar tumors seemed desirable, both as bearing on the general problem of tumor etiology and as covering the possibility that the neutralizing power of the serum of rabbit 5-79, with large transplantation cancers, represented a non-specific response to epithelial proliferation as such.

Experiment 2, Table II.—9 gray-brown domestic rabbits that had long been tarred[1] were selected from a large group as providing a pronounced contrast in its effects. All had been kept in a separate room, rigidly isolated from contact with the Shope virus. The ears of the rabbit with the largest papillomas (4-58) had been tarred 3 times a week for 12 months, and growths had been present on them for more than 3 months. The remaining 8 rabbits had been tarred thrice a week for 5½ months. 3 of these carried multiple large papillomas (maximum diameter about 2 cm.) of several weeks duration. A fourth had multiple, smaller papillomas up to 1 cm. across. The remaining 4 animals served as controls, no papillomas having appeared on their ears or only minute ones very recently. Serum specimens from all were mixed in equal part with Berkefeld-filtered 1 and 0.2 per cent extracts, respectively, of virus material W. R. 1240. After incubation for 2 hours the materials were inoculated into 3 brown-gray test rabbits. Table II records the results.

None of the sera from the tarred rabbits possessed any neutralizing power whatever save that from 4-58, which had been tarred for many more months than the others, and had larger papillomas of much longer duration. The effect of its serum was slightly more pronounced than that of the generality of specimens from normal animals.

The tarring was continued, the papillomas grew more numerous and larger, and other animals of the group developed them, some of which were included in a second test. In several of the instances with "large" papillomas the crowded, pedunculated growths filled the hollow of the ears, but in none had cancer manifested itself.

Experiment 3 (Tables III and IV).—The sera were procured 9 weeks after those of the first test. All of the animals had tar papillomas now, though in some they were few, minute, and of but a few weeks duration. These are termed "small" in the tables. Sera from 6 normal brown-gray rabbits were taken as controls. Two mixtures were made, with 1 per cent and 0.2 per cent filtered extracts of virus W. R. 1240 respectively. The number of the test mixtures was so great as to preclude the inoculation of all into the same rabbits and hence 3 groups of 3

[1] Horizontal retort tar of the Oster-Gasfabrik of Amsterdam was employed throughout. It was generously given us by Dr. Karl Landsteiner.

TABLE III

Neutralization Tests with the Serum of Rabbits Carrying Tar Papillomas

		1/5 per cent virus, 0.5 cc. + 0.5 cc. serum																			
Rabbits.....		A 4-58*	6-47	6-48	6-45	6-49	6-56	6-46	B 6-51	1	2	3	4	5	6	AB 6-60	A 6-54	6-63	6-58	B 6-50	+ 0.5 cc. Tyrode
Tar papillomas..		Large								Controls						Small					
Test rabbit																					
18 days	1	+	0	+	0	0	0	0	0	0	+−	0	+−	0	0	0	0	0	0	0	+−
	2	+	0	0	0	0	+	+	++	0	0	0	+−	++−	++−	+−	+	+	+−	0	0
	3	+	+	++	0	+−	+	0	0	0	++	0	+	0	0	0	0	+−	++	+−	++
21 days	1	+	+	++	++−	0	+	0	+−	0	++−	0	+	+−	+	+−	+−	+	++−	0	++
	2	++−	++−	++−	+	++	++−	++	++	0	0	0	++	++	++	++	++	++	+−	+−	0
	3	++−	+	++	++−	++	++	++−	+	++−	++	+	++	++−	++−	+	0	+−	++	+	+++−
25 days	1	++	++−	+++−	++	0	++	+−	++−	+	++	++−	++	+	++	++−	++	+	++	+−	++
	2	++	++	++	+	+++	++	+++−	+++−	++−	++	++	+++	+++	+++	++	++	+++	+−	+	+−
	3	++	+++−	+++	++	++	+++	++	++	++	+++	++	+++	++	++−	++	++	++−	+++	++−	+++

A = ascites. B = papillomas of short duration.

* Papillomas of over 5 months duration.

test animals each were employed, one receiving only mixtures with 0.2 per cent virus (Table III) while the other mixtures were apportioned as shown in Table IV. The group first mentioned were of Dutch belted stock, and papillomas appeared slightly later in them than in the others, which were of brown-gray breed.

Three of the tarred rabbits had pronounced ascites due to liver cirrhosis consequent on the tarring. With these exceptions they all appeared to be in good health.

The tests yielded no evidence of serum changes referable to the presence of the tar papillomas. Indeed the specimens from some of the animals carrying them appeared more favorable to the virus than those from some of the controls, though the differences were well within the normal range. Rabbit 4-58, with papillomas now filling both ears, yielded a serum that had none of the neutralizing power evident on previous test. The findings as a whole demonstrate that epidermal proliferation, as such, does not cause the serum to become neutralizing for the Shope virus.

Tests with the Sera of Rabbits Carrying the Brown-Pearce Tumor

In further elucidation of the findings serum specimens were studied from rabbits in which the Brown-Pearce tumor was growing or had retrogressed. This transplantable tumor is a swiftly growing carcinoma derived from an epidermal element, presumably of a hair follicle (4). In a previous paper the fact has been reported that animals in which large Brown-Pearce tumors had recently retrogressed developed papillomatosis on broadcast inoculation of the Shope virus at the same time and to the same extent as did normal controls (5). Nevertheless serum tests seem desirable since inoculation with the Shope virus is sometimes successful in rabbits carrying the papilloma, even when their blood possesses some antiviral power.

Experiment 4.—8 normal gray-brown domestic rabbits, from the same source and of approximately the same size, were inoculated with a heavy suspension of freshly prepared Brown-Pearce tumor in Tyrode. 4 others were kept as controls. The tumor was broken up with sand, pressed through a sterile wire mesh, and suspended in 0.9 per cent NaCl. 4 intradermal injections of 0.2 cc. each were made into the loose skin of the back of the neck of each rabbit, as well as intramuscular injections of 0.3 cc. each into the flexor and extensor muscles of both thighs. Both the inoculated animals and the normal ones were kept isolated and in individual cages. The size and character of the tumors were recorded twice weekly. It was

Neutralization Tests with

		1 per cent virus, 0.5 cc. + 0.5 cc. serum								
Rabbits.....		A 4-58*	6-49	6-46	B 6-51	1	2	3	AB 6-60	
Tar papillomas....		Large				Controls				
Test rabbit										
15 days	1	0	+	0	0	0	0	0	+++−	
	2	+−	+−	0	+++−	?	+	0	++	
	3	++	++	+++−	++−	++−	+−	+	+++−	
18 days	1	+	++	++−	+	++−	++	++−	+++−	
	2	++	++	++−	+++	++	++	0	++	
	3	+++	+++−	++++−	+++−	++	+	++	+++	+
21 days	1	++	+++−	++	++−	++	+++−	++	+++	−
	2	++	+++−	++	+++	++	++	++−	+++−	
	3	++++−	++++−	++++−	++++−	+++−	++	+++−	+++	−
Rabbits.....		6-47	6-48	6-45	6-56	4	5	6	6-60	
Tar papillomas....		Large				Controls				
Test rabbit										
15 days	1	0	?	0	0	0	0	0	0	
	2	0	+−	0	0	+	0	0	++	
	3	+	+	+−	++−	++−	+−	0	+	
18 days	1	++−	++	++−	++−	++−	++	+	+	
	2	++	+	+−	++	++	+−	++	++	
	3	++−	++−	++	++	++	++−	++−	++	+
21 days	1	++	++	++	++	++	++	++	++−	
	2	+++−	++−	++−	++	++	++−	+++−	+++	+
	3	++	++	++−	+++	++	++	++	+++−	

A = ascites. B = papillomas of short duration.

* Papillomas of over 5 months duration.

ıbbits Carrying Tar Papillomas

		1/5 per cent virus, 0.5 cc. + 0.5 cc. serum										
	+ 0.5 cc. Tyrode	A 4-58*	6-49	6-46	6-51	1	2	3	AB 6-60	6-58	B 6-50	+ 0.5 cc. Tyrode
		Large				Controls			Small			
	?											
	+											
	+++											
	++	0	+−	+−	++	++	++−	0	++−	++	+−	++−
	++	++	+	+−	++	++	++−	++	+++−	+−	0	++
	++++−	++	+++	+	+++−	+	++	++	++	++−	+−	++−
	++	+	++−	+	+++−	+++−	++	+	++	++	+	++
	++	+++−	+	+−	++	++	++	++	+++	++−	0	++
	++++	++	+++	++−	+++	++−	++	+++−	++	++	+	++
	Tyrode	6-47	6-48	6-45	6-56	4	5	6	6-60	A 6-54	6-63	Tyrode
		Large				Controls			Small			
	++−											
	++											
	++−											
	+++−	++−	++	++−	++	++−	0	+	+	++−	0	+−
	++	++	++−	++	++	+	++−	++−	++	++	+	++
	+++−	++−	+	++	++−	+	+−	++	++	++−	+	+
	+++	++−	++	++−	++	++	0	++−	++−	++−	+−	+−
	+++	++	++−	+++−	++	+	++	++	+++−	++	++−	++−
	+++	++	+	++	++	++−	+−	+++−	++	++−	++−	++−

thought that implantations in the unfavorable skin sites might be followed by early retrogression which in turn would lead to retrogression of the leg growths. Whether for this reason or another, that was the actual course of events in several cases.

Serum specimens were taken from 5 of the implanted rabbits after 50 days. One of these (N 15) had large tumors at all sites, which continued to grow, causing death on the 63rd day. 2 other animals (N 20 and 22) had tumors which had grown well for the first 4 to 5 weeks, attaining a diameter of 2 cm. to 5 cm. in the legs, but then had dwindled and were much smaller at the time of test. In 2 other individuals (N 16 and 18) the tumors reached a fair size in the first 3 to 4 weeks (2 cm. to 4 cm. in the legs) and then rapidly retrogressed, disappearing completely about a week before the test. This was carried out in the usual way. The serum specimens, together with those of the 4 control rabbits, were mixed in equal parts with 1 and 0.2 per cent filtered extracts of virus W. R. 1240. After incubation for 2 hours, inoculation was done into 3 rabbits in the usual way. The findings are summarized in Table V.

The course taken by the Brown-Pearce tumors is expressed symbolically: $\hat{P}$ = growing tumors, death later; $\overset{\vee}{R}$ = good sized, retrogressing tumors; $\overset{\vee}{R}$ = fair sized retrogressing tumors; $\overset{\vee}{N}$ = negative now, after the retrogression of fair sized tumors.

From Table V it will be seen that of the 9 sera tested, namely those of 5 hosts of the Brown-Pearce tumor and 4 normal controls, only one had any power to neutralize the virus and this derived from a control. The power was but slight, only transiently evident where the mixtures with 1 per cent virus had been inoculated, but it sufficed for the complete neutralization of 1/5 per cent virus.

In a further experiment (Table VI) the serum of other animals carrying the Brown-Pearce tumor proved devoid of antiviral power.

Tests with the Serum of Rabbits Carrying Brown-Pearce Tumors Deriving from Cells Mixed with Shope Virus

It will be recalled that the serum of the rabbit with large carcinomas, consequent on transplantation of a cancer arising from a virus-induced papilloma, had a distinct neutralizing effect upon the virus. The serum of another rabbit of the same transplantation series with but a single small nodule had a slight antiviral influence. These findings appeared to indicate the presence of the Shope virus in the cancers.

Levaditi (6) and Rivers and Pearce (7) have demonstrated that

TABLE V

Neutralization Tests with the Serum of Rabbits Carrying or Recently Recovered from the Brown-Pearce Tumor

	1 per cent virus, 0.5 cc. + 0.5 cc. serum										*1/5 per cent virus*, 0.5 cc. + 0.5 cc. serum									
Rabbits.	15	22	20	18	16	C 1	C 2	C 3	C 4	+ 0.5 cc. Tyrode	15	22	20	18	16	C 1	C 2	C 3	C 4	+ 0.5 cc. Tyrode
Tumor course.	P	R	R	N	N	Control rabbits					P	R	R	N	N	Control rabbits				
Test rabbit																				
16 1	+−	++−	?	+	?	?	0	+−	+−	+−										
days 2	+	?	++−	?	++	?	+	++−	++	++−										
3	++−	++−	++	++	++	?	++−	++	++−	++										
19 1											0	0	0	0	0	0	+−	+−	+−	?
days 2											+−	+−	+−	0	0	0	0	+	+	+
3											0	+−	+−	+−	+	0	+−	+	+−	+−
21 1	+	++−	+	++−	++−	++−	+	+	++−	+										
days 2	+++−	++	++−	++	+++	++	++	+++−	+++	+++−										
3	+++	++	+++	+++−	+++	++	++	+++−	+++	+++										
23 1											0	0	0	0	+	0	+−	+−	+	+
days 2											+−	+−	+−	+	0	0	0	++	++−	++
3											0	+	+	+	++	0	+−	+	++	++−
26 1	++	+++−	+++−	+++−	++	++	++	++	++	++	0	0	+−	+−	+	0	+−	+−	+	+
days 2	++++−	+++	+++−	++	++++−	++	+++	+++	++++−	++++−	+	+	+	++−	0	0	0	++	++	++
3	++++−	+++	++++−	+++	++++−	+++−	+++	++++−	++++−	++++	+	+	+	+	++	0	+−	+	++	++−

0 = negative. +− = 1 or 2 small papillomas. + = a few small papillomas. ++ = many small papillomas. +++ = semiconfluent and discrete papillomas. ++++ = confluent papillomas.

wholly extraneous, necrotizing viruses, vaccinia (chicken pox), and Virus III, will flourish after experimental introduction into rat, mouse, chicken, and rabbit tumors, endure long after the host has become immune to reinfection with these viruses, and undergo transfer when the growths are transplanted. Such observations suggest that the Shope virus might ride into the cancers deriving from the papillomas it induces, and persist in them, though no longer in a causative relation. As bearing upon this possibility an experiment was carried out to see whether the virus would persist in association with Brown-Pearce tumor cells exposed to infection with it. Incidentally a second test was made of the neutralizing power of serum specimens procured from one of the 2 rabbits above mentioned which carried transplanted cancers deriving from virus-induced papillomas.

Experiment 5.—A suspension of Brown-Pearce tumor cells prepared as in Experiment 4 was mixed in equal volume with 5 per cent filtered Shope virus fluid (W. R. 1240), and with Tyrode solution respectively. The two mixtures were allowed to stand at room temperature for 1 hour, and then 0.5 cc. of each was inoculated into the fore and hind legs of 3 normal, brown-gray rabbits. The injections were made into the upper leg muscles through skin slits held wide, those of the same inoculum on the same side of the animal. On withdrawing the needle the inoculation fluids were purposely allowed to come in contact with the skin wounds; and wherever they had contained virus, a skin papilloma developed later. None appeared where the mixture with Tyrode had been introduced.

One animal was killed on the 18th day. Brown-Pearce tumors had developed at all of the implantation sites, those derived from the cells exposed to virus being much the smaller. The second rabbit was killed on the 31st day. It too had tumors at all of the inoculation sites. The combined weight of those from the cells exposed to virus was 4.8 gm., whereas the tumors from the Tyrode-soaked cells weighed 18.0 gm. The last animal was killed on the 35th day. Again the tumors engendered by the material containing virus were much the smaller, weighing 17 gm. as compared with 54 gm. for the controls. Metastases were present in the axillary and groin lymph nodes on both sides. All of the growths had the same histological character.

The tumors from the first animal were utilized for further transplantation. Those from the two sides were suspended separately in Tyrode and inoculated with the same technique as before into the legs of 4 normal brown-gray rabbits. In addition, 0.2 cc. of the injection material presumably containing virus was inoculated intradermally at 4 sites in the loose skin over the nape of the neck.

Tumors appeared in all of the new hosts. By the 46th day, when serum tests were made, they had reached a diameter of 2 to 4 cm. in one animal (N 57). In another (N 59) they had grown as large but were now retrogressing, while in the

TABLE VI

Neutralization Tests with the Serum of (a) a Rabbit with Transplanted Cancers Derived from Papillomas, (b) Rabbits with Brown-Pearce Tumors Deriving from Cell Material Exposed to the Papilloma Virus

	5 per cent virus 0.5 cc. +			*1 per cent virus* 0.5 cc. +										*1/5 per cent virus* 0.5 cc. +									
Serum.. Tyrode.	0.5 cc. −	0.2 cc. 0.3 cc.	− 0.5 cc.	0.5 cc. −	0.5 cc. −	− 0.5 cc.	0.5 cc. serum							0.5 cc. −	0.5 cc. −	− 0.5 cc.	0.5 cc. serum						
	(a)			(a)			(b)							(a)			(b)						
Rabbit..	L	L	−	L	N 1	−	5-7	5-9	5-8	5-6	C 5	C 6		L	N1	−	5-7	5-9	5-8	5-6	C 5	C 6	
Tumor course.							P	R	N	N	Control rabbits						P	R	N	N	Control rabbits		
Test rabbit																							
13 1	0	0	++																				
days 2	0	0	+++																				
17 1	?	0	+++−	?	+++−	+−	+−	0	+	+	+	0		0	+−	+−	0	0	+	+−	+−	+−	
days 2	+−	++−	++++	+−	+++−	++	++	+	++	+++−	++−	+		+−	++−	+	+	+−	+−	+	+−	+	
20 1	+−	+	+++	+−	+++−	++−	+++−	+−	++	++−	+	+−		0	+	+	+−	+	+	+	+	+−	
days 2	+−	+++−	++++	+−	+++	+++−	+++−	+	+++−	+++	+++−	+		+−	++	+	+	+	+	+	+	+−	
32 1	++−	+++−	++++	+−	++++	+++	++++	+−	++++	+++	++	++−		0	++	++	+	+	++	++	++−	+−	
days 2	+−	++++−	++++	+	++++	++++−	++++−	+++−	++++−	++++	++++−	+++		+−	+++	++	++	+	++	++	++	++−	

(*a*) L = with large cancers derived from a virus-induced papilloma. N 1 = implanted with negative outcome.

(*b*) 5-6 to 5-9 = with growing, retrogressing, or retrogressed Brown-Pearce tumors, as indicated. C 5 and 6 = normal controls.

remaining 2 hosts (N 56 and 58) they had attained a diameter of 2 to 4 cm. at an early period but then had rapidly dwindled, disappearing a few days before the sera were procured. The size of the tumors produced by the 2 inocula did not differ noticeably, and no papillomas appeared where the skin had been slit for inoculation.

Rabbits 5-79 and 5-83, to which a cancer deriving from a papilloma had been transplanted, were also bled for serum. The growths of 5-79 had enlarged since the serum test 50 days before (Table I, L), but no tumors had ever appeared in 5-83. It had been amongst the controls of the previous test.

Mixtures were made of each of the sera, and of those of 2 normal, brown-gray controls, with an equal part of 1 per cent and 1/5 per cent filtered virus fluid respectively, and with a 1/5 per cent fluid made up from the 1 per cent material (strain 1240). After 2 hours incubation all were inoculated into the skin of 3 normal brown-gray rabbits, one of which died of intercurrent causes a few days later. The growths appearing in the others were recorded as usual (Table VI).

In this experiment the tumors resulting from the implantation of Brown-Pearce cell material mixed with Tyrode and with virus respectively were transplanted to a second set of hosts, and the sera of the latter were tested for antiviral power in due course. They proved wholly devoid of it.

The initial activity of the virus was sufficiently shown by the development of papillomas where the skin was injured during implantation of the mixture containing it together with Brown-Pearce tumor cells; and the growths deriving from this mixture were smaller than those produced by the implantation of cells unexposed to virus, although no histological differences were discernible. On further transplantation no papillomas arose at the sites of skin injury,[2] and the tumors did not differ from the controls in size or aspect. The findings corroborated those of Experiment 4, in which the serum of animals carrying the Brown-Pearce tumor, or in which it had recently retrogressed, was found to have no neutralizing effect for the papilloma virus, and they go further, proving that the virus does not persist sufficiently long in Brown-Pearce tumors to be carried over into new hosts together with the cells of these growths. The findings differed essentially from those in the case of the rabbit with large transplanted carcinomas derived

[2] This failure of papillomas to develop cannot be considered significant, since virus of the strain employed has never been recovered in active form from the papillomas engendered therewith in domestic rabbits.

from a virus-induced papilloma. The serum of this animal once again gave evidence that the Shope virus had been transferred together with the tumor material.

DISCUSSION

The cancers deriving from the papillomas have proved difficult to transplant to other animals of impure breed,—in which respect they resemble the tar cancers,—and in only one case has the implanted material given rise to large, indubitable carcinomas. In this instance the possibility that papilloma cells had been carried over into the new host was excluded by the utilization of a metastasis for the transfer. The serum of this host proved neutralizing for the papilloma virus on two occasions separated by an interval of more than 7 weeks. Its titer was low, however, as measured by the standard described in Paper I; it neutralized not more than 20 virus units when mixed with virus suspension in equal amount, whereas the blood of some rabbits carrying papillomas rendered inactive 3000 units or more. But it may be recalled that the serum of D. R. 3-19 (Chart 1, Paper I) had no neutralizing power whatever, at a time when it supported much more papillomatous tissue than the cancer animal did of malignant tissue in the curious cystic carcinomas. The blood of a second rabbit of the same group as the latter, with a small nodule of problematic character, resulting from transplantation, exhibited some neutralizing power.

Even in the case of the rabbit with large cancers the findings mean no more than that the Shope virus underwent transfer with the cancer, an expected finding, since wholly alien, necrotizing viruses often thrive in tumors into which they are experimentally introduced, and they can be transferred therewith. In view of this fact it is remarkable that the papilloma virus did not persist in Brown-Pearce tumors exposed to infection with it, for these growths are certainly of epithelial, and presumably of epidermal, nature. The virus, if active, could scarcely have been outgrown and left behind, for it attends the growth of the papilloma which proliferates with great rapidity. The remarkable specificity of the virus, which will not "take" on the gums and tongue of rabbits, though highly effective on the neighboring epidermis, may explain its disappearance.

The findings with serum specimens procured from animals carrying tar papillomas or the Brown-Pearce tumor speak decisively against the possibility that these growths are caused by viruses antigenically related to the one causing papillomas. Yet this does not exclude a virus causation for them, since the sera of fowls with Chicken Tumor I and Fujinami sarcoma respectively, though possessed of neutralizing power for the virus causing the growth carried by the host, have no cross-neutralizing effect whatever (8).

SUMMARY

The serum of a rabbit with large cancers resulting from the transplantation of a squamous cell carcinoma that had arisen from a virus-induced papilloma, possessed the power to neutralize the virus, and so too in less degree did that of an animal of the same transplantation series in which a small nodule had developed. The sera of rabbits carrying tar papillomas or the Brown-Pearce carcinoma proved wholly devoid of effect on the virus. The implantation of Brown-Pearce tumor material mixed with virus did not lead to an enduring establishment of the latter in the resulting growths, nor to any immediate changes in their morphological character.

The significance of the facts is discussed.

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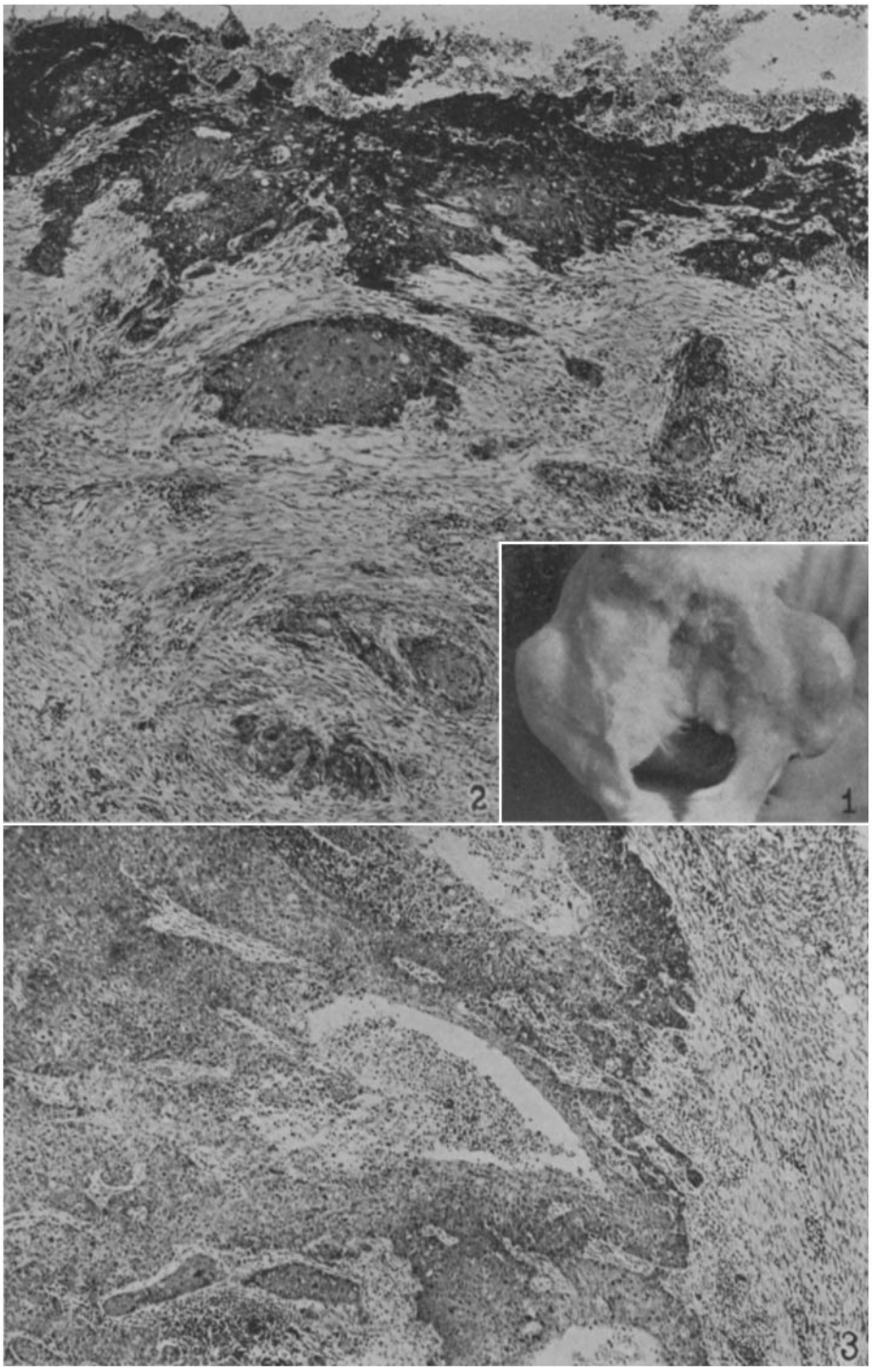
EXPLANATION OF PLATE 6

FIG. 1. Cancers consequent on transplantation to the fore legs of D. R. 5-79: 190th day. ×⅓.

FIG. 2. Inner portion of the wall of one of the cystic growths shown in Fig. 1. Methylene blue and eosin. ×30.

FIG. 3. Growing edge of the axillary metastasis utilized for transplantation to D. R. 5-79. Methylene blue and eosin. ×30.



Photographed by Louis Schmidt and Joseph B. Haulenbeek

(Kidd *et al.*: Virus causing rabbit papillomas. II)