A DISTINCTIVE SUBSTANCE ASSOCIATED WITH THE BROWN-PEARCE RABBIT CARCINOMA*

I. Presence and Specificity of the Substance as Determined by Serum Reactions

By JOHN G. KIDD, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

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An immunologically active substance with highly distinctive properties is present in extracts of the virus-induced papillomas of rabbits, which is closely associated or identical with the virus responsible for the growths, as studies already reported have shown (1). A substance with remarkably similar general traits but having specific characteristics of its own has now been found in a transplanted tumor of unknown cause (the Brown-Pearce carcinoma of rabbits). The present paper describes the results of complement fixation reactions which demonstrate the presence of the substance and its specificity. The properties of the substance which render it distinctive in type will be taken up in the succeeding paper along with the general significance of the findings.

Materials and Methods

The procedures employed in the work were identical for the most part with those used in the immunological study of the virus-induced rabbit papilloma. They have been fully described (1), but will be recapitulated briefly.

Transplantation of the Brown-Pearce Tumor.—The tumor, provided by Dr. J. B. Murphy and Mr. Ernest Sturm, has been transplanted in series in normal, adult rabbits of various breeds, mostly blue-cross or agouti hybrids. It has the features described originally by Brown and Pearce (2).

Vigorously growing tumors in testicles or muscles, or metastases in omentum, mesentery, retroperitoneal lymph nodes or diaphragm, provided the material for routine transplantation. The growths were procured with aseptic technique, and the healthiest portions were selected, hashed with knives, then pressed through a 40 mesh monel metal sieve and suspended in Tyrode's or Locke's solution. 1 cc. of the dense suspension was injected with syringe and needle into both testicles and 6 leg muscles (posterior muscles of all four legs and anterior muscles of the thighs) of the new hosts.

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Five to 12 rabbits were used in each of the routine transplantations. The course of the resulting tumors varied greatly from animal to animal, even when these were blood relatives, a finding already emphasized by others (3). One or two rabbits of each experiment generally developed large tumors which grew progressively, often metastasizing, and causing death after 4 or 5 weeks. Others developed large or medium sized or small tumors that retrogressed after variable periods. Some failed to develop palpable nodules. The rabbits with large, vigorously growing tumors were often killed after 10 to 40 days, to obtain material for further transplantation or as a source of antigen. Most of the rabbits that were kept 3 weeks or longer after the implantations were bled from the heart or ear vein at one time or another to procure serum for use in the complement fixation tests.

Representative blocks were taken of every tumor used for transplantation or as a source of antigen. The sections revealed no significant deviations from the histological findings of Brown and Pearce (2), the growths in every animal consisting of compact, healthy tumor tissue at the periphery of the grafts with larger or smaller necrotic or seminecrotic areas toward the center. None showed inflammation, or purulence, or other evidence of bacterial infection. Dr. P. K. Olitsky searched many sections for characteristic inclusion bodies in the cells of the tumor. He found none.

Many of the growths were cultured on agar, blood agar, glucose-serum-broth, and a variety of other media, incubated under aerobic and anaerobic conditions, but no organisms were recovered from them.

Materials and Procedure of the Complement Fixation Test.—The antigen extracts were prepared from tumor tissue procured with aseptic precautions, freed as far as possible from necrotic material, and stored for various lengths of time in Petri dishes at −20° to −25°C., or in 50 per cent glycerol-Locke's solution at about 4°C. The preserved tissue was ground in a mortar, suspended in isotonic saline or in dilute phosphate buffer (about M/200—pH 7.2), let stand overnight in the refrigerator, spun at 3500 to 4400 R.P.M. in the 51° angle head centrifuge for 5 minutes and the supernatant liquid spun a second time at one or the other speed for 10 or 20 minutes. The final supernatant liquids, slightly opalescent or clear, were heated at 56°C. for 30 minutes immediately prior to use in the complement fixation tests, to reduce anticomplementary effect and to inactivate any complement that might be present. The suspensions which had been made up in dilute buffer were made isotonic with concentrated saline.

The preserved tumor tissue was found to retain its complement-fixing potency over long periods. Glycerolated material was still potent in this respect after 13 months in the refrigerator, and so too was frozen tissue that had been kept 21 months, the longest periods tested. In general, extracts of tumor tissue that had been kept frozen had considerably greater potency in the tests than those of tissue that had stood long in glycerol.

Two hemolytic units of guinea pig complement, titrated immediately beforehand with four hemolytic doses of amboceptor, were used in almost all of the tests, 3 units in a few.

The sera investigated were stored without preservatives in the refrigerator. Many of the specimens were used repeatedly; and all were found to retain their full effectiveness for periods up to 16 months. They were used in the tests after dilution with isotonic saline to 1:4 or more. Like the antigens they were heated at 56°C. for 30 minutes immediately before use.

In setting up the tests, the antigen or serum was put in first; the complement was

always added second. 0.2 cc. of each reagent was used. Control tubes were set up in every experiment to test the hemolytic system, which consisted of 4 units of amboceptor (anti-sheep rabbit serum) and 5 per cent washed sheep cells. Tubes were also set up to test for non-specific anticomplementary effect with each antigen and serum used in an experiment; these contained twice the amount of the material that was used in the test. 1:10 or 1:20 saline extracts of the tumors were only rarely found to be anticomplementary, except those made from tumors situated in the testes, which were often anticomplementary in dilutions up to 1:20, as were also 1:20 extracts of normal rabbit testes. Sera in dilutions of 1:4 or more as a rule showed no anticomplementary action. 2 hours at room temperature was allowed for fixation of the complement, after which time the sensitized cells were added. Readings were made after 30 minutes at 37°C. and again after the tubes had stood overnight in the refrigerator, according to the following scale: ++++ = complete fixation (no hemolysis), +++ = about 75 per cent fixation (about 25 per cent hemolysis), ++ = about 50 per cent fixation (about 50 per cent hemolysis), + = about 25 per cent fixation (about 75 per cent hemolysis), ± = about 10 per cent fixation (about 90 per cent hemolysis), 0 = no fixation (complete hemolysis). No postzone or prozone reactions were noted in the work with the Brown-Pearce antigen and its specific antiserum, nor has visible flocculation or agglutination occurred upon admixture of the antigen and antiserum under a variety of conditions.

Demonstration of a Complement-Fixing Antigen in Extracts of the Brown-Pearce Tumor

It was known that complement fixation would take place if saline extracts of virus papillomas were mixed with sera of rabbits immune to the papilloma virus (1), and it seemed possible that fixation might also occur if extracts of the Brown-Pearce carcinoma were mixed with sera procured from rabbits bearing that tumor. In a first experiment to test the possibility, antigens consisting of saline extracts of Brown-Pearce tumors and of virus papillomas were mixed with sera from rabbits bearing growths of each sort.

Experiment 1.—The glycerolated peritoneal metastases of the Brown-Pearce tumors of one domestic rabbit (D.R. 69-S) and the diaphragmatic metastases of another (D.R. 38-S) were extracted 1:20 in saline, centrifugalized as usual, and filtered through Berkefeld V candles. In the same way extracts of the glycerolated papillomas of 2 cottontails (W.R. 8-N and W.R. 1240), which had yielded much virus on many previous occasions, were made and filtered. The extracts were tested as already described for capacity to fix complement upon admixture with specimens of serum obtained from 4 domestic rabbits bearing the Brown-Pearce tumor or in which it had recently retrogressed, and also with serum obtained from four domestic rabbits that had borne large virus-induced papillomas for many weeks.

The experiment yielded striking results, as Table I shows. The sera from the rabbits with Brown-Pearce tumors fixed complement in mixture with the antigens derived from those growths, but not when mixed with extracts of the virus papillomas, while the sera of the rabbits with virus

papillomas reacted with extracts of the growths of this sort but not with extracts of the Brown-Pearce tumors.

Manifestly, complement fixation may occur when saline extracts of the Brown-Pearce tumor are mixed with sera from rabbits bearing the growth, or in which it has recently retrogressed. Is the reaction specific, as the results of Experiment 1 appear to indicate? A second experiment was done to broaden the findings, and especially to learn whether saline

TABLE I

Complement Fixation Tests with Extracts of Brown-Pearce Tumors and Virus Papillomas in Mixture with Sera from Rabbits Bearing the Growths

Source of sera		Source of extracts				
Rabbits	Rabbit No.	Brown-Pearce tumors		Virus papillomas		Controls (no antigen)
		D.R. 69-S	D.R. 38-S	W.R. 8-N	W.R. 1240	
With Brown-Pearce carcinomas	71-S	++++	++	0	0	0
	73-S	++++	++	0	0	0
	4-69	+++±	+	0	0	0
	33-S	+++±	±	0	0	0
With virus papillomas	2-54	0	0	++++	++++	0
	2-55	0	0	++++	++++	0
	5-19	0	0	+++±	++±	0
	2-10	0	0	+++	0	0
Controls (no serum)		0	0	0	0	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 Berkefeld V filtrates of tumor tissue as indicated.

++++ = complete fixation (no hemolysis).
0 = no fixation (complete hemolysis)—see scale in text.

W.R. = wild rabbit; D.R. = domestic rabbit.

extracts of the Brown-Pearce tumor are regularly effective as complement-fixing antigens, whether made from implanted tumors in muscle or testicle or from distant metastases.

Experiment 2.—Brown-Pearce tumors from 4 animals were used. Two were implantation growths in muscle and testicle, the others metastases; some of the tumor specimens had been preserved frozen, the remainder in glycerol, for periods from 28 to 70 weeks. 1:20 saline extracts of the growths were made as usual and tested with sera obtained from 2 rabbits in which Brown-Pearce tumors had recently retrogressed, from 2 others carrying large virus-induced papillomas, and from 2 normal rabbits.

The findings (Table II) confirm and extend those of Experiment 1. Saline extracts of the Brown-Pearce tumor, procured from various situations in several rabbits and preserved frozen as well as in glycerol, fixed complement completely when mixed with sera from rabbits in which the Brown-Pearce tumor had recently retrogressed, but gave no reaction with the sera of normal rabbits or of those bearing virus-induced papillomas.

The results of the two experiments suggest that saline extracts of the Brown-Pearce tumor may be regularly effective as complement-fixing antigens when mixed with sera obtained from certain rabbits carrying the

TABLE II

Complement Fixation Tests with Extracts of Brown-Pearce Tumors and Sera from Rabbits (a) with Brown-Pearce Tumors and (b) with Virus-Induced Papillomas

Sera		Extracts						
Rabbits	Rabbit No.	Frozen peritoneal metastasis D.R. 35	Frozen peritoneal metastasis D.R. 69	Glyc. peritoneal metastasis D.R. 38	Glyc. testicle D.R. 15	Glyc. muscle D.R. 35	Glyc. peritoneal metastasis D.R. 35	Controls (no antigen)
With Brown-Pearce tumors	71	++++±	++++	++++	++++	++++	++++	0
	73	++++	++++	++++	++++	++++	++++	0
With virus-induced papillomas	2-10	0	0	0	0	0	0	0
	2-54	0	0	0	0	0	0	0
Normal	3-04	0	0	0	0	0	0	0
	3-06	0	0	0	0	0	0	0
Controls (no serum).		0	0	0	±	±	0	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 saline extracts of Brown-Pearce tumors from sources designated.

Brown-Pearce tumor, or in which this has retrogressed. And this is the case, as many subsequent tests have shown. More than 85 extracts of Brown-Pearce tumors,—implantation growths in muscle or testicle or metastases in lymph nodes, omentum, mesentery, diaphragm, or lungs,—from 28 rabbits in all have been tested. Every extract fixed complement specifically in mixture with the sera of certain rabbits bearing the Brown-Pearce tumor or in which this had retrogressed, with a single exception.[1]

[1] The one extract that failed to fix complement when tested had been made from a tumor which proved to be almost wholly necrotic on microscopic section. It was tested as a Berkefeld V filtrate.

In a more recent test a saline extract of the necrotic material from the center of a

Incidence of Complement-Fixing Antibody in the Serum of Rabbits Implanted with the Brown-Pearce Tumor

An experiment was next done to learn how frequently the complement-fixing capacity develops in the serum of rabbits implanted with the Brown-Pearce tumor, and under what conditions. Twelve related domestic rabbits were bled for serum and then implanted with the tumor. Samples of the sera were again procured 31 days after the implantations, and the serum specimens of both bleedings were tested for capacity to fix complement. The results were analyzed in relation to the size of the tumor in the different animals.

Experiment 3.—The rabbits employed were young adult males of hybrid English variety (blue-cross), taken from an inbred colony at The Rockefeller Institute. They were bled 10 cc. from an ear vein for serum, then implanted with the Brown-Pearce tumor, which had been procured from the vigorously growing testicle and muscle grafts of a single animal. The tumor was hashed and pressed through the 40 mesh monel metal sieve and suspended in Tyrode as usual. 1 cc. of the dense suspension was injected into both testicles and 6 leg muscles of all of the rabbits.

Ten of the dozen rabbits developed palpable tumors, 2 (D.R. 5-08 and 5-00) having none when examined on the 10th day and developing none later. In 4 individuals (D.R. 5-06, 5-09, 4-99, and 5-03) tumors appeared at every implanted situation and enlarged progressively, metastasizing and killing the hosts on the 45th, 34th, 54th, and 32nd days, respectively. Another individual (D.R. 5-04) developed 8 large growths which retrogressed very rapidly, dwindling from an average size of 3.0 cm. on the 27th day to nothing on the 36th; while still another (D.R. 5-01) had 2 smaller tumors that also retrogressed abruptly, vanishing between the 27th and 36th days. The remaining 4 rabbits of the experiment (D.R. 5-02, 5-10, 5-07, and 5-05) developed 2 to 8 tumors each, which had dimensions of from 2.0 to 4.0 cm. by the 21st day, retrogressing slowly thereafter, some nodules being still palpable as late as the 109th day after implantation.

Table III shows the results of tests with the serum specimens procured prior to the tumor implantations and 31 days thereafter, along with the number and average size of the tumors present in the various animals on the 31st day. Three antigens were used in the tests, these consisting of extracts of the Brown-Pearce tumors (implantation. growths in muscles) of 3 animals. The tumors of D.R. 5-03 had been procured and extracted the day prior to the test, those of D.R. 472-S had been kept frozen at −25°C.

Brown-Pearce tumor with dimensions of 4.0 by 3.0 by 2.5 cm. was tested in various dilutions for capacity to fix complement in mixture with a Brown-Pearce antiserum of known potency. It proved notably effective, quite as much so, in fact, as a similar extract prepared from the living tissue at the periphery of the same tumor. The finding raises the question whether the active substance in the exceptional instance noted above had been retained by the Berkefeld candle used. Results to be described in Paper II, which show that the substance may be retained by some Berkefeld filters, make this seem a likely possibility.

for 26 days, while the muscle growths of D.R. 35 had been kept 394 days in glycerol. 1:20 saline extracts of the growths of all 3 animals were made and centrifuged as usual the day prior to the serum tests. The results with the 3 antigens were consistent, as Table III makes clear, though there were considerable quantitative differences.

None of the sera procured prior to the implantations had any ability to react with the antigens in the test (Table III), whereas the sera of 10 of

TABLE III

Complement Fixation Tests with the Serum of Rabbits Bled before and after Implantation with the Brown-Pearce Tumor

Rabbit No.	Serum procured before implantations				Outcome of implantations (8 situations)	Serum procured 31 days after implantations			
	Antigens*			Controls (no antigen)	Number and approximate average size of tumors	Antigens*			Controls (no antigen)
	5-03	4-72	35			5-03	4-72	35	
5-04	0	0	0	0	Eight, 2.5 × 1.5 × 1.5 cm.	++++	++++	++++	±
5-01	0	0	0	0	Two, 0.2 × 0.2 × 0.2 cm.	++++	++++	++++	±
5-08	0	0	0	0	No palpable tumors	++++	++++	++++	0
5-00	0	0	0	0	" " "	++++	++++	++++	0
5-06	0	0	0	0	Eight, 3.5 × 2.0 × 2.0 cm.	++++	++++	0	±
5-02	0	0	0	0	Two, 2.5 × 1.2 × 1.2 cm.	++++	++++	+	0
5-09	0	0	0	0	Eight, 3.5 × 2.0 × 2.0 cm.	+++±	+	+	±
4-99	0	0	0	0	Eight, 3.5 × 2.5 × 2.5 cm.	+++±	+±	0	0
5-10	0	0	0	0	Two, 2.0 × 1.0 × 1.0 cm.	+++±	++	+	±
5-03	0	0	0	0	Eight, 4.0 × 3.0 × 3.0 cm.	++	±	0	±
5-07	0	0	0	0	Five, 3.0 × 1.5 × 1.5 cm.	0	0	0	±
5-05	0	0	0	0	Four, 2.0 × 1.0 × 1.0 cm.	0	0	0	0
Controls (no serum)..	0	0	0			0	0	0	

2 units of complement in all tubes.

Sera diluted 1:4.

* Antigens, 1:20 saline extracts of Brown-Pearce tumors from 3 rabbits.

the 12 rabbits had developed the capacity to fix complement in greater or less degree in mixture with the same antigens by the 31st day after the implantations. The complement-fixing capacity had developed in the blood of rabbits in which the tumors had appeared at every implanted situation and had reached large size (D.R. 5-04, 5-06, 5-09, and 4-99), as well as in the blood of other rabbits in which the tumors had grown poorly (D.R. 5-01, 5-02, 5-10) or had been overcome before the 10th day (D.R. 5-08, 5-00). No detectable complement-fixing capacity was evident in

the serum of 2 rabbits that carried 4 or 5 fair sized growths each (D.R. 5-07 and 5-05).

The fact that 10 of the 12 rabbits of Experiment 3 developed the capacity to fix complement in mixture with antigens derived from the Brown-Pearce tumor gave reason to suppose that almost all of the rabbits serving as hosts for the growth would do likewise. But further experience has shown that while, as a rule, more than half of the rabbits implanted with the Brown-Pearce tumor in any one experiment developed the complement-fixing capacity, in certain experiments few or none of them did so in any noteworthy titer. Indeed, in one experiment 11 blue-cross rabbits, procured from the same inbred colony as those of Experiment 3, and bearing 6 to 8 large or medium sized tumors when bled on the 23rd day after implantation, all yielded sera devoid of complement-fixing antibody in detectable titer. On the 41st day, when most of the tumors had begun to retrogress, the sera of 9 out of 11 of these rabbits had some complement-fixing capacity (giving + to +++ fixation reactions with each of 2 potent antigens); but when tested again on the 48th and 59th days, after retrogression of most of the tumors had taken place, the sera again manifested little or no complement-fixing capacity.

Sera have been tested from 65 rabbits in all bearing Brown-Pearce tumors of various size and duration. Of these, 52 (80 per cent) had the capacity to fix complement specifically in greater or less degree when mixed with antigens consisting of saline extracts of the growths.[2]

These findings, which show that the complement-fixing capacity varies greatly from host to host and may not infrequently be absent, accord with those previously obtained with the sera of rabbits carrying virus-induced

[2] A further study of the incidence and titer of the complement-fixing antibody in rabbits implanted with the Brown-Pearce tumor and injected with extracts of it has been made recently with the collaboration of Dr. Ian MacKenzie. The results of this study, which will be reported in detail in a later communication, confirm and extend the findings just cited. The complement-fixing antibody appeared fairly regularly and often in high titer (1:512 or higher) in the blood of rabbits developing good sized Brown-Pearce tumors that retrogressed abruptly, and almost as regularly, though in not such high titer, in rabbits that overcame the growth early. The antibody was usually present, though in comparatively low titer, as a rule, in the sera of animals with progressively enlarging growths. Often it failed to appear in detectable amount in the blood of rabbits carrying indolent tumors. Like the circulating antibodies directed against the chicken tumor viruses (4) and the rabbit papilloma virus (5), the complement-fixing antibody for the Brown-Pearce tumor antigen has no perceptible influence on the course of the Brown-Pearce tumors *in vivo*, these enlarging or retrogressing irrespective of the presence or absence of the antibody.

papillomas; indeed only a small proportion of domestic rabbits bearing virus-induced papillomas have complement-fixing antisera of noteworthy titer (1).

Specificity of the Reaction

In the experiments considered thus far the sera of normal rabbits or of those with virus-induced papillomas failed to fix complement when mixed with extracts of the Brown-Pearce tumor; whereas the sera of certain rabbits bearing the transplanted growth, or in which it had retrogressed, gave notably strong reactions with the Brown-Pearce tumor antigens, but not with those derived from the virus papillomas. To test the specificity of the reaction still further, the sera of a number of rabbits with various other diseases were now mixed with extracts of Brown-Pearce carcinoma, and complement-fixing antisera of known potency from Brown-Pearce tumor rabbits were tested in mixture with extracts of other tissues of rabbits.

Tests with Materials from Rabbits Immune to Vaccine Virus.—In the following experiment the sera of 5 rabbits bearing the Brown-Pearce tumor, or in which it had retrogressed, of 5 others immune to vaccine virus as result of previous infection therewith, and of 5 normal rabbits, were tested with saline extracts of the Brown-Pearce tumor and with extracts of rabbit testicles infected with vaccine virus.

Experiment 4.—The vaccinia-immune sera were procured from 5 rabbits 21 to 36 days after intracutaneous inoculations with this virus for titration purposes. All 5 had developed characteristic lesions and had proved immune to reinfection with a potent suspension of vaccine virus in a dilution of 10^{-2}, 7 days before the present bleeding. Antigens were made by extracting with saline and centrifuging as usual the frozen Brown-Pearce tumors of 2 rabbits (D.R. 69 and 35) and the vaccinia-infected testicles of 2 others (D.R. V-I and V-II). A potent suspension of vaccine virus had been injected into the testicles of these latter animals, and after 4 days the swollen testicles were removed and kept frozen until used 21 days later. Extracts of the testicular tissue contained vaccine virus in high titer, as subsidiary tests proved.

It will be seen from Table IV that the test antigens made from the Brown-Pearce tumors reacted specifically with the sera of 3 of the rabbits with Brown-Pearce tumors, but not with the sera of normal rabbits or those immune to vaccine virus. The test antigens made from the testicles infected with vaccine virus reacted specifically only with the sera of the vaccinia-immune rabbits, although one of the antigens derived from the vaccinia testicles was somewhat anticomplementary and gave incomplete, non-specific fixation with normal sera as well as with the sera of the tumor rabbits.

Tests with Materials from Rabbits Immune to Virus III.—A similar experiment was next done to learn whether the sera of rabbits immune to Virus III will fix complement in mixture with extracts of the Brown-Pearce tumor, and whether extracts of rabbit testicles infected with the virus will react with the antisera obtained from rabbits with the Brown-Pearce tumor.

TABLE IV

Complement Fixation Tests with Materials from Rabbits (a) with Brown-Pearce Tumors and (b) with Vaccinia

Source of sera		Source of extracts				
Rabbits	Rabbit No.	Brown-Pearce tumors		Testicles infected with vaccine virus		Controls (no antigen)
		D.R. 69	D.R. 35	D.R. V-II	D.R. V-I	
With Brown-Pearce tumors	67	0	0	0	±	0
	71	++++	++++	0	+±	0
	73	++++	++++	0	+	0
	1-26	++	+±	0	±	0
	1-27	+	±	0	++	0
Immune to vaccine virus	3	0	0	++++	++++	0
	11	0	0	++++	++++	0
	12	0	0	++++	++++	0
	13	0	0	+++	++++	0
	14	0	0	++++	++++	0
Normal	1-07	0	0	0	0	0
	1-08	0	0	0	±	0
	3-04	0	0	0	+±	0
	3-05	0	0	0	++±	0
	3-06	0	0	0	++	0
Controls (no serum).....		0	0	0	+	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 saline extracts of frozen tissues.

Experiment 5.—The Virus III immune sera were obtained from 4 rabbits that had been injected in both testicles with 1 cc. of a 10 per cent suspension of rabbit testicle containing Virus III, supplied by Dr. Rivers. All developed fever and swollen testicles within 2 or 3 days, but these manifestations had subsided by the 9th day. Then they were again inoculated intratesticularly with 1 cc. of a potent preparation of Virus III, all proving immune. 5 days later the rabbits were bled to procure serum for use in the present experiment. As test antigens, 1:20 Berkefeld V filtrates in saline were made as usual of the Virus III infected testicles of 2 rabbits (D.R. 24 and 25). These organs were procured 5 days after injection into them of a potent suspension of Virus III. They

had been kept 18 days in glycerol. Berkefeld filtrates were made in the same way of Brown-Pearce tumors which had grown in the testicles of 3 rabbits (D.R. 69, 36, and 38). Sera were employed that were known to react with antigens derived from the Brown-Pearce tumor. The sera and test antigens were heated as usual at 56°C. for 30 minutes immediately before use in the tests.

As Table V shows, the sera of the rabbits immune to Virus III did not react in mixture with the antigens made from the Brown-Pearce tumors,

TABLE V

Complement Fixation Tests with Filtrates of the Brown-Pearce Tumor and with Filtrates of Testicles Infected with Virus III

Source of sera		Source of antigens					
Rabbits	Rabbit No.	Brown-Pearce tumors			Testicles infected with Virus III		Controls (no antigen)
		D.R. 69	D.R. 36	D.R. 38	D.R. 24	D.R. 25	
With Brown-Pearce tumors	67	++++	++	0	0	0	0
	71	++++	++++	+++±	0	0	0
	73	++++	++++	+++±	0	0	0
	4-69	+++	++	±	0	0	0
Immune to virus III	16	0	0	0	0	0	0
	30	0	0	0	0	0	0
	31	0	0	0	0	0	0
	32	0	0	0	0	0	0
Normal	1-07	0	0	0	0	0	0
	1-08	0	0	0	0	0	0
	3-99	0	0	0	0	0	0
	18	0	0	0	0	0	0
Controls (no serum)....		0	0	0	0	0	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 Berkefeld V filtrates of glycerolated tissues.

nor for that matter with the antigens derived from the testicles infected with the virus; and the latter gave no reaction in mixture with the Brown-Pearce antisera. Several other similar tests made with widely various dilutions of the same and other Virus III antigens and sera yielded identical findings. The failure of the Virus III antigens to give complement fixation in mixture with corresponding immune serum is hardly surprising when it is recalled that Virus III is unstable and that but a small bulk of it is present in extracts of infected testicles (6).

Tests with Sera from Rabbits with Spontaneous and Transplanted Uterine

Cancers.—An experiment was next made to determine whether sera from rabbits bearing a tumor of another sort will fix complement in mixture with antigens made from the Brown-Pearce tumor. The sera of rabbits bearing spontaneous or transplanted uterine cancers (7) were made available by Dr. H. S. N. Greene.

Experiment 6.—Four of the sera had been procured from rabbits with large primary cancers of the uterus, all of several months' duration. Eight others had come from

TABLE VI

Complement Fixation Tests with the Sera of Rabbits with Uterine Cancers

Source of serum	Rabbit No.	Brown-Pearce tumor antigens		Controls (no antigen)
		D.R. 35	D.R. 69	
Rabbits with primary cancer of the uterus	G 1	0	0	0
	G 2	0	0	0
	G 3	0	0	0
	G 4	0	0	0
Rabbits with eye transplants of uterine cancer	G 5	0	0	0
	G 6	0	0	0
	G 7	0	0	0
	G 8	0	0	0
Rabbits with testicular and eye transplants of uterine cancer	G 9	0	0	0
	G 10	0	0	0
	G 11	0	0	0
	G 12	0	0	0
Rabbits with Brown-Pearce tumors	71	++++	++++	0
	73	++++	++++	0
Controls (no serum)		0	0	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 saline extracts of Brown-Pearce tumors from 2 rabbits.

rabbits with large transplanted tumors of the same sort situated in the anterior chamber of the eye in 4 cases, and in the testicle as well as the eye in 4. The transplanted growths had been present for periods ranging from 4 to 13 months. Two known Brown-Pearce antisera were included for comparison. After heating at 56°C. for 30 minutes, all were tested for capacity to fix 2 units of complement in mixture with 1:20 saline extracts of the peritoneal metastases of the Brown-Pearce tumors of 2 rabbits (D.R. 35 and 69).

From Table VI it will be seen that none of the 12 sera from the rabbits with uterine cancers fixed complement in mixture with the Brown-Pearce

tumor antigens, while the 2 Brown-Pearce antisera fixed it completely when tested concurrently.

The experiments leave little room for doubt that the serum reaction is specific. In the tests described, and in others which need not be given in detail, a total of 102 sera have been tested from domestic rabbits other than those carrying Brown-Pearce tumors. These were procured from

TABLE VII

Complement Fixation Tests with Extracts Derived from Various Tissues of Rabbits

Extracts		Complement fixation tests							
Tissue	Rabbit No.	Brown-Pearce antisera					Normal sera		Controls (no serum)
		71	5-00B	5-01B	5-04B	5-08B	5-00A	5-01A	
Skin	4-19	0	0	0	0	0	0	0	0
	4-20	0	0	0	0	0	0	0	0
	4-21	0	0	0	0	0	0	0	0
Spleen	4-19	0	0	0	0	0	0	0	0
	4-20	0	0	0	0	0	0	0	0
	4-21	0	0	0	0	0	0	0	0
Liver	4-19	0	0	0	0	0	0	0	0
	4-20	0	0	0	0	0	0	0	0
	4-21	0	0	0	0	0	0	0	0
Muscle	4-19	0	0	0	0	0	0	0	0
	4-20	0	0	0	0	0	0	0	0
	4-21	0	0	0	0	0	0	0	0
Brown-Pearce tumor	4-72	++++	++++	++++	++++	++++	0	0	0
	4-76	++++	++++	++++	++++	++++	0	0	0
	5-09	++++	++++	++++	++++	++++	0	0	0
Controls (no antigen)....		0	0	0	0	0	0	0	

2 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 saline extracts.

normal and pregnant domestic rabbits, from rabbits with experimental syphilis or uterine cancers or virus papillomas, and from rabbits immune to Virus III, fibroma virus, vaccine virus, or herpes virus. None has fixed complement specifically upon admixture with antigens derived from the Brown-Pearce tumor, whereas, as already stated, 52 of the 65 sera drawn from rabbits carrying the Brown-Pearce tumor or in which it had recently retrogressed have done this in concurrent tests.

Tests with Antigens Made from Other Normal and Neoplastic Tissues of Rabbits.—Saline extracts of the skin, spleen, liver, and muscle of normal rabbits were next compared with extracts of the Brown-Pearce tumor for capacity to react with the known complement-fixing antisera. These

TABLE VIII

Complement Fixation Tests with Antigens Derived from Brown-Pearce Tumors and from Uterine Cancers, and Sera from Rabbits with Growths of One Sort or the Other

Source of serum	Rabbit No.	Antigens				Serum controls (no antigen)
		Brown-Pearce tumors		Uterine cancers		
		D.R. 4-72	D.R. 5-03	D.R. 383-3	D.R. E-182*	
Rabbits with primary or transplanted uterine cancer	5812-3	0	0	±	±	0
	16-05	0	0	±	±	0
	383-3	0	0	0	0	0
	578-2*	0	0	0	0	0
	180-02	0	0	0	0	0
	62-79	0	0	0	0	0
	E-182*	0	0	0	0	0
	8554-1*	0	0	0	0	0
Brown-Pearce tumor rabbits	71	++++	+++±	0	0	0
	5-01	++++	++++	0	0	0
	5-04	++++	++++	0	0	0
	5-08	++++	+++±	0	0	0
Normal rabbits	2-60	0	0	0	0	0
	2-61	0	0	0	0	0
	3-06	0	0	0	0	0
	4-20	0	0	0	0	0
Antigen controls (no serum)		0	0	0	0	0

3 units of complement in all tubes.
Sera diluted 1:4.
Antigens, 1:20 saline extracts.
Sera and antigens had been heated at 56°C. immediately prior to use.
* Transplanted cancers.

failed to react, as Table VII shows, while the antigens derived from the Brown-Pearce tumors of 3 rabbits gave complete fixation.

A similar test was next made, using extracts derived from the transplanted Brown-Pearce tumors of 2 rabbits and from the naturally occurring uterine cancers of 2 others, and sera from rabbits with growths of one sort or the other. Table VIII shows the results. As in the preceding

experiments, the reactions were highly specific, no complete fixation taking place except in the mixtures containing the antigens made from the Brown-Pearce tumor and sera from rabbits with growths of this sort. It will be observed that there was a slight amount of fixation, which appeared to be specific in character, in the mixtures containing the uterine cancer antigens and 2 of the sera from 2 rabbits with primary uterine cancers. This amount of fixation has several times been observed in similar tests with sera procured from rabbits with primary or transplanted uterine cancers in mixture with extracts of these growths, but repeated tests, some of them made in collaboration with Dr. Ian MacKenzie, failed to yield consistently positive results with such materials. The fact will be commented upon in the discussion to be found in the accompanying paper.

Many other experiments have been made to test further the specificity of the reaction, using antigens consisting of extracts of normal testicles (9 rabbits), normal rabbit embryos (4 rabbits), testicles and skin infected with fibroma virus (2 rabbits), and virus-induced papillomas (8 rabbits). The results of these need not be tabulated, for none of the extracts had the capacity to fix complement specifically in mixture with antisera of known potency obtained from rabbits with the Brown-Pearce tumor.

SUMMARY AND COMMENT

A substance is regularly present in saline extracts of the Brown-Pearce rabbit carcinoma growth which is capable of fixing complement specifically in mixture with the sera of certain rabbits bearing the tumor or in which this has recently retrogressed, as the foregoing experiments have shown. The substance was not demonstrable in extracts of the normal tissues, virus papillomas, or uterine cancers of rabbits, nor in extracts of rabbit tissues infected with certain viruses (vaccine virus, Virus III, fibroma virus). The sera of normal rabbits, of those immune to a variety of infectious diseases, including syphilis, vaccinia, fibromatosis, and Virus III, and of others with uterine cancers or virus-induced papillomas, failed to fix complement specifically in mixture with extracts containing the antigen of the Brown-Pearce tumor.

The significance of the findings will be discussed in the succeeding paper, after consideration has been given to some of the properties of the serologically active material derived from the Brown-Pearce tumor.

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