INDUCED ANTIBODIES THAT REACT IN VITRO WITH SEDIMENTABLE CONSTITUENTS OF NORMAL AND NEOPLASTIC TISSUE CELLS

PRESENCE OF THE ANTIBODIES IN THE BLOOD OF RABBITS CARRYING VARIOUS TRANSPLANTED CANCERS

BY WILLIAM F. FRIEDEWALD, M.D., AND JOHN G. KIDD, M.D.

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

(Received for publication, March 21, 1944)

A distinctive sedimentable substance, identifiable by means of its reaction *in vitro* with a specific antibody, can be regularly extracted from the cells of the Brown-Pearce rabbit carcinoma, as previous studies have shown (1). To learn the nature and significance of this cell component, and whether other cancers contain substances of similar sort, have been the aims of further investigations. A preceding paper has dealt with one of the complexities encountered in these studies,—namely, a natural antibody which is usually to be found in the blood of normal adult rabbits and which reacts *in vitro* with a sedimentable constituent of normal and neoplastic tissue cells (2). The present report is concerned with induced antibodies having similar affinities which appear occasionally in the blood of rabbits carrying various transplanted cancers.

Methods and Materials

The induced antibodies and the sedimentable tissue constituents with which these react have been studied by means of a standardized complement fixation test (2). "Healthy" normal and neoplastic tissues were used as a source of antigens; these were procured with aseptic precautions and extracted while fresh or after storage for periods up to several months at −22° C. The saline extracts were made fresh for each experiment by grinding tissues in sterile mortars and adding 20 or more volumes of salt solution; they were cleared in the centrifuge and used unheated. Control tests for anticomplementary effects were always made, using double volumes of every serum and antigen. These were negative without exception in the reported experiments.

In serological work with the virus-induced papilloma (Shope) it had proved necessary to produce a great mass of papilloma tissue on the skin of each animal if sera with high titers of antiviral antibody were to be procured (3). Assuming that the same might be true with other tumors, we have made multiple implantations of the transplantable cancers used in the present study, in order to have a large amount of neoplastic tissue growing in each animal.

Four transplantable rabbit cancers were employed—two carcinomas and two sarcomas. The Brown-Pearce carcinoma is well known (4) and serological studies of it have already been reported (1). The V2 carcinoma—a squamous cell carcinoma which originated in a virus-induced papilloma—was recently described (5). Neither of the sarcomas has heretofore been studied in this country. The Rabbit Sarcoma I (RSI), generously sent by Dr. C. H. Andrewes of the National Institute of Health in London, originated in 1936 in the leg muscles

of a rabbit at a site where the Shope fibroma virus had localized in tissue cells previously exposed to the influence of tar (6). A spindle cell sarcoma, it forms huge tumors but is slow to metastasize and kill. The Kato sarcoma has been transplanted during a number of years in Japan.[1] It is a rapidly growing, anaplastic sarcoma, which forms immense masses when put into the leg muscles or subcutaneous tissues, and often spreads widely and frequently kills its host within 3 to 4 weeks.

Three of the four transplanted cancers undergo extensive central necrosis as the tumor nodules enlarge. The Kato sarcoma is not so prone to this. But Brown-Pearce tumors that have attained a diameter of 3.0 cm. or more (and this often happens within 2 to 3 weeks after implantation) are largely necrotic as a rule; they have rinds of pale-pink, "healthy-looking" tumor tissue, 1–3 mm. thick, while all the rest is necrotic, buff-colored or brownish-gray, only slightly moist, usually close-textured and friable, rarely pultaceous. The RSI is similar in gross aspect, though it generally grows less rapidly, and extensive necrosis does not occur until after a longer interval. It attains a diameter of 10.0 cm. or more after 6 or 8 weeks, and by then has usually become necrotic except for a thin, living rind, and often partly liquefied. The V2 carcinoma soon undergoes a central necrosis followed by an accumulation of fluid which results in large cysts. This usually happens 3–5 weeks after implantation, when the tumor nodules have attained a diameter of 2.0 cm. or more. The walls of the cysts, 1.0–10.0 cm. or more across, are composed of proliferating tumor tissue which breaks down on the inner side and here has the aspect of giant granulations. The fluid, often under considerable tension, is glairy and tenacious, sometimes brown or red from hemorrhage, and it may be thick like candle grease or contain yellowish gouts of pultaceous matter.

Transplantation of the four cancers was carried out as in preceding experiments (1), by implanting hashed or sieved tumor tissue in young adult domestic rabbits, usually market-bought agouti hybrids. Six or eight situations were implanted as a rule, usually the muscles of both forelegs and of both anterior and posterior thighs and the testicles also of the males. Sometimes the Kato sarcoma was implanted subcutaneously in a single situation in an attempt to slow its growth and stay the death of its host. Microscopic sections were examined of many of the tumor materials used for transfer or as a source of antigen to make certain that they were characteristic and free from conspicuous bacterial infection.

Heat-Labile and Heat-Resistant Antibodies in the Serum of Rabbits Carrying the V2 Carcinoma

Antibodies of several sorts may be encountered in the blood of rabbits carrying the transplanted V2 carcinoma, as the following experiments show.

In the first experiment the sera of two normal rabbits, known from previous tests to have appreciable titers of the natural tissue antibody, were used as controls in a test of the sera of eight rabbits carrying V2 carcinomas. Specimens from the tumor rabbits had all come from animals with large, cystic V2 carcinomas of several weeks' duration; and all had been previously tested in experiments done for other purposes, and had been found to fix complement in high titer in mixture with antigens consisting of saline extracts of the V2 carcinoma. Previous work had shown that 65°C. for 30 minutes inactivates the natural antibody in rabbit serum without affecting induced ones, whereas 56° C. has no deleterious effect on either antibody (2); hence specimens of the various sera were heated for 30 minutes at 56° C. and 65° C., respectively, and tested for capacity to fix complement in mixture with two antigens,—1:20 saline extracts of frozen normal rabbit liver (D.R. 5-73) and frozen V2 carcinoma tissue (D.R. 4-39), respectively.

[1] Rabbits bearing the sarcoma were generously sent from Osaka by Dr. Kinosita in 1940. We are also indebted to Dr. Kawachi, surgeon of the Horoku Maru, who transplanted the growth in mid-Pacific.

Table I shows the results of the tests. Complement fixation took place in all of the mixtures containing the sera heated at 56° C. The sera from the normal rabbits (D.R. 5-74 and 5-75) fixed complement in dilutions as high as 1:16 in mixture with the normal rabbit liver antigen and slightly less well with the V2 carcinoma antigen. The sera of the rabbits carrying V2 carcinomas in general reacted in somewhat higher dilutions with the liver antigen and in much higher dilutions with the V2 carcinoma antigen. The results were very different with the serum specimens heated at 65° C. The normal rabbit sera (which contained the natural antibody) now failed to react with either antigen, and the sera of the V2 rabbits 14-66 and 15-54 had lost entirely their capacity to react with the liver antigen but had retained practically undiminished their ability to fix complement in mixture with the V2 carcinoma antigen. Much the same was true of the 65° C. specimens from V2 rabbits 13-47 and 14-69, which now reacted only slightly with the liver antigen but about as well as before with the V2 carcinoma antigen. The sera of the rest of the V2 rabbits (8-50, 14-64, 12-57, 15-43) still fixed complement in mixture with the liver antigen after heating at 65°, though they did this less well than the corresponding specimens heated at 56° and, like the other V2 sera heated at 65° C., these reacted with the V2 carcinoma antigen as well as did the specimens heated to only 56°.

In a second experiment of similar sort, tests were made with serum specimens procured from all of the rabbits with progressively enlarging V2 carcinomas of the 19th tumor generation (Table II). Specimens from three normal rabbits, included as controls, all fixed complement in mixture with the normal liver and V2 carcinoma antigens when heated at 56° C. for 30 minutes but not after 65° C. Without exception they reacted better with the normal liver antigen than with that of the V2 carcinoma. The V2 carcinoma sera, by contrast, reacted in high titer with the V2 carcinoma antigen and their capacity to do so was not notably diminished by 65° C. for 30 minutes. As in the experiment of Table I, the V2 sera also reacted with the normal liver antigen in varying degree; in some instances (specimens 15-37, 15-54, 15-35) the ability to react with the normal tissue antigen was largely or completely abolished upon heating at 65° C. for 30 minutes, whereas in others (15-43, 15-50) the heating had no appreciable effect.

To broaden the observation, tests were now made with several normal and neoplastic tissue antigens in mixture with sera procured from several kinds of rabbits before and after implantation with the V2 carcinoma. The results, set down in Table III, confirm and extend those of the preceding experiments. All of the serum specimens had been heated at 65° C. for 30 minutes immediately prior to use in the tests (Table III). The sera which had been procured before the implantation failed without exception to react with any of the antigens, which were made from normal rabbit kidney, liver, and spleen tissues and from the Brown-Pearce epithelioma and the V2 carcinoma, respectively. All of the specimens obtained on the 27th and 54th days after implantation, however, reacted with one or another of the antigens except that from rabbit 5-75, a host in which the carcinoma had grown only briefly. In general, the larger the tumors and the longer their duration the greater was the capacity of the host serum to react with the test antigens. For example, the sera of rabbits 5-74, 5-66, 5-71, and 5-72, drawn on the 54th day when the tumors had been large for several weeks, all reacted in greater or less titer with the various normal and neoplastic tissue antigens; whereas the specimens procured from the same hosts on the 27th day failed to react with some of the antigens, though doing so in various titers with others. It will be noted that most of the sera reacted with the antigens in the following order of diminishing titer: V2 carcinoma, spleen, kidney, liver, and Brown-Pearce carcinoma,—though several sera reacted somewhat better with spleen than with the V2 carcinoma antigen. More will be said later about this fact.

From the experiments just described (Tables I to III) it would appear that the sera of rabbits carrying transplanted V2 carcinomas may contain more than one type of antibody. Like the sera of most normal adult rabbits, they usually

TABLE I

Heat-Labile and Heat-Resistant Antibodies in Sera of Rabbits Carrying the Transplanted V2 Carcinoma

Sera			Complement fixation tests												
			Liver antigen D.R. 5-73, 1:20							V2 carcinoma antigen D.R. 4-39, 1:20					
			Serum dilution							Serum dilution					
Heated (30 min.)	Procured from	Rabbit No.	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	Normal rabbits	5-74	++++	++++	+++	0	0	0	0	+++±	+++	±	0	0	0	0
		5-75	++++	++++	++++	0	0	0	0	++++	++++	+++	0	0	0	0
56° C.	Rabbits carrying the V2 carcinoma	14-66	++++	++++	++++	+++	0	0	0	++++	++++	++++	++++	++++	+++±	+++
		15-54	+++±	+++	0	0	0	0	0	++++	++++	++++	++++	++++	++++	+++
		13-47	++++	++++	++++	+++	0	0	0	++++	++++	++++	++++	++++	++++	++++
		14-69	++++	++++	++++	+	0	0	0	++++	++++	++++	++++	++++	+++±	±
		8-50	++++	++++	+++	0	0	0	0	++++	++++	++++	++++	++++	+++±	0
		14-64	++++	++++	++++	++++	+++	0	0	++++	++++	++++	++++	++++	++++	+++
		12-57	++++	++++	++++	+++±	0	0	0	++++	++++	++++	++++	++++	++++	++++
		15-43	++++	++++	++++	++++	++++	+++	0	++++	++++	++++	++++	++++	++++	++++
	Normal rabbits (same as above)	5-74	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		5-75	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65° C.	Rabbits carrying the V2 carcinoma (same as above)	14-66	0	0	0	0	0	0	0	++++	++++	++++	++++	++++	+++±	+++
		15-54	0	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++	+
		13-47	++	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++	++++
		14-69	+++	0	0	0	0	0	0	++++	++++	++++	++++	+++±	++±	0
		8-50	++++	+++±	0	0	0	0	0	++++	++++	++++	++++	++++	+++	0
		14-64	++++	++++	++++	+++±	±	0	0	++++	++++	++++	++++	++++	+++±	++
		12-57	++++	++++	++++	++++	±	0	0	++++	++++	++++	++++	++++	++++	++++
		15-43	++++	++++	++++	++++	+++±	0	0	++++	++++	++++	++++	++++	++++	++++

2 units of complement in all tubes, as also in the tests of the tables to follow.

Antigens, saline extracts of frozen rabbit tissues as indicated.

The V2 sera had come from rabbits of different tumor generations, all of which carried large cystic tumors of several weeks' duration.

None of the sera proved anticomplementary when tested concurrently in double volume, nor did the antigens. The same held true for the experiments of the later tabulations.

TABLE II

Heat-Labile and Heat-Resistant Antibodies in Sera of Rabbits Carrying Transplanted V2 Carcinoma

Heated (30 min.)	Procured from	Rabbit No.	Diameter of tumors (six situations)	Days since implantation	Liver antigen D.R. 5-69, 1:40					V2 carcinoma antigen D.R. 4-39, 1:40				
					Serum dilutions					Serum dilutions				
					1:4	1:8	1:16	1:32	1:64	1:4	1:8	1:16	1:32	1:64
			cm.											
	Normal rabbits	5-69			++++	++++	++++	++++	++±	+++	+++±	+++	0	0
		5-71			++++	++++	++++	+++±	++	+++	+++	+++	+±	0
		5-75			++++	++++	++++	+++±	±	+++	+++	+++	0	0
	Rabbits carrying	15-37	10-9-8-10-5-5	46	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
56° C.	V2 carcinomas	15-54	4-5-6-8-8-6	63	++++	++++	++++	+	0	++++	++++	++++	+++±	+++
	(19th generation)	15-35	5-7-9-14-8-4	69	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
		15-48	8-6-10-8-4-6	41	+++	+++±	+++	+±	0	++++	++++	++++	++++	+++
		15-36	8-8-8-11-9-10	69	+++±	++++	+++±	+++	0	++++	++++	++++	++++	++++
		15-51	7-6-10-8-8-6	41	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
		15-33	6-8-10-14-6-8	69	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++
		15-50	5-4-7-8-4-1.5	63	++++	++++	++++	+++±	+++	++++	++++	++++	+++±	+++
		15-43	5-8-10-10-4-8	59	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	Normal rabbits	5-69			0	0	0	0	0	0	0	0	0	0
	(same as above)	5-71			0	0	0	0	0	0	0	0	0	0
		5-75			0	0	0	0	0	0	0	0	0	0
	Rabbits carrying	15-37	As above		0	0	0	0	0	++++	++++	++++	++++	+++±
65° C.	V2 carcinomas	15-54			+±	0	0	0	0	++++	++++	++++	+++±	+++
	(19th generation) (same as above)	15-35			+++	++	±	0	0	++++	++++	++++	++++	++++
		15-48			+++±	++±	0	0	0	++++	++++	++++	++++	+++
		15-36			++++	+++	+	0	0	++++	++++	++++	++++	++++
		15-51			+++±	+++±	+++	0	0	++++	++++	++++	++++	++++
		15-33			++++	++++	++++	+++	+	++++	++++	++++	++++	++++
		15-50			++++	++++	++++	++++	++±	++++	++++	++++	++++	++++
		15-43			++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

TABL

Tests with Serum Procured from Rabbits before

(21st Tumor Generation—

Source of Serum: Rabbit No. and breed	Outcome of intramuscular implantations (six situations)	Antigens	Prior to implantation: Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64
5-75 (Blue-cross hybrid)	Three tumors measuring up to 1.0 cm. on the 12th day; negative on the 27th day and thereafter	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-77 (Blue-cross hybrid)	Three tumors up to 1.5 cm. on the 12th day; six tumors up to 5.0 cm. on the 27th day; killed on the 54th day, six tumors up to 6.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-63 (Gray-brown agouti hybrid)	Four tumors up to 2.0 cm. on the 12th day; six tumors up to 4.0 cm. on the 27th day; negative on the 54th day and thereafter	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-62 (Gray-brown agouti hybrid)	Four tumors up to 2.0 cm. on the 12th day; six tumors up to 7.0 cm. on the 27th day; killed on the 54th day, six tumors up to 10.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-74 (Blue-cross hybrid)	Three tumors up to 1.0 cm. on the 12th day; six tumors up to 6.0 cm. on the 27th day; killed on the 54th day, six tumors up to 8.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-66 (New Zealand)	Four tumors up to 2.0 cm. on the 12th day; six tumors up to 7.0 cm. on the 27th day; killed on the 54th day, six tumors up to 8.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-71 (Chinchilla)	Six tumors up to 1.2 cm. on the 12th day; all enlarged, up to 4.0 cm. on the 27th day; killed on the 54th day, six tumors up to 8.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0
5-72 (Chinchilla)	Six tumors up to 1.5 cm. on the 12th day; all enlarged, up to 4.0 cm. on the 27th day; killed on the 54th day, six tumors up to 8.0 cm.	Kidney	0	0	0	0	0	0
		Liver	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0
		V2 carcinoma	0	0	0	0	0	0

Sera heated at 65° C. for 30 minutes immediately prior to use.
Antigens, 1:40 saline extracts of frozen rabbit tissues as indicated.

E III

and after Implantation with the V2 Carcinoma

Hosts of Various Breeds)

Complement fixation tests Serums procured											
27 days after implantation Serum dilutions						54 days after implantation Serum dilutions					
1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4	1:8	1:16	1:32	1:64
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	+±	+	±	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
+	±	0	0	0	0	++++	++++	++++	++±	0	0
0	0	0	0	0	0	0	0	0	0	0	0
++++	++++	++++	+++±	+	0	++++	++++	++++	++++	++	0
++++	++++	+++	+	0	0	+	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
++++	++++	++++	+++	+	0	++++	++++	++++	+	0	0
±	0	0	0	0	0	0	0	0	0	0	0
++++	++++	++++	++++	++++	+++±	++++	++++	++++	++++	++++	++±
0	0	0	0	0	0	++++	++++	++++	±	0	0
0	0	0	0	0	0	0	0	0	0	0	0
+++	++	++	0	0	0	++++	++++	++++	++++	++++	+++±
0	0	0	0	0	0	+++±	+±	0	0	0	0
++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
±	0	0	0	0	0	++++	++++	++++	++	0	0
±	0	0	0	0	0	++++	++++	+++	0	0	0
++++	++++	++++	++++	++++	+++±	++++	++++	++++	++++	++++	+++±
0	0	0	0	0	0	+±	+	0	0	0	0
++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	+++
++	±	0	0	0	0	++++	++++	++	0	0	0
0	0	0	0	0	0	±	0	0	0	0	0
++++	++++	+++	+	0	0	++++	++++	++++	++++	+++±	++±
+++	+	0	0	0	0	++++	++++	+++±	±	0	0
++++	++++	++++	++++	+++±	0	++++	++++	++++	++++	++++	++++
0	0	0	0	0	0	++++	++++	++++	++++	+++±	±
0	0	0	0	0	0	++++	++++	++++	+	0	0
++++	++++	++++	++++	++++	+++±	++++	++++	++++	++++	++++	++++
++++	++++	++++	+	0	0	++++	++++	++++	++++	++++	++±
++++	++++	++++	+++±	±	0	++++	++++	++++	++++	++++	++++
++++	+++	±	0	0	0	++++	++++	++++	++++	++++	++++
0	0	0	0	0	0	++++	++++	++++	++++	++++	±
++++	++++	++	±	0	0	++++	++++	++++	++++	++++	++++
++++	+±	0	0	0	0	++++	++++	++++	++++	++++	++
++++	++++	++++	++++	+++±	0	++++	++++	++++	++++	++++	++++

contain the natural tissue antibody which is heat-labile (inactivated upon heating at 65° C. for 30 minutes) and which reacts *in vitro* with saline extracts of various normal and neoplastic tissues (2). In addition, they frequently develop antibodies of a second type—the special object of this study. These, like the natural tissue antibody, react with saline extracts of various normal and neoplastic tissues, but they differ notably from the natural tissue antibody in that heating at 65° C. for 30 minutes has no effect upon them, while furthermore they are absent from the serum of normal rabbits. The heat-resistant antibodies will henceforth be referred to as induced tissue antibodies, to distinguish them from the natural tissue antibody.

The presence of induced tissue antibodies can be discerned especially well in the sera of V2 rabbits 15-43, 12-57, 14-64, and 8-50 of Table I, which after the heating at 65° C. still reacted in high titer in mixture with the normal rabbit liver antigen. Heat-resistant antibodies were apparently absent from the sera of rabbits 14-66 and 15-54 and were present in relatively small titer in the sera of rabbits 13-47 and 14-69, though all four of these sera contained the natural tissue antibody, three of them much of it. So also in Table III, all of the rabbits that carried large tumors on the 54th day after implantation had developed induced tissue antibodies capable of reacting with one or another or all three of the normal tissue antigens employed.

The data of Tables I to III yield evidence that still another type of antibody, also induced and heat-resistant, may likewise be present in the blood of rabbits carrying V2 carcinomas, which reacts with saline extracts of that tumor though not with extracts of normal tissues. This antibody was present in high titer in most of the V2 sera of Tables I to III, for these specimens reacted with V2 carcinoma antigens about as well after heating at 65° C. as after 56° C. Its titer does not run parallel with that of the antibodies that react with normal tissue antigens, as the tables show, and it sometimes exists in sera that contain little or none of the induced tissue antibodies just mentioned. The specimens of rabbits 14-66, 15-54, 13-47, and 14-69 of Table I, for example, reacted in high titer with the V2 carcinoma antigen though not at all or only slightly with the normal liver antigen; and likewise the sera of rabbits 5-77 and 5-62 of Table III, procured 27 days after implantation, reacted well with the V2 carcinoma antigen though not with the normal kidney and liver antigens and hardly at all with that of the spleen. The antibody appears to react specifically with a distinctive sedimentable constituent of V2 carcinoma cells. In further tests, not here described, it has regularly failed to react with saline extracts of virus papillomas of the sort from which the V2 carcinoma originally sprang.[2]

[2] Still another antibody also appears regularly in the blood of rabbits carrying large V2 carcinomas. It reacts specifically with the Shope papilloma virus but has no affinity whatever for other sedimentable substances derived from normal or neoplastic rabbit tissues and fails to react with saline extracts of the V2 carcinoma, as many experiments have shown (5).

Induced Tissue Antibodies in the Blood of Rabbits Carrying Transplanted Cancers of Other Types

The findings already given have made it plain that induced heat-resistant tissue antibodies are frequently present in the blood of rabbits carrying the transplanted V2 carcinoma, as not in that of normal rabbits. Tests were now made with serum from rabbits with transplanted cancers of other sorts. All of the specimens were heated at 65° C. for 30 minutes prior to the tests, to inactivate natural antibodies.

Table IV records the results of tests with sera from nine rabbits implanted with the Brown-Pearce carcinoma in mixture with antigens made from normal rabbit kidney, liver, and spleen, respectively, and from the Brown-Pearce carcinoma. None of the sera procured prior to the tumor implantations manifested any ability to react with the test antigens, and the specimens from three rabbits with regressing tumors (4-14, 4-21, and 4-18) were likewise negative on the 45th day after implantation. The sera of rabbits 4-13, 4-15, 4-16, and 4-17, all of which had carried large tumors for several weeks when they were bled on the 45th day after implantation, had developed the capacity to react to some extent with all of the test antigens, best in general with spleen and kidney and in lesser degree with the Brown-Pearce tumor and normal rabbit liver antigens. Rabbit 4-20, which had growths that had regressed several weeks before the bleeding on the 45th day, provided serum with slight ability to react with the normal tissue antigens but not with the Brown-Pearce carcinoma antigen. Rabbit 4-19, a blue-cross-hybrid in which Brown-Pearce tumors had grown briefly and then regressed abruptly, provided a serum on the 45th day which reacted in high titer with the Brown-Pearce carcinoma antigen but not at all with the normal tissue antigens,—a finding of special interest which will be mentioned again further on.

In the experiment of Table V, tests were made with sera procured from a number of rabbits implanted with the sarcoma of Andrewes and Ahlström (RSI) and with antigens made from various normal and neoplastic rabbit tissues. The specimens from three rabbits (28, 42, and 43) failed to react with any of the test antigens, and those from two others (16-00 and 16-01) reacted irregularly and poorly; but the rest (rabbits 2-93, 3-45, 3-50, 8-13, 15-95) provided sera that reacted about equally with the various antigens, if anything somewhat better with the one derived from normal kidney than with those from the various neoplasms. It may be noted that the reacting sera all came from rabbits with progressively enlarging tumors of several weeks' duration, whereas two of the three negative sera came from rabbits with regressing growths (28, 43).

From Table VI it will be seen that rabbits with large Kato sarcomas of several weeks' duration (2-73, 5-22) provided sera that reacted with saline extracts of various normal and neoplastic rabbit tissues, while other rabbits (J-1, 2-95, 3-08, 3-29), also with progressively enlarging growths though of shorter duration, furnished specimens that failed to react in concurrent tests.

In sum, heat-resistant antibodies capable of reacting with saline extracts of various normal and neoplastic tissues were encountered in the blood of five of nine rabbits implanted with the Brown-Pearce carcinoma (Table IV), in that of seven of ten rabbits implanted with Sarcoma I of Andrewes and Ahlström (Table V), and in that of two of six rabbits implanted with the Kato sarcoma (Table VI). Such antibodies were invariably absent from the serum of rabbits

TABLE IV

Tests for Induced Tissue Antibodies in the Blood Serum of Rabbits Implanted with the Brown-Pearce Carcinoma

Source of serum: Rabbit No. and breed	Outcome of intramuscular implantations (six situations)	Antigens	Complement fixation tests: Serums procured Before implantation, Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64	Complement fixation tests: Serums procured 45 days after implantation, Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64
4-13 (Chinchilla)	Four tumors up to 3.0 cm. on the 11th day; six tumors up to 5.0 cm. on the 24th day; six tumors up to 6.0 cm. on the 45th day, with slow regression thereafter	Kidney	0	0	0	0	0	0	+++±	+++	±	0	0	0
		Liver	0	0	0	0	0	0	++	0	0	0	0	0
		Spleen	0	0	0	0	0	0	++++	+++±	+±	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	++++	+++±	+++	+	0	0
4-14 (Chinchilla)	Six tumors up to 4.0 cm. on the 11th day; all had enlarged up to 6.0 cm. on the 24th day and dwindled to 4.0 cm. on the 45th day, with slow regression thereafter	Kidney	0	0	0	0	0	0	0	0	0	0	0	0
		Liver	0	0	0	0	0	0	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
4-15 (Chinchilla)	Six tumors up to 4.0 cm. on the 11th day; all enlarged up to 6.0 cm. on the 24th day; moribund when bled on the 45th day, with six tumors up to 8.0 cm. and many omental and glandular metastases	Kidney	0	0	0	0	0	0	++++	+++±	++	0	0	0
		Liver	0	0	0	0	0	0	+++	+	±	0	0	0
		Spleen	0	0	0	0	0	0	++++	++++	+++±	+++	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	++++	++++	+	0	0	0
4-19 (Blue-cross hybrid)	Four tumors up to 2.5 cm. on the 11th day; two softish nodules 1.0 cm. on the 24th day; negative on the 45th day	Kidney	0	0	0	0	0	0	0	0	0	0	0	0
		Liver	0	0	0	0	0	0	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
4-20 (Blue-cross hybrid)	Six tumors up to 3.5 cm. on the 11th day; one softish nodule 2.5 cm. on the 24th day; negative on the 45th day	Kidney	0	0	0	0	0	0	+	0	0	0	0	0
		Liver	0	0	0	0	0	0	±	0	0	0	0	0
		Spleen	0	0	0	0	0	0	++++	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
4-21 (Blue-cross hybrid)	Four tumors up to 2.5 cm. on the 11th day; negative on the 24th day and thereafter	Kidney	0	0	0	0	0	0	0	0	0	0	0	0
		Liver	0	0	0	0	0	0	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	0	0	0	0	0	0

4-16 (Gray-brown agouti hybrid)	Six tumors up to 3.0 cm. on the 11th day; all had enlarged up to 6.0 cm. on the 24th day; about the same on the 45th day, with slow regression thereafter	Kidney	0	0	0	0	0	0	++++	+++	++	+	0	0
		Liver	0	0	0	0	0	0	++	+	±	0	0	0
		Spleen	0	0	0	0	0	0	+++±	+++±	++	+	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	++++	++++	+++±	++	0	0
4-17 (Gray-brown agouti hybrid)	Six tumors up to 3.5 cm. on the 11th day; all had enlarged up to 7.0 cm. on the 24th day; about the same on the 45th day; killed for material on the 50th day, with many omental, glandular, and visceral metastases	Kidney	0	0	0	0	0	0	++++	++++	+++	+	0	0
		Liver	0	0	0	0	0	0	++	+	0	0	0	0
		Spleen	0	0	0	0	0	0	++++	++++	+++±	++	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	++++	++	±	0	0	0
4-18 (Gray-brown agouti hybrid)	Four tumors up to 2.0 cm. on the 11th day; two softish nodules, 1.0 cm., on the 24th day; negative on the 45th day	Kidney	0	0	0	0	0	0	0	0	0	0	0	0
		Liver	0	0	0	0	0	0	0	0	0	0	0	0
		Spleen	0	0	0	0	0	0	0	0	0	0	0	0
		Brown-Pearce carcinoma	0	0	0	0	0	0	0	0	0	0	0	0

Sera heated at 65° C. for 30 minutes immediately prior to use.
Antigens, 1:30 saline extracts of frozen rabbit tissues as indicated.

TABLE V

Tests for Induced Tissue Antibodies in the Blood Serum of Rabbits Implanted with Sarcoma I of Andrewes and Ahlström

Source of serum — Rabbit No.	Outcome of intramuscular implantations	Day bled	Antigens	Complement fixation tests — Serum dilutions 1:2	1:4	1:8	1:16	1:32
28	Four tumors up to 3.0 cm. on the 8th day; all slightly larger on the 15th day; dwindled to 2.5 cm. and less on the 25th day; negative on the 46th day and thereafter	49th	Liver	0	0	0	0	0
			Kidney	0	0	0	0	0
			RSI	0	0	0	0	0
			Kato sarcoma	0	0	0	0	0
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	0	0	0	0	0
42	Three tumors up to 1.2 cm. on the 11th day; all enlarged to 5.0 cm. on the 66th day; moribund, with three huge tumors and regional metastases, when bled on the 154th day	154th	As above	All negative				
43	Three tumors up to 1.4 cm. on the 9th day; slightly larger (up to 2.0 cm.) on the 17th day; negative on the 25th day and thereafter	49th	" "	" "				
2-93	Two tumors, 3.0 cm., on the 11th day; both 8.0 cm. on the 22nd day; moribund, with tumors 12.0 cm. across and iliac metastases, 7.0 cm., when bled on the 52nd day	52nd	Liver	++++	+++	±	0	0
			Kidney	++++	++++	++++	+++	+
			RSI	++++	++++	++++	+++	±
			Kato sarcoma	++++	++++	+++	++	±
			V2 carcinoma	++++	++++	++++	++	0
			Brown-Pearce carcinoma	++++	++++	++++	+++	0
3-45	Two tumors, 8.0 and 10.0 cm., on the 15th day; moribund, with tumors 11.0 and 12.0 cm. and metastases in retroperitoneal glands, when bled on the 29th day	29th	Liver	+++	++	0	0	0
			Kidney	++++	+++	+	0	0
			RSI	++++	+++±	++±	+	0
			Kato sarcoma	++++	+++	++	++	0
			V2 carcinoma	++++	++±	+	0	0
			Brown-Pearce carcinoma	++++	++++	++±	0	0
3-50	Two tumors, 3.5 and 1.5 cm. on the 11th day; moribund, with cystic tumors 14.0 and 11.0 cm. across, when bled on the 61st day	61st	Liver	+++	++	0	0	0
			Kidney	++++	+++	+	0	0
			RSI	++++	+++±	++±	+	0
			Kato sarcoma	++++	+++	++	0	0
			V2 carcinoma	++++	++±	+	0	0
			Brown-Pearce carcinoma	++++	++++	+++	0	0
8-13	One tumor enlarging progressively to 8.0 cm. when killed for material on the 45th day	45th	Liver	++++	++++	+++	0	0
			Kidney	++++	++++	++++	+++±	++
			RSI	++++	++++	+++	++	0
			Kato sarcoma	++	++	++	+±	0
			V2 carcinoma	+±	0	0	0	0
			Brown-Pearce carcinoma	++++	++++	++±	+±	0

TABLE V—*Concluded*

Source of serum / Rabbit No.	Outcome of intramuscular implantations	Day bled	Antigens	Complement fixation tests, Serum dilutions 1:2	1:4	1:8	1:16	1:32
15-95	Two tumors enlarging progressively to 12.0 cm. when bled on the 55th day	55th	Liver	++++	++++	+++	0	0
			Kidney	++++	++++	++++	++++	++
			RSI	++++	++++	++++	+++	+±
			Kato sarcoma	++++	++++	++++	+++	++
			V2 carcinoma	++++	++++	+++	+±	0
			Brown-Pearce carcinoma	++++	++++	++++	+++±	+±
16-00	Two tumors enlarging progressively to 8.0 and 14.0 cm. on the 55th day, moribund, metastases 3.5 cm. in regional glands	55th	Liver	0	0	0	0	0
			Kidney	++++	++++	+±	0	0
			RSI	+	0	0	0	0
			Kato sarcoma	0	0	0	0	0
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	++++	+++	±	0	0
16-01	Two tumors slowly enlarging to 4.0 cm. when bled on the 55th day	55th	Liver	0	0	0	0	0
			Kidney	0	++±	0	0	0
			RSI	0	+	±	0	0
			Kato sarcoma	0	++±	+++	++	±
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	0	++	+±	0	0

All sera heated at 65° C. for 30 minutes immediately prior to use.
The sera of three normal rabbits, tested concurrently, were negative with all antigens.
All rabbits were gray-brown agouti hybrids.
Antigens, 1:40 saline extracts of frozen rabbit tissues as indicated.

prior to implantation (Table IV), and from that of normal control rabbits, as previous experiments had shown (2).

Tests for Induced Tissue Antibodies in the Serum of Rabbits Carrying Autochthonous Papillomas and Cancers and in That of Animals Recovered from Vaccinia and Fibromatosis

In the preceding experiments tests were made with sera got from domestic rabbits carrying various transplanted cancers. Table VII summarizes the results of tests with the sera of domestic and cottontail rabbits having autochthonous growths. It will be seen that none of the specimens procured from ten domestic rabbits carrying virus papillomas and squamous cell cancers deriving from them possessed the ability to react with saline extracts of rabbit kidney, spleen, V2 carcinoma, and Brown-Pearce carcinoma. The results were likewise negative with the sera of three wild cottontails having growths of similar sort, and with specimens procured from two domestic rabbits carrying papillomas and cancers induced with methylcholanthrene. By contrast, sera from rabbits 5-22 and 11-54, which had been implanted with Brown-Pearce and V2 carcino-

mas, respectively, both fixed complement in mixture with the normal and neoplastic tissue antigens.

TABLE VI

Tests for Induced Tissue Antibodies in the Blood Serum of Rabbits Implanted with the Kato Sarcoma

Source of serum — Rabbit No.	Outcome of intramuscular implantations	Day bled	Antigens	Complement fixation tests — Serum dilutions 1:2	1:4	1:8	1:16	1:32
J-1	Two tumors enlarging progressively to 8.0 and 12.0 cm. in diameter, moribund, with innumerable metastases up to 2.0 mm. in lungs, liver, and kidneys, when bled on the 56th day	56th	Liver	0	0	0	0	0
			Kidney	0	0	0	0	0
			RSI	0	0	0	0	0
			Kato sarcoma	0	0	0	0	0
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	0	0	0	0	0
2-73	One tumor enlarging progressively to 12.0 cm. when bled on the 90th day	90th	Liver	++++	++	±	0	0
			Kidney	++++	++++	++++	++++	++++
			RSI	++++	++++	++++	++++	++++
			Kato sarcoma	++	+++	+++±	++++	+++±
			V2 carcinoma	+±	+++	+++±	+++±	+±
			Brown-Pearce carcinoma	++++	++++	++++	++++	++++
2-95	One tumor enlarging progressively to 8.0 cm. when bled and killed for material on the 48th day	48th	As above	All negative				
3-08	One tumor enlarging progressively to 3.5 cm. when bled and killed on the 30th day because of intercurrent pox	30th	" "	" "				
3-29	One tumor enlarging progressively to 6.0 cm. when bled on the 30th day; died on the 47th day with miliary metastases	30th	" "	" "				
3-22	One tumor enlarging progressively to 8.0 cm. when bled and killed for material on the 68th day; no metastases	68th	Liver	++++	++++	++	0	0
			Kidney	++++	++++	+++	±	0
			RSI	++++	±	0	0	0
			Kato sarcoma	++++	+	±	0	0
			V2 carcinoma	+±	0	0	0	0
			Brown-Pearce	++++	+±	0	0	0

The experiment was done concurrently with that of Table V and in the same way.

In other experiments similar tests were made with sera from rabbits recently recovered from virus-induced fibromatosis (ten animals, all of which had received multiple inoculations of fibroma virus, with result in many large lesions in each case, which were regressing or had disappeared when the animals were bled 3 to 6 weeks after the inoculations). None of these sera manifested any

ability to react with normal and neoplastic rabbit tissue antigens (kidney, spleen, Brown-Pearce carcinoma, V2 carcinoma) when tested as in the experiment of Table VII, though specimens from five rabbits with transplanted V2 carcinomas, included for comparison, all did so. Sera from five rabbits recently recovered from multiple vaccinial lesions were likewise negative in mixture with the antigens named. A possible inference to be drawn from the findings will be mentioned further on.

Sedimentability of the Cell Constituents with Which the Induced Antibodies React

An experiment was next made to learn whether the induced tissue antibodies will react with the constituents of normal and neoplastic cells that prove readily sedimentable in the high speed centrifuge.

Sera from rabbits carrying large transplanted growths of the four types under study were tested as usual in mixture with antigens made from various normal and neoplastic rabbit tissues and from those of the chick embryo as well. For comparison, serum specimens were included from a normal rabbit known to have a high titer of the natural tissue antibody. The antigens were made by extracting frozen tissues in 0.9 per cent saline as usual, clearing the extracts by centrifugation at 4,400 R.P.M. for 10 minutes, and then spinning them at 25,000 R.P.M. for 1 hour, with removal of the supernatant liquids for use as such, and careful resuspension of the sedimented particles in 0.9 per cent saline according to a method previously described (1).

The results are set down in Table VIII. It may be seen that the sera from the three V2 carcinoma rabbits reacted with the sedimented fractions of all of the tissue antigens (normal rabbit liver, kidney, spleen, V2 and Brown-Pearce carcinomas, Sarcoma I, chick embryo), whereas they failed to react, or did so in only slight degree, with any of the corresponding supernatant liquids. The sera from the Brown-Pearce, RS, and Kato animals also reacted with the sedimented fractions of several or all of the various antigens, and in general they failed to react with the supernatant liquids, though the Kato specimens proved exceptional in giving moderately strong reactions with certain of the supernatant liquids, notably those of the spleen extract and of the two carcinomas. As in previous work (2), the serum of a normal rabbit, heated at 56° C. for 30 minutes to inactivate complement but not the natural tissue antibody, reacted in varying degrees with the sedimented materials from all of the tissues though it failed to react with the overlying liquids. The same normal serum, however, heated at 65° C. for 30 minutes, failed to react with any of the test antigens.

In sum, the findings show that the induced tissue antibodies present in the sera of rabbits carrying transplanted cancers of various sorts, reacted quite well in general with those constituents of various normal and neoplastic tissues that prove readily sedimentable in the high speed centrifuge, though they failed as a rule to react with the "soluble" substances present in the supernatant liquids.

SUMMARY AND COMMENT

Antibodies were found in the blood of certain rabbits carrying one or another of four transplanted cancers (Brown-Pearce and V2 carcinomas; RSI and Kato sarcomas) which will fix complement *in vitro* in mixture with saline extracts of

TABLE VII

Tests for Induced Antibodies in the Blood Serum of Rabbits with Autochthonous Papillomas and Cancers

Source of serum	Rabbit No.	Character and duration of growths	Antigens	Complement fixation tests, Serum dilutions 1:2	1:4	1:8	1:16	1:32
Domestic rabbits with virus papillomas and derivative cancers	10-98	Confluent papillomas on both sides, becoming cancerous during 12th mo. and metastasizing to lymph node. 6 autoplasts of cancer, 12th mo. These had enlarged to 4.0 cm. at the bleeding in the 16th mo.	Kidney	0	0	0	0	0
			Spleen	0	0	0	0	0
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	0	0	0	0	0
	11-04	Papillomas 7 × 5 cm. on both sides, becoming cancerous during 13th mo. Two huge foul cancers when bled, 25th mo.	As above	All negative				
	11-81	Papillomas 3 × 4 cm. on both sides; 16th mo. growth on right cancerous. Bled 17th mo.	" "	" "				
	12-10	Papillomas 7 × 3 cm. on both sides. Cancers originating in growths of both sides, 10th mo., which steadily enlarged till the bleeding, in 16th mo. Autoplasts of cancer up to 3 × 2 cm., 10th to 16th mos. Animal moribund then. Many metastases at death	" "	" "				
	14-07	Papillomas 8 × 5 cm. on both sides, which had become wholly cancerous at the bleeding in the 7th mo.	" "	" "				
	14-09	Papillomas 6 × 4 cm. on both sides, cancerous during 9th mo. Bled, 10th mo.	" "	" "				
	16-89	Confluent papillomas on both sides, cancerous during 7th mo. Bled, 10th mo.	" "	" "				
	16-98	Confluent papillomas on both sides, cancerous during 6th mo. Bled, 10th mo.	" "	" "				

TABLE VII—*Concluded*

Source of serum	Rabbit No.	Character and duration of growths	Antigens	Complement fixation tests, Serum dilutions 1:2	1:4	1:8	1:16	1:32
As above	16-99		As above	All negative				
	17-00	Large papillomas on both sides, cancerous with metastasis in regional gland, 7th mo. Six cancerous autoplasts up to 2.0 cm. (3 wks.' duration) when bled during 8th mo.	" "	" "				
	17-01	Huge foul cancers on both sides and large precancerous papillomas. Bled, 10th mo.	" "	" "				
Wild cottontail rabbits with virus papillomas and derivative cancers	4-11	Small benign papillomas and one discoid cancer 2.8 cm. across at bleeding. Duration not recorded	" "	" "				
	5-03	Several benign papillomas and four cancers, up to 4.0 cm. across, duration not recorded. Autoplasts up to 2.5 cm., 6 wks.' duration, when bled	" "	" "				
	5-13		" "	" "				
Domestic rabbits with autochthonous papillomas and cancers produced with methylcholanthrene	10-59		" "	" "				
	15-29	Several onion-like papillomas and a cancer which had eaten away about 2 cm. of the tip of the ear. Duration more than 2 mos.	" "	" "				
Domestic rabbit with transplanted Brown-Pearce carcinomas	5-22	Six progressively enlarging muscle growths up to 6.0 cm.; moribund when bled on the 44th day; many metastases	Kidney	++++	++++	++++	++++	+++
			Spleen	++++	+++	++±	±	0
			V2 carcinoma	0	0	0	0	0
			Brown-Pearce carcinoma	++++	++++	++++	++++	+
Domestic rabbit with transplanted V2 carcinomas	11-54	Four progressively enlarging intramuscular growths up to 6.0 cm. when bled on the 40th day	Kidney	++++	++++	++++	+++	0
			Spleen	++++	++++	++++	++	0
			V2 carcinoma	++++	++++	++++	++++	±
			Brown-Pearce carcinoma	+++±	+++±	+++	±	0

Antigens, 1:40 saline extracts of frozen rabbit tissues as indicated.

TABLE VIII

Sedimentability of the Cell Constituents with Which the Induced Antibodies React

Antigens			Source of sera										
			V2 carcinoma rabbits			Brown-Pearce carcinoma rabbits		Sarcoma I rabbits		Kato sarcoma rabbits		Normal rabbits	
Tissue	Fraction tested after high speed centrifugation	Dilution	5-71	16-17	16-19	16-67	16-70	2-89	2-93	2-73	14-97	20-45	20-45*
Normal rabbit liver	Supernatant	1:10	0	0	+	0	0	0	0	++	+++	0	0
		1:20	0	0	0	0	0	0	0	0	++	0	0
	Sediment	1:10	++++	++++	++++	++++	+++	++++	+++±	+++±	++++	++++	0
		1:20	++++	++++	++++	++++	0	+++	+++±	+++±	+++	++++	0
		1:40	++++	++++	++++	+++	0	0	+++	+++±	±	++++	0
		1:80	++++	+++	++++	±	0	0	+++	+++±	0	++++	0
Normal rabbit kidney	Supernatant	1:10	0	0	0	0	0	0	0	0	+++	0	0
		1:20	0	0	0	0	0	0	0	0	0	0	0
	Sediment	1:10	++++	++++	++++	++++	++	++++	++++	++++	+++	++++	0
		1:20	++++	++++	++++	++++	±	++++	++++	++++	++	++++	0
		1:40	++++	++++	+++±	0	0	+++	++++	++++	0	++++	0
		1:80	++++	±	±	0	0	0	+++	+++	0	++++	0
Normal rabbit spleen	Supernatant	1:10	0	0	0	0	0	0	0	++±	++±	0	0
		1:20	0	0	0	0	0	0	0	+±	0	0	0
	Sediment	1:10	++++	++++	+++	+	0	+++	++±	0	0	+	0
		1:20	++++	++	+±	0	0	+	+±	0	0	0	0
		1:40	++++	0	0	0	0	0	0	0	0	0	0
		1:80	+++	0	0	0	0	0	0	0	0	0	0
V2 carcinoma	Supernatant	1:10	0	0	+++	0	0	0	0	++++	+++±	0	0
		1:20	0	0	++	0	0	0	0	++++	++	0	0
	Sediment	1:10	++++	++++	++++	0	0	+++	+++±	++++	0	++++	0
		1:20	++++	++++	++++	0	0	+	++	±	0	++±	0
		1:40	+++±	++++	++++	0	0	0	0	0	0	+±	0
		1:80	+	+	++++	0	0	0	0	0	0	0	0
Brown-Pearce carcinoma	Supernatant	1:10	0	0	0	0	0	0	0	++++	++±	0	0
		1:20	0	0	0	0	0	0	0	+++	+±	0	0
	Sediment	1:10	++++	++++	++++	+++	++++	++++±	++++	++++	+	+++	0
		1:20	+++±	+++±	+++	0	+	++±	+++±	++++	0	++	0
		1:40	++	0	+±	0	0	0	++±	+	0	+	0
		1:80	0	0	0	0	0	0	0	0	0	0	0
Sarcoma I	Supernatant	1:10	0	0	0	0	0	0	0	0	+	0	0
		1:20	0	0	0	0	0	0	0	0	0	0	0
	Sediment	1:10	++	+++	+++	0	0	+++	++++	0	0	+	0
		1:20	0	0	+±	0	0	+±	++	0	0	0	0
		1:40	0	0	0	0	0	0	±	0	0	0	0
		1:80	0	0	0	0	0	0	0	0	0	0	0
Chick embryo (8 days)	Supernatant	1:10	0	0	0	0	0	0	0	0	0	0	0
		1:20	0	0	0	0	0	0	0	0	0	0	0
	Sediment	1:10	0	±	++++	0	0	+++	++++	0	+±	++++	0
		1:20	0	0	+++	0	0	+++	++++	0	0	+++	0
		1:40	0	0	0	0	0	++	+++±	0	0	++	0
		1:80	0	0	0	0	0	+	+++	0	0	0	0

All sera diluted 1:4. All heated at 65° C. for 30 minutes, with one exception as noted. See text for details of preparation and centrifugation of antigens.

* Heated at 56° C. for 30 minutes instead of 65° C.

various normal and neoplastic rabbit tissues—including liver, kidney, spleen, and the four tumors mentioned—and chick embryo tissue as well. These antibodies, which have been called induced tissue antibodies, are similar to the natural antibodies previously described (2) in that they react with those constituents of the various tissue cells that prove readily sedimentable in the high speed centrifuge; they differ from the natural antibodies in being absent from the blood of normal rabbits and in withstanding 65° C. for 30 minutes.

Certain quantitative differences suggest that the induced tissue antibodies have somewhat various affinities, depending in part upon the type of neoplasm carried by the host. They may perhaps be consequent on antigenic differences between the sedimentable constituents of the tumor cells and those of the new hosts; for they were not found in the blood of rabbits carrying papillomas and cancers composed of the animals' own cells, and not in that of rabbits in which multiple vaccinia or fibroma virus lesions had recently regressed.

The characters of the sedimentable constituents of normal and neoplastic tissue cells, as revealed thus far by chemical, morphological, and serological studies, have recently been discussed (2, 8). In this relation, it has seemed essential to recognize the induced antibodies here described, particularly since they may complicate serological studies aimed at disclosing distinctive sedimentable substances in tissue cells. In an associated paper experiments are reported which bear upon the relation between the induced tissue antibodies and an antibody that reacts specifically with a distinctive sedimentable constituent of Brown-Pearce carcinoma cells (7).

BIBLIOGRAPHY

1. Kidd, J. G., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38,** 292; *J. Exp. Med.*, 1940, **71,** 335, 351; *J. Bact.*, 1940, **39,** 349.
2. Kidd, J. G., and Friedewald, W. F., *J. Exp. Med.*, 1942, **76,** 543, 557.
3. Kidd, J. G., *J. Exp. Med.*, 1938, **68,** 703, 725, 737.
4. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37,** 601.
5. Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71,** 813.
6. Andrewes, C. H., and Ahlström, C. G., *J. Path. and Bact.*, 1938, **47,** 87.
7. MacKenzie, I., and Kidd, J. G., *J. Exp. Med.*, 1945, **82,** 41.
8. Claude, A., in Frontiers in cytochemistry, (N. L. Hoerr, editor). Biological symposia, Vol. **10,** Lancaster, The Jaques Cattell Press, 1943, 111.