INCIDENCE AND SPECIFICITY OF THE ANTIBODY FOR A DISTINCTIVE CONSTITUENT OF THE BROWN-PEARCE TUMOR

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Attempts to demonstrate specific substances in tumor cells by serological means have often yielded complex and ambiguous results, especially when materials derived from normal and neoplastic tissues have been injected into animals of alien species. For under such circumstances the injected materials have proved antigenically complex, as a rule, and the antibodies stimulated by them have reflected the differences in species, to such an extent usually as to mask any intrinsic difference present (1). A study of the Brown-Pearce rabbit carcinoma has shown, however, that this tumor regularly yields a distinctive substance which has not been detectable in extracts of normal rabbit tissues or in those of other rabbit cancers and which can be identified through its reactions *in vitro* with an antibody that appears in the blood of certain rabbits implanted with the tumor; species differences can have played no part in the findings, for the materials employed all came from domestic rabbits (2). More recent investigations have shown that various normal and neoplastic rabbit tissues, including the Brown-Pearce carcinoma, will yield other substances which will react *in vitro* with certain natural and induced antibodies of rabbits' blood and which, like the distinctive constituent of the Brown-Pearce tumor, can be readily thrown down in the high speed centrifuge (3). With a view to learning whether a relation exists between the distinctive constituent of the Brown-Pearce carcinoma cell and the sedimentable substances present in other rabbit tissue cells both normal and neoplastic, the incidence and specificity of the Brown-Pearce antibody have been scrutinized in the present work, and parallel observations have been made with the natural and induced antibodies previously described (3).

Materials and Methods

The materials and methods were essentially those previously employed (2, 3), hence they will be described only briefly. A standardized complement fixation reaction has been utilized to study the distinctive constituent of the Brown-Pearce carcinoma and the antibody that reacts specifically with it. Frozen tissues were utilized as a source of antigens, and the extracts were made fresh for each experiment: they were used unheated, since submission to 56° C. for 30 minutes to reduce "non-specific" fixation, as employed earlier (2), has proved unnecessary. The sera were heated at 65° C. for 30 minutes immediately prior to use, except

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in a few designated instances, in order to inactivate the natural antibody which is present in the serum of nearly all adult rabbits and which reacts with a sedimentable constituent of normal rabbit tissues (3). Such heating has no effect on the induced antibodies in rabbit blood, and in particular none on the Brown-Pearce tumor antibody (3).

Much use has been made of a special breed of rabbits, which provides the specific Brown-Pearce antibody in high titer (2),—"blue-cross" hybrids (Rockefeller Institute strain), propagated by means of fertile hybrids derived originally from crossing rabbits of English and Lilac breeds.

Incidence and Titer of the Specific Antibody for the Distinctive Substance of the Brown-Pearce Tumor

In previous experiments (2), tests had been made for the presence of the specific Brown-Pearce antibody in the serum of 167 rabbits, including sixty-five implanted with the tumor. The specimens from fifty-two of these latter fixed complement in greater or less degree in mixture with extracts of the Brown-Pearce tumor (2); whereas control sera never did so, though specimens from 102 rabbits were tested, including some from animals carrying other types of transplanted cancers, many with virus-induced papillomas, a few with experimental syphilis, several with necrotizing virus infections, and many normal animals. In subsequent work along the same lines, Cheever made use of the sera of sixteen normal rabbits in forty tests, getting no specific fixation in mixtures with antigens derived from the Brown-Pearce tumor (4). He also tested the sera of thirty-five rabbits that carried Brown-Pearce tumors in varying stages of enlargement or regression and found that the specimens from five of them showed some degree of fixation in mixture with antigens made from the Brown-Pearce tumor. Jacobs and Houghton, in a few experiments of similar sort, got indeterminate results (5), sometimes obtaining fixation in mixtures of normal rabbit sera and extracts of the Brown-Pearce tumor, though usually getting none in similar mixtures containing the sera of rabbits implanted with the Brown-Pearce tumor. It is noteworthy that neither Cheever nor Jacobs and Houghton made multiple intramuscular and intratesticular implantations of the Brown-Pearce tumor as had been done in the original work (with the aim to elicit antibodies in high titer by having a great mass of tumor tissue in the host) (2), and they did not make use of the special breed of "blue-cross" rabbits which had been found to provide the specific Brown-Pearce antibody in high titer.

To study further the incidence and titer of the antibody for the distinctive substance of the Brown-Pearce tumor, and to learn more about its specificity, three groups of rabbits of various breeds were implanted at different times with the Brown-Pearce tumor, by injecting a heavy suspension of it, prepared as previously described (2), into six leg muscle situations in each animal and into both testes as well in the males. The outcome of the implantations was recorded and the rabbits were bled for serum at various intervals. Later the sera, heated at 65° to inactivate the natural tissue antibody, were tested for capacity to fix complement in mixture with saline extracts of normal rabbit tissues and of the Brown-Pearce tumor. Table I provides a summary of the results of the serum tests in correlation with the outcome of the tumor implantations in the various animals. The course of the tumors in relation to the development of the antibodies will be discussed in a later section; meanwhile the results of the serum tests will be analyzed in detail.

There were great differences in the capacity of the various sera to react with the two antigens (normal rabbit kidney and Brown-Pearce tumor) in the complement fixation tests, as Table I shows. The majority of the sera exhibited no such ability, or only very little. Several specimens, some from each group and breed, gave considerable fixation in mixture with both antigens (*e.g.*, 4-23 and 4-27 from group I; 5-16, 5-24, and 5-25 from group II; and 5-45, 5-53, and 6-66 from group III); while the specimens from four animals (4-19 of group I and 5-47, 5-51, and 5-53 of group III) all reacted in high titer (1:32 or better) with the Brown-Pearce tumor antigen but not at all with the normal rabbit kidney antigen.

When the findings of Table I are considered in the light of those reported in an associated paper (3) they are seen to be consistent with the view that the sera that reacted with the normal kidney antigen must all have contained the induced, heat-resistant antibody, which, like the natural heat-labile antibody previously described, has an affinity for a sedimentable constituent of normal and neoplastic tissues. Some of the sera—notably the ones that reacted in higher titer with the Brown-Pearce tumor antigen than with the normal kidney antigen (*e.g.*, specimens 4-27 of group I, 5-16 and 5-24 of group II, and 5-53 of group III)—may well have contained the specific Brown-Pearce tumor antibody in addition; for, whereas the latter has a strict affinity for a constituent of the Brown-Pearce tumor, the natural and induced tissue antibodies are known to react as well or better with antigens made from normal kidney and liver tissues than with those derived from neoplasms (3). The sera that reacted in high titer with the Brown-Pearce tumor antigen and not at all with the normal kidney antigen (4-19, 5-47, 5-51, and 5-53) apparently contained only the antibody that is specific for the distinctive constituent of the Brown-Pearce tumor, as further observations will make clear.

Tests of sera from a single bleeding of the implanted rabbits are recorded in Table I. In order to follow more closely the development of antibodies in relation to the course of the tumors, several bleedings were made of the rabbits of group II at intervals after the tumor implantations. Table II gives a detailed analysis of the course of events in these animals.

None of the sera drawn immediately prior to the implantations (1st day) had any capacity to react with either of the test antigens; and the sera of several rabbits proved completely devoid of this capacity, or almost so, at all of the several bleedings (5-48, 5-49, 5-50, 5-54, 5-70). The rest of the rabbits, however, provided sera with striking abilities to fix complement in mixture with one or another or both of the antigens. This was especially noteworthy in the case of rabbits 5-47, 5-51, and 5-53, for they yielded sera at all of the bleedings after the implantations which regularly fixed complement in mixture with the Brown-Pearce tumor antigen, often in high titer, though not reacting at all with the antigen made from normal rabbit kidney. Rabbit 5-52 provided a similar case, except that the specimens procured on the 18th, 28th, and 46th days reacted also in mixture with the normal kidney antigen, though the reactions with this antigen were slight as compared with those of the same specimens in mixture with the Brown-Pearce tumor antigen. Rabbits 5-45, 5-52, 6-66, 6-67, 6-68, 6-69, and 6-70 all yielded at one time or another sera that reacted with the normal tissue antigen as well as with the Brown-Pearce tumor antigen.

TABLE I

Complement Fixation Tests with Sera of Rabbits Implanted with the Brown-Pearce Tumor

Source of sera			Outcome of implantations with Brown-Pearce tumor (6 to 8 situations)	Complement fixation tests								
				Normal rabbit kidney antigen, 1:40				Brown-Pearce tumor antigen, 1:40				
				Serum dilution				Serum dilution				
Implantation group	Rabbit No.	Breed		1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	1:32
I	4-13	Chinchilla	8 tumors up to 6.0 cm., enlarged progressively	0	0	0	0	0	0	0	0	0
	4-14F*	"	6 " " " 6.0 " " "	0	0	0	0	0	0	0	0	0
	4-15	"	8 " " " 8.0 " " "	0	0	0	0	0	0	0	0	0
	4-19	Blue-cross	4 " " " 2.5 " , regressed abruptly	0	0	0	0	++++	++++	++++	++++	++++
	4-20	"	6 " " " 3.5 " " "	0	0	0	0	0	0	0	0	0
	4-21F	"	3 " " " 2.5 " " "	0	0	0	0	0	0	0	0	0
	4-22	Agouti	8 " " " 10.0 " , enlarged progressively	0	0	0	0	0	0	0	0	0
	4-23	"	5 " " " 8.0 " " "	+++±	++	0	0	++	+	0	0	0
	4-24	"	8 " " " 8.0 " " "	0	0	0	0	0	0	0	0	0
	4-25	"	5 " " " 3.0 " , regressed abruptly	0	0	0	0	0	0	0	0	0
	4-26	"	8 " " " 8.0 " , enlarged progressively	++±	±	0	0	±	0	0	0	0
	4-27	"	8 " " " 8.0 " " "	++++	++±	+	±	++++	++++	+++	+±	0
II	5-11	Chinchilla	7 tumors up to 1.5 cm., regressed abruptly	0	0	0	0	±	0	0	0	0
	5-12	"	8 " " " 7.0 " , enlarged progressively	0	0	0	0	0	0	0	0	0
	5-13	"	8 " " " 4.5 " , regressed abruptly	++++	±	0	0	0	0	0	0	0
	5-14	"	8 " " " 6.0 " , enlarged progressively	++++	++	0	0	+	0	0	0	0
	5-15	"	7 " " " 6.0 " " "	0	0	0	0	0	0	0	0	0
	5-16	"	5 " " " 2.0 " , regressed abruptly	+++±	+++	0	0	+++±	+++±	++±	0	0
	5-17	Blue-cross	6 " " " 4.0 " " "	0	0	0	0	0	0	0	0	0
	5-18F	"	4 " " " 1.0 " " "	0	0	0	0	0	0	0	0	0
	5-19F	"	Negative	0	0	0	0	0	0	0	0	0
	5-20	"	5 tumors up to 3.0 " " "	0	0	0	0	0	0	0	0	0
	5-21	Agouti	7 " " " 6.0 " , enlarged progressively	++++	++++	++++	+±	+++	++±	±	0	0
	5-22	"	8 " " " 8.0 " " "	++++	+++	0	0	++	+	0	0	0
	5-23	"	5 " " " 6.0 " " "	0	0	0	0	0	0	0	0	0
	5-24	"	6 " " " 7.0 " " "	+++±	++±	++±	+	++++	++++	+++±	+++	+±
	5-25	"	6 " " " 7.0 " " "	+++	++±	+±	±	+++±	+++	++±	±	0
	5-26	"	8 " " " 8.0 " " "	0	0	0	0	0	0	0	0	0
III	5-45	Blue-cross	8 tumors up to 6.0 cm., regressed abruptly	++++	++++	+++	±	++++	++++	++++	++++	+++
	5-47	"	4 " " " 4.0 " " "	0	0	0	0	++++	++++	++++	++++	++++
	5-48F	"	Negative	0	0	0	0	0	0	0	0	0
	5-49F	"	4 tumors up to 3.0 " " "	0	0	0	0	0	0	0	0	0
	5-50	"	4 " " " 4.0 " " "	0	0	0	0	0	0	0	0	0

5-51F	"	5	"	"	"	4.0 "	"	"	0	0	0	0	++++	++++	++++	++++	++++
5-52F	"	5	"	"	"	5.0 "	"	"	++++	++++	++±	0	++++	++++	++++	++++	++++
5-53	"	6	"	"	"	3.0 "	"	"	0	0	0	0	++++	++++	++++	++++	++++
5-54	"	Negative							0	0	0	0	0	0	0	0	0
5-55	"	8 tumors up to				6.0 "	, enlarged progressively		0	0	0	0	0	0	0	0	0
6-66	Agouti	8	"	"	"	6.0 "	"	"	++++	++++	++±	+±	++++	++++	++++	++++	++++
6-67	"	8	"	"	"	5.0 "	"	"	++++	++++	+++±	+±	++++	++++	+++	±	0
6-68	"	8	"	"	"	4.0 "	, regressed slowly		++	±	0	0	++++	+++	+±	0	0
6-69	"	8	"	"	"	6.0 "	, enlarged progressively		+±	0	0	0	0	0	0	0	0
6-70	"	8	"	"	"	4.0 "	"	"	++±	±	0	0	++±	±	0	0	0

The animals of group I were bled on the 32nd day following the implantations, those of group II on the 35th day, and those of group III on the 28th day.

All of the serum specimens had been heated at 65°C. for 30 minutes immediately prior to use in the tests, in order to inactivate the natural tissue antibody (2). Except where noted otherwise, the same holds true in the experiments that follow.

The chinchillas and agouti hybrids were market-bought, while the blue-cross rabbits were fertile F1 hybrids (English × Lilac) propagated in the breeding rooms of The Rockefeller Institute.

None of the sera were anticomplementary in concurrent tests, nor were the antigens. This also held true in the experiments that follow.

++++ = complete fixation; 0 = no fixation (complete hemolysis).

* F = female; all the rest were males.

TABLE II

Complement Fixation Tests with Rabbit Sera Procured at Intervals After Implantation of the Brown-Pearce Tumor

Rabbit No.	Outcome of implantations with Brown-Pearce tumor (6 to 8 situations)	Bled	Complement fixation tests											
			Normal rabbit kidney antigen 2-73, 1:40						Browne-Pearce tumor antigen 6-73, 1:40					
			Serum dilution						Serum dilution					
			1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4	1:8	1:16	1:32	1:64
		day												
5-45	Seven tumors, 4.0-4.0-5.5-6.0-3.0-N-5.0-6.0 cm., on 10th day; about same on 18th day; all dwindled abruptly to ?-1.5-?-1.0-N-N-5.0-4.0 cm., softish, on 28th day; negative on 46th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	+	0	0	0	0	0	++++	++++	+++±	±	0	0
		28th	++++	++++	+++±	+	0	0	++++	++++	++++	++++	+++±	±
		46th	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
		67th	++++	++++	++++	++++	+++±	±	++++	++++	++++	++++	++++	+++±
5-47	Four tumors, N-1.0-3.0-4.0-N-N-N-1.5 cm., on 10th day; negative on 18th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
		28th	0	0	0	0	0	0	++++	++++	++++	++++	++++	+++±
		46th	0	0	0	0	0	0	++++	++++	++++	++++	++++	+++
		67th	0	0	0	0	0	0	++++	++++	++++	+++±	0	0
5-48F	Two tumors, 1.5-2.0-N-N-N-N cm., on 10th day; negative on 18th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	0	0	0	0	0	0	++	0	0	0	0	0
		46th	0	0	0	0	0	0	0	0	0	0	0	0
		67th	0	0	0	0	0	0	0	0	0	0	0	0
5-49F	Four tumors, 2.0-3.0-2.0-2.0-N-N cm., on 10th day; negative on 18th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	0	0	0	0	0	0	0	0	0	0	0	0
		46th	0	0	0	0	0	0	0	0	0	0	0	0
		67th	0	0	0	0	0	0	0	0	0	0	0	0
5-50	Four tumors, 1.5-3.0-3.0-4.0-N-N-N-N cm., on 10th day; two tumors, N-1.5-N-1.0-N-N-N-N cm., on 18th day; negative on 28th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	0	0	0	0	0	0	0	0	0	0	0	0
		46th	0	0	0	0	0	0	0	0	0	0	0	0
		67th	0	0	0	0	0	0	0	0	0	0	0	0

5-51F	Five tumors, 4.0-4.0-4.0-4.0-4.0-N cm., on 10th day; negative on 18th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	++++	++++	++++	++++	++++	+++±
		28th	0	0	0	0	0	0	++++	++++	++++	++++	++++	+++
		46th	0	0	0	0	0	0	++++	++++	++++	++++	++±	0
		67th	0	0	0	0	0	0	++++	++++	++++	±	0	0
5-52F	Four tumors, 3.0-4.0-4.0-5.0-N-N cm. on 10th day; five nodules, 3.0-4.0-2.0-2.0-1.0-N cm., softish, on 18th day; negative on 28th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	++++	++	0	0	0	0	++++	++++	++++	++++	++++	++++
		28th	++++	++++	++±	0	0	0	++++	++++	++++	++++	++++	++++
		46th	±	0	0	0	0	0	++++	++++	++++	++++	++++	++++
		67th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
5-53	Six tumors, 1.0-2.0-3.0-3.0-N-N-0.2-1.3 cm., on 10th day; negative on 18th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
		28th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
		46th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
		67th	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++
5-54	Negative at all situations on 10th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	0	0	0	0	0	0	0	0	0	0	0	0
		46th	0	0	0	0	0	0	0	0	0	0	0	0
		67th	0	0	0	0	0	0	0	0	0	0	0	0
5-55	Seven tumors, 3.0-4.0-6.0-5.0-4.0-?-5.5-5.0 cm., on 10th day; all enlarged progressively until death on the 29th day, with metastases in omentum, liver, lungs	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	+++±	++	+	0	0	0
		28th	±	0	0	0	0	0	+++±	+++	+	0	0	0
6-66	Seven tumors, 3.0-3.5-5.0-4.0-2.0-?-0.2-0.2 cm., on 10th day; all enlarged progressively until death on the 34th day, with metastases in omentum, liver, lungs	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	+++±	+±	0	0	0	0
		28th	++++	++++	+++±	++±	0	0	++++	++++	++++	++++	++++	++++
6-67	Seven tumors, 4.0-3.0-6.0-5.0-3.0-N-3.0-0.2 cm., on 10th day; all enlarged to 5.0-6.0-8.5-9.0-6.0-3.0-6.0-0.3 cm. on 18th day; dwindled slowly to 4.0-5.0-5.0-3.5-4.0-3.0-4.0-1.5 cm. on 28th day; N-1.5-1.0-?-N-N-N-0.3 cm., softish, on 46th day; negative on 67th day	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	++++	++++	++++	++	0	0	++++	++++	++++	++	0	0
		46th	+±	0	0	0	0	0	++++	+++	0	0	0	0
		67th	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II—*Concluded*

Rabbit No.	Outcome of implantations with Brown-Pearce tumor (6 to 8 situations)	Bled	Complement fixation tests: Normal rabbit kidney antigen 2–73, 1:40; Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64	Brown-Pearce tumor antigen 6–73, 1:40; Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64
		day												
6-68	Seven tumors, 4.0-3.5-4.0-4.0-1.5-N-3.0-3.0 cm., on 10th day; about same on 18th day; thereafter dwindled to 1.5-1.0-1.0-N-N-N-N-N cm. on 28th day; negative on 46th day and thereafter	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	++±	+±	0	0	0	0	+++	+++±	+	0	0	0
		28th	+++	+	0	0	0	0	++++	++++	+±	0	0	0
		46th	++++	++++	++++	++++	+++±	+	++++	++++	++++	++++	+++±	+
		67th	++++	+++±	++±	±	0	0	++++	+++±	++±	+	0	0
6-69	Seven tumors, 3.0-4.0-3.5-4.0-1.5-N-2.0-3.0 cm., on 10th day; slightly larger on 18th day and still larger on 28th day; then all dwindled slowly to N-2.5-N-1.5-0.4-N-N-2.0 cm., softish, on 46th day; negative on 67th day	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	++±	+	±	0	0	0	0	0	0	0	0	0
		46th	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
		67th	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
6-70	Four tumors, 2.0-3.0-5.0-1.5-N-N-N-N cm., on 10th day; 2.0-6.0-7.0-2.0-N-2.0-1.0-0.3 cm. on 18th day; enlarged slowly to 3.0-4.0-5.0-3.0-N-4.0-3.0-4.0 cm. on 46th day; moribund when bled on 46th day, omental metastases.	1st	0	0	0	0	0	0	0	0	0	0	0	0
		18th	0	0	0	0	0	0	0	0	0	0	0	0
		28th	++±	±	0	0	0	0	+++	+±	±	0	0	0
		46th	+	+	±	0	0	0	0	±	+	±	0	0

The rabbits of this table are those of implantation group II of Table I, q.v.

The findings as a whole would seem consistent with those of Table I; and they are more definitive in showing that an antibody capable of reacting in high titer with a saline extract of the Brown-Pearce tumor and not at all with a similar extract of normal rabbit kidney may appear in the blood of rabbits following implantation with the cancer.

Specific Affinity of the Brown-Pearce Antibody: Tests with Various Antigens

The results of further tests of the specific affinity of the Brown-Pearce antibody are set down in Table III.

The sera of five of the rabbits of Tables I and II were tested in mixture with antigens made from various normal and neoplastic rabbit tissues and from purulent exudates as well. It will be seen that four of them (4-19, 5-47, 5-51, 5-53), procured 32 to 35 days after implantation of the animal with the Brown-Pearce tumor, reacted in high titer with the Brown-Pearce tumor antigen but not at all with those made from normal tissues, with those derived from neoplasms other than the Brown-Pearce carcinoma, or with those made from the purulent exudates. These were sera which, according to the analysis of the results of Tables I and II, contained only the specific Brown-Pearce antibody. The sera from rabbit 5-45, procured on the 67th day following implantation, presumably contained the induced tissue antibody (and perhaps the specific Brown-Pearce tumor antibody also, for in the preceding test it had reacted with the Brown-Pearce tumor antigens as well as with the normal tissue antigens). In the experiment of Table III it reacted with the normal tissue antigens, with those made from three other transplanted rabbit cancers (the V2 carcinoma and the RSI and Kato sarcomas), and with those made from purulent rabbit exudates as well. In this respect it was somewhat less potent than the serum of a normal rabbit (7-97), which, heated at 56° C. for 30 minutes instead of at 65° C., as were the rest, had been included for comparative purposes since it was known from previous tests to contain the heat-labile natural antibody.

The findings with the antigens made from the purulent exudates (Table III) have special interest in relation to the work of Dmochowski. In a series of papers dealing with the antigens present in tuberculous and other purulent tissues, following the work of Hirszfeld *et al.* (6), Dmochowski recorded in a preliminary note (7) that the sera of rabbits injected with either unheated or boiled watery extracts of the Brown-Pearce tumor (or with similar materials derived from rabbit pus) would fix complement in mixture with extracts made from the tumor, though not with extracts of normal tissues. In a later, more detailed report (8), the results of complement fixation tests are recorded with the sera of two rabbits that had been injected with unheated, phenolized, saline extracts of the Brown-Pearce tumor, and of two others similarly injected with boiled, phenolized extracts of the growth, along with specimens procured from several rabbits and guinea pigs injected with extracts of phenolized rabbit pus. The protocols show that the sera of the four rabbits injected with the unheated or the boiled saline extracts of the Brown-Pearce tumor reacted almost precisely alike, giving fixation in mixtures containing phenolized saline extracts of the Brown-Pearce tumor (both unheated and boiled); they reacted also, though perhaps slightly less well, with unheated, phenolized, saline extracts of rabbit pus but not at all or only slightly in the dilutions tested in mixture with boiled, phenolized extracts of rabbit pus. No record is given of tests with the tumor-immune sera in mixture with saline extracts of normal rabbit tissues. It is not possible to decide from the protocols whether anticomplementary effects may have played a part in the reactions, or whether natural or induced tissue antibodies (3) may have been involved; for control tests with normal sera are not recorded, nor is the temperature of serum "inactivation" given.

TABLE III

Tests with Sera Procured from Rabbits Implanted with the Brown-Pearce Carcinoma in Mixture with Antigens Made from Various Rabbit Tissues

Source of sera	Rabbit No.	Source of antigens (rabbit tissues)	Complement fixation tests Dilution of antigen 1:20	1:40	1:80	1:160	1:320	1:640	Remarks
Rabbits implanted with the Brown-Pearce carcinoma (see Tables I and II)	4-19	Normal kidney	0	0	0	0	0	0	Reacts only with the Brown-Pearce tumor antigen,—contains only the specific Brown-Pearce antibody
		" liver	0	0	0	0	0	0	
		" spleen	0	0	0	0	0	0	
		" bone marrow (red)	0	0	0	0	0	0	
		Pus-NaCl	0	0	0	0	0	0	
		" -BCG	0	0	0	0	0	0	
		" -aleuronat	0	0	0	0	0	0	
		" -saponin	0	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	++++	++++	
		V2 carcinoma	0	0	0	0	0	0	
		RSI sarcoma	0	0	0	0	0	0	
		Kato "	0	0	0	0	0	0	
	5-47	Normal kidney	0	0	0	0	0	0	" "
		" liver	0	0	0	0	0	0	
		" spleen	0	0	0	0	0	0	
		" bone marrow (red)	0	0	0	0	0	0	
		Pus-NaCl	0	0	0	0	0	0	
		" -BCG	0	0	0	0	0	0	
		" -aleuronat	0	0	0	0	0	0	
		" -saponin	0	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	++++	+	
		V2 carcinoma	0	0	0	0	0	0	
		RSI sarcoma	0	0	0	0	0	0	
		Kato "	0	0	0	0	0	0	
	5-51	Normal kidney	0	0	0	0	0	0	" "
		" liver	0	0	0	0	0	0	
		" spleen	0	0	0	0	0	0	
		" bone marrow (red)	0	0	0	0	0	0	
		Pus-NaCl	0	0	0	0	0	0	
		" -BCG	0	0	0	0	0	0	
		" -aleuronat	0	0	0	0	0	0	
		" -saponin	0	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	++++	+++	
		V2 carcinoma	0	0	0	0	0	0	
		RSI sarcoma	0	0	0	0	0	0	
		Kato "	0	0	0	0	0	0	
	5-53	Normal kidney	0	0	0	0	0	0	" "
		" liver	0	0	0	0	0	0	
		" spleen	0	0	0	0	0	0	
		" bone marrow (red)	0	0	0	0	0	0	
		Pus-NaCl	0	0	0	0	0	0	
		" -BCG	0	0	0	0	0	0	
		" -aleuronat	0	0	0	0	0	0	
		" -saponin	0	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	++++	+++	
		V2 carcinoma	0	0	0	0	0	0	
		RSI sarcoma	0	0	0	0	0	0	
		Kato "	0	0	0	0	0	0	

TABLE III—*Concluded*

Source of sera	Rabbit No.	Source of antigens (rabbit tissues)	Complement fixation tests: Dilution of antigen 1:20	1:40	1:80	1:160	1:320	1:640	Remarks
	5-45	Normal kidney	++++	++++	++++	++++	++++	0	Reacts with antigens made from normal, purulent, and neoplastic tissue antigens, —contains the induced tissue antibody (see the associated paper (3))
		" liver	++++	++++	++++	++++	+±	0	
		" spleen	++++	++++	++++	++±	0	0	
		" bone marrow (red)	+++	0	0	0	0	0	
		Pus-NaCl	++++	±	0	0	0	0	
		" -BCG	+++	±	0	0	0	0	
		" -aleuronat	+++	0	0	0	0	0	
		" -saponin	0	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	+±	0	
		V2 carcinoma	++++	++++	+++±	0	0	0	
		RSI sarcoma	+++±	±	0	0	0	0	
		Kato "	++++	++++	+++±	+	0	0	
Normal rabbit (serum known to contain the natural tissue antibody)	7-97	Normal kidney	++++	++++	++++	++++	++++	++++	Reacts much as did the serum of rabbit 5-45 with antigens made from normal, purulent, and neoplastic tissues, —contains the natural tissue antibody (see a preceding paper (3))
		" liver	++++	++++	++++	++++	++++	++++	
		" spleen	++++	++++	++++	++++	+++±	+	
		" bone marrow (red)	+++	±	0	0	0	0	
		Pus-NaCl	++++	++++	++++	+++	+	0	
		" - BCG	++++	+++±	++	0	0	0	
		" -aleuronat	+++	+±	0	0	0	0	
		" -saponin	++±	0	0	0	0	0	
		Brown-Pearce carcinoma	++++	++++	++++	++++	+++±	++±	
		V2 carcinoma	++++	++++	++++	++++	+++±	+±	
		RSI sarcoma	++++	++++	++++	+++	+	0	
		Kato "	++++	++++	++++	+++±	++	0	

The sera were all diluted 1:4 with saline. The specimen from rabbit 7-97 was heated at 56°C. for 30 minutes immediately prior to the test to inactivate complement; the rest were heated at 65°C. for the same period to destroy any natural tissue antibody that may have been present.

The antigens were made fresh as usual from frozen tissues. 0.9 per cent NaCl, aleuronat and starch, and 0.1 per cent saponin, respectively, had been injected intraperitoneally into normal rabbits to elicit pus cells, which had been harvested by washing out the cavities with 3.8 per cent sodium citrate after 24 to 48 hours. BCG pus was procured by repeatedly injecting the organism, generously provided by Dr. Jules Freund, subcutaneously into rabbits.

On the whole it seems unlikely that the findings with the specific Brown-Pearce antibody have any relation to those recorded by Dmochowski. For this antibody has no affinity for extracts of rabbit pus, as Table III shows, while the distinctive tumor constituent with which it does react is rapidly destroyed by temperatures greater than 65° C., as previous observations have made plain (2).

In other experiments similar to that of Table III, saline extracts of Brown-Pearce tumor tissues procured from more than seventy different rabbits have been tested in mixture with sera containing the specific antibody. They reacted without exception, usually in titers of 1:320 to 1:1280 or more, whereas extracts of normal liver, kidney, spleen, voluntary and involuntary muscle, brain, or testicle tissues from more than fifty rabbits have all failed to do so in concurrent tests.

Specific Absorption of the Antibody for the Distinctive Constituent of the Brown-Pearce Tumor

The findings of previous papers (2) and those of Tables I to III would seem to indicate that the specific Brown-Pearce tumor antibody reacts only with a distinctive sedimentable constituent of the tumor; whereas the induced tissue antibody, like the heat-labile natural antibody, will react not only with a sedimentable constituent of normal rabbit tissues but also, though to lesser extent, with a similar, perhaps identical, material derived from various rabbit neoplasms, including the Brown-Pearce carcinoma. This being true, it should prove possible to absorb the specific Brown-Pearce antibody with a suspension of the sedimented material from the Brown-Pearce tumor, but not with similar materials derived from normal rabbit tissues; whereas the induced tissue antibody should be absorbed by sedimented materials derived from both normal and neoplastic tissues. The results of an actual absorption test fit such expectations, as Table IV shows.

The sera of rabbits 5-47 and 5-51, procured on the 46th day following implantation with the Brown-Pearce tumor, had reacted in the experiments of Tables I and II with extracts of the Brown-Pearce tumor as not with those of normal rabbit tissues; whereas those of rabbits 6-68 and 5-45, got the same day, had reacted with antigens of both types. The four sera were absorbed with sedimented substances derived from normal rabbit liver (D.R. 6-77), normal rabbit kidney (D.R. 4-94), and the Brown-Pearce tumor (omental metastases of D.R. 4-29), respectively. The tissues, which had been preserved frozen at −22° C., were ground as usual and suspended in physiological saline buffered with phosphate (approximately M/100, pH 7.4). After the suspensions had been spun at 3,000 R.P.M. for 5 minutes to throw down gross tissue fragments, the densely opalescent supernatant fluids were again centrifuged, this time at 25,000 R.P.M. for 1 hour in the air-driven machine. The clear supernatant liquids were poured off and discarded and the sedimented materials resuspended in 1/5th the original volume of buffered saline. All three materials yielded copious sediments, the Brown-Pearce tumor a good deal less than the normal tissues. The resuspended preparations were all densely opalescent, and all contained numerous aggregates that could not be broken up with the stirring rod. These were allowed to settle out and the suspensions decanted from them.

Mixtures were made to contain equal parts of each serum (diluted 1:2 with buffered saline) and the three tissue suspensions respectively. These, along with saline control mixtures for each serum, were kept at 37° C. for 2 hours, then at about 4° C. for 4 hours; all were then spun at 25,000 R.P.M. for 1 hour to remove the absorbing substances. The supernatant liquids were tested for capacity to fix complement in mixture with antigens made from the Brown-Pearce tumor (muscle implantations, D.R. 6-69) and from normal rabbit liver (D.R. 2-74).

Table IV shows the results of the tests. The unabsorbed sera of rabbits 5-47 and 5-51, as in the preceding experiments, reacted with the Brown-Pearce tumor antigen but not with the normal tissue antigen; the specimens from these animals that has been absorbed with the normal liver and kidney substances reacted precisely as did the controls, whereas the ones absorbed with the Brown-Pearce tumor substance were devoid of complement-fixing ability. The control specimen of rabbit 6-68 reacted with both the Brown-Pearce tumor antigen and with the normal liver antigen, slightly better with the latter; the absorbed ones failed to fix complement in mixture with either antigen. The serum of rabbit 5-45, unabsorbed, also

TABLE IV

Absorption of Sera Procured from Rabbits Implanted with the Brown-Pearce Tumor When Mixed with Sedimentable Substances Derived from Normal Rabbit Liver, Normal Rabbit Kidney, and the Brown-Pearce Tumor, Respectively

Serum from rabbit No.	Absorbed with*	Complement fixation tests: Normal rabbit liver antigen D.R. 2-73, 1:40 — Serum dilution 1:4	1:8	1:16	1:32	1:64	Brown-Pearce tumor antigen D.R. 6-49, 1:40 — Serum dilution 1:4	1:8	1:16	1:32	1:64	Inferences from the findings
5-47	Nil, control	0	0	0	0	0	++++	++++	++++	+++±	±	Contained specific Brown-Pearce antibody only: it did not react with the normal tissue antigen and was not absorbed by the normal tissue substances, but was completely absorbed by the Brown-Pearce tumor substance
	Normal liver substance	0	0	0	0	0	++++	++++	++++	+++±	±	
	" kidney "	0	0	0	0	0	++++	++++	++++	+++±	±	
	Brown-Pearce tumor "	0	0	0	0	0	0	0	0	0	0	
5-51	Nil, control	0	0	0	0	0	++++	++++	+++±	±	0	" "
	Normal liver substance	0	0	0	0	0	++++	++++	+++±	±	0	
	" kidney "	0	0	0	0	0	++++	++++	+++±	±	0	
	Brown-Pearce tumor "	0	0	0	0	0	0	0	0	0	0	
6-68	Nil, control	++++	++++	++++	+++±	+	++++	++++	++++	++	0	Contained induced tissue antibody only; absorbed by all three materials
	Normal liver substance	0	0	0	0	0	0	0	0	0	0	
	" kidney "	0	0	0	0	0	0	0	0	0	0	
	Brown-Pearce tumor "	0	0	0	0	0	0	0	0	0	0	
5-45	Nil, control	++++	++++	++++	+++±	±	++++	++++	++++	+++±	±	Contained antibodies of both kinds; the induced tissue antibody was completely absorbed by the liver and kidney substances and partially by the Brown-Pearce tumor substance, which partially absorbed the specific Brown-Pearce antibody also
	Normal liver substance	0	0	0	0	0	++++	+++±	+++	±	0	
	" kidney "	0	0	0	0	0	++++	++++	+++±	±	0	
	Brown-Pearce tumor "	++++	++++	++++	++±	0	++++	+++±	++±	0	0	

* For description of the materials and method of absorption, see the text.

reacted with both antigens; absorption with the liver and kidney substances removed completely its capacity to fix complement in mixture with the normal liver antigen and diminished somewhat its ability to react with the Brown-Pearce tumor antigen; while absorption with the Brown-Pearce tumor substance diminished considerably its ability to react with the tumor antigen and slightly lowered its reactivity with the normal tissue one.

Development of the Specific Brown-Pearce Antibody in Normal Rabbits Injected Intraperitoneally with Cell-Free, Watery Extracts of the Tumor

Does the development of the specific Brown-Pearce antibody depend upon the growth (and resorption?) of Brown-Pearce tumor cells or can it be elicited by cell-free extracts of them?

Brown-Pearce tumor tissue was procured aseptically from small, "healthy" intramuscular growths of several animals and from the omental metastases of others. This was trimmed free from as much necrotic and connective tissue as possible and kept frozen at −22° C. until required, often for several months. Saline suspensions were made as usual by grinding the frozen tissue, adding 9 volumes of dilute phosphate buffer (approximately M/200, pH 7.3), and spinning the suspension at 4400 R.P.M. for 5 minutes, the superantant being spun again at the same speed for 15 minutes. In several experiments, further purification was effected by spinning the extract in the air-driven centrifuge at 25,000 R.P.M. for 1 hour, the supernatant fluid being discarded and the sediment resuspended in the original volume of dilute buffer for injection. Microscopic examinations of the final preparations showed that they were free from visible cells, and normal control animals into which the materials were injected intratesticularly and intramuscularly remained free from palpable tumors without exception, as did also the experimental animals. For the immunizations 10 cc. was usually injected intraperitoneally into each rabbit on the 1st, 5th, and 9th days; occasionally a fourth injection was made on the 13th day. Serum was procured for test 7 or 8 days after the last injection.

Table V shows the results of an experiment in which sedimented Brown-Pearce tumor material was injected intraperitoneally into sixteen "blue-cross" hybrid rabbits (Rockefeller Institute strain). Serum specimens procured 8 days after the fourth injection were tested with antigens made from the Brown-Pearce tumor and from three normal rabbit tissues—liver, kidney, and spleen. It will be seen that sera from four normal controls of the same stock failed to react with any of the antigens, and so too did specimens from six of the injected rabbits (8-46, 8-47, 8-48, 8-50, 8-52, 8-57), while a 7th rabbit (8-59) provided serum that gave only slight reactions with the normal kidney and spleen antigens. Five of the rabbits (8-45, 8-51, 8-53, 8-56, 8-58) had blood that manifested only the specific Brown-Pearce antibody, their sera giving fixation in dilutions from 1:18 to 1:64 in mixture with the Brown-Pearce antigen though failing to react at all with the normal tissue antigens. The specimens from four other rabbits (8-49, 8-54, 8-55, 8-60) reacted with the Brown-Pearce tumor antigen and in lesser degree with one or another or all three of the normal tissue antigens.

From these results it appears that cell-free saline suspensions of the Brown-Pearce tumor may elicit the specific antibody in a considerable proportion of

TABLE V

Complement Fixation Tests with Sera from Rabbits Injected Intraperitoneally with Cell-Free Extracts of the Brown-Pearce Tumor

Source of sera	Rabbit No.	ANTIGENS: Brown-Pearce tumor, 1:40 — Serum dilutions 1:2	1:4	1:8	1:16	1:32	1:64	Normal rabbit liver, 1:40 — Serum dilutions 1:2	1:4	1:8	1:16	Normal rabbit kidney, 1:40 — Serum dilutions 1:2	1:4	1:8	1:16	1:32	Normal rabbit spleen, 1:40 — Serum dilutions 1:2	1:4	1:8	1:16
Blue-cross hybrid	8-45	++++	++++	++++	++++	+++±	0	0	0	0	0	0	0	0	0	0	0	0	0	0
rabbits injected	8-46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
with cell-free sa-	8-47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
line suspensions	8-48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
of sedimented	8-49	++++	++++	++++	++++	+++±	0	++±	+±	0	0	++++	++++	++++	++++	±	++++	++++	++++	±
material from the	8-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown-Pearce tu-	8-51	++++	++++	++++	++++	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mor. 10 cc. of	8-52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:10 suspensions	8-53	++++	++++	+±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
injected intra-	8-54	++++	++++	++++	++++	++++	0	0	0	0	0	++	±	0	0	0	±	0	0	0
peritoneally on	8-55	++++	++++	++++	++++	+++±	0	0	0	0	0	++	+	±	0	0	+	0	0	0
the 1st, 5th, 9th,	8-56	++++	++++	++++	++++	++++	+++	0	0	0	0	0	0	0	0	0	0	0	0	0
and 13th days;	8-57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sera procured on	8-58	++++	++++	++++	++++	+++±	+	0	0	0	0	0	0	0	0	0	0	0	0	0
the 21st day	8-59	0	0	0	0	0	0	0	0	0	0	+±	±	0	0	0	+±	+	±	0
	8-60	++++	++++	++++	+++	0	0	0	0	0	0	++	+	±	0	0	+	0	0	0
Normal control	8-61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
rabbits of same	8-62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
stock	8-63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	8-64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The materials injected intraperitoneally were thrown down in the high speed centrifuge and resuspended in the original volume of dilute buffer solution. They were free from cells visible under the microscope, and they failed to elicit tumors when implanted intratesticularly and intramuscularly in normal rabbits.

Serum specimens procured from all of the rabbits prior to intraperitoneal injections failed to react with the same test antigens in subsidiary experiments.

"blue-cross" hybrid rabbits, and occasionally also the induced tissue antibody in low titer. Further experiments have confirmed the findings: cell-free saline extracts of the Brown-Pearce tumor have been injected repeatedly as described into forty-four of the normal inbred hybrid rabbits; serum tests showed that sixteen of these developed the specific antibody as result, usually without any detectable induced tissue antibody (3). In contrast, however, similar cell-free saline extracts, injected several times intraperitoneally as in the experiments already described, have regularly failed to stimulate the specific antibody in more than fifty Dutch, chinchilla, and agouti hybrid rabbits.

A Specific Anamnestic Response

Table VI shows the results of an experiment in which four normal young adult "blue-cross" rabbits and five of those of Table I, which had developed the specific Brown-Pearce antibody as a result of implantations with the tumor many months before, were bled for serum and then injected repeatedly with watery extracts of the Brown-Pearce tumor. The injections had no effect on the sera of three of the normal animals (A, B, C), the specimens procured from these both before and after the injections being devoid of ability to fix complement in mixture with the normal liver and Brown-Pearce tumor antigens. Normal rabbit D, however, provided serum after the injections that reacted in a dilution of 1:64 with the Brown-Pearce tumor antigen though not at all with the liver antigen.

The findings with the sera of the Brown-Pearce tumor animals (4-19, 5-47, 5-51, 5-52, 5-53) were very different. The titer of the specific antibody had fallen to low levels at the time the injections were begun, the sera then fixing complement in mixture with the Brown-Pearce tumor antigen in dilutions of 1:8 or less. After the injections, however, the rabbits provided sera that reacted in dilutions of 1:256 or more with the Brown-Pearce antigen though not at all with the liver antigen. It is noteworthy that the antibody titers following the intraperitoneal injections were as high as were those resulting earlier from the implantations with living tumor cells (Tables I and II) and in two cases at least (rabbits 5-47 and 5-51) even higher.

In further tests it proved impossible to stimulate the specific Brown-Pearce antibody by means of repeated intraperitoneal injections of watery extracts of the growth in "blue-cross" rabbits that had failed to develop it earlier as result of the growth and regression of the tumor. Repeated injections of extracts of the tumor, however, sometimes stimulated low titers of the induced tissue antibodies that react with normal antigens (3). This was notably the case in animals that had previously manifested the induced tissue antibodies as result of the growth of the tumor. Conversely, repeated intraperitoneal injections of watery extracts of normal rabbit tissues (liver, kidney, spleen) failed without exception to stimulate development of the specific Brown-Pearce antibody,

TABLE VI

Tests with the Sera of Rabbits Injected Intraperitoneally with Extracts of the Brown-Pearce Tumor

Source of sera	Complement fixation tests with sera procured before the injections (1st day)							Complement fixation tests with sera procured after the injections (16th day)										
	Normal liver antigen, 1:40			Brown-Pearce tumor antigen 1:40				Normal liver antigen, 1:40			Brown-Pearce tumor antigen, 1:40							
	Serum dilution			Serum dilution				Serum dilution			Serum dilution							
	1:2	1:4	1:8	1:2	1:4	1:8	1:16	1:4	1:8	1:16	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Normal rabbit A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
" " B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
" " C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
" " D	0	0	0	0	0	0	0	0	0	0	++++	++++	++++	++++	+++±	0	0	0
Brown-Pearce rabbit 4-19	0	0	0	++++	+++±	±	0	0	0	0	++++	++++	++++	++++	++++	+++±	±	0
" " " 5-47	0	0	0	+++±	+++	0	0	0	0	0	++++	++++	++++	++++	++++	++++	++++	+±
" " " 5-51	0	0	0	+++	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++	++++	+++
" " " 5-52	0	0	0	++++	++++	++		0	0	0	++++	++++	++++	++++	++++	++++	++++	++
" " " 5-53	0	0	0	++++	+++±	+		0	0	0	++++	++++	++++	++++	++++	++++	+++±	+

10 cc. of a twice centrifuged saline extract of frozen Brown-Pearce tumor tissue (1:10) was injected intraperitoneally into each rabbit on the 1st, 5th, and 9th days. Rabbits A–D, inclusive, were normal young adult blue-crosses; 4-19 had been implanted with the Brown-Pearce tumor 277 days prior to this experiment; 5-47 to 5-53, inclusive, had been implanted with the Brown-Pearce tumor 164 days previously,—see Tables I and II for outcome of the implantations.

even in rabbits that were capable of manifesting the specific response to injections of extracts of the tumor.

Development of the Specific Brown-Pearce Antibody in Relation to the Outcome of Tumor Implantations

The development of the specific Brown-Pearce tumor antibody in rabbits implanted with the growth will now be considered in relation to the course of the implanted tumors and to the development of the induced tissue antibody.

Tables I and II provide pertinent data. Mention has already been made of the fact that a dense suspension of finely minced Brown-Pearce tumor was implanted into six or eight situations in each animal in order to have a great mass of tumor tissue growing in each host and thus to elicit antibodies in high titer. The growths in any one animal enlarged or regressed together as a rule, but there were differences in their course from one animal to another, and especially noteworthy differences in the behavior of the implanted tumor in animals of differing breeds. For, whereas at the time the bleedings were made the tumors had enlarged progressively in all except one of the sixteen market-bought agouti hybrids, and they likewise had enlarged in six of the nine market-bought chinchillas, they had not become palpable or had regressed abruptly in all except one of the seventeen "blue-cross" hybrids procured from the breeding colony of The Rockefeller Institute.[1]

A number of the rabbits of all three varieties yielded sera that failed to fix complement in mixture with antigens made from either normal tissues or the Brown-Pearce tumor, this in spite of the fact that most of them when bled had carried several large tumors for 10 days or longer (see Tables I and II). No reason for this can now be advanced.

Special interest centers in the rabbits that developed the specific Brown-Pearce antibody but not the induced tissue antibody,—the ones, that is, with sera capable of fixing complement in mixture with Brown-Pearce tumor antigens but not in mixture with normal tissue antigens. These animals (4-19, 5-47, 5-51, 5-53 of Tables I and II) were all "blue-cross" hybrids, and all had developed several tumors, each 1.0 to 4.0 cm. across that had regressed abruptly. None of the agouti hybrids and only two of the chinchillas had growths of comparable size and behavior. Seven other "blue-crosses," however, had growths of similar size that also regressed abruptly; yet the sera of these rabbits (4-20, 4-21, 5-17, 5-18, 5-20, 5-49, 5-50) remained completely devoid of complement-fixing antibodies.

It is interesting to compare the variations in titer of the specific Brown-Pearce antibody with the course of the tumor in rabbits 5-47, 5-51, 5-52, and 5-53 (see Table II). The antibody was present in high titer in the blood of all four animals on the 18th day following the implantations; the tumors had already vanished in three of the animals at this time and were dwindling in the fourth (5-52). The titer of the specific antibody was less at each of the successive bleedings in rabbits 5-47 and 5-51.

The induced tissue antibody was present in the serum of twelve of the eighteen agouti hybrids of Table I and in that of three of the nine chinchillas, as evidenced by the reactions of these specimens in mixture with the normal tissue antigens. Only two of the seventeen inbred "blue-cross" rabbits, however, had sera containing the inducd tissue antibody, and specimens from both of these animals (5-45 and 5-52) appeared to contain the specific Brown-

[1] Others have previously noted great differences in the behavior of the Brown-Pearce tumor in rabbits of various breeds (12); and the course of transplanted cancers is notoriously variable in hybrid hosts, as many observations attest.

Pearce antibody also. It is noteworthy that the tumors in these two animals were somewhat larger than those in most of the other "blue-crosses," and they persisted longer.

The fluctuations in titer of the induced tissue antibody in some of the animals are worthy of note. In rabbit 5-45 of Table II, for example, the induced antibody was at its highest titer soon after the growths had been resorbed, and this proved true also in the case of rabbits 5-52 and 6-68. It was present, though in comparatively low titer, in the blood of two rabbits with progressively enlarging tumors and metastases (5-55, 6-66).

From these findings it can be seen that the market-bought hybrid rabbits—agoutis and chinchillas—implanted in several situations with the Brown-Pearce carcinoma, developed growths which enlarged progressively as a rule and often metastasized widely, bringing about death of the host. Their sera were apt to contain the induced antibody that reacts with sedimentable constituents of normal and neoplastic tissues, as described in the associated paper (3), though frequently the Brown-Pearce antibody seemed also to be present, as was indicated by the fact that the sera reacted in higher titer with extracts of the Brown-Pearce tumor than with extracts of normal tissues, whereas the reverse relation obtains with sera containing only the induced tissue antibody (3).[2] The outcome was otherwise in the "blue-cross" rabbits. For these animals, implanted in the same way with the Brown-Pearce carcinoma, and often with the same material, frequently resisted its growth and usually overcame it after a time; and a considerable proportion of them developed high titers of the antibody that reacts specifically with the distinctive constituent of the carcinoma, while usually manifesting little or none of the induced antibody that reacts with the sedimentable constituents of other tissues. It should be mentioned, however, that many of the "blue-cross" rabbits failed to develop the specific Brown-Pearce antibody, though implanted in the same way with the same material and having growths of comparable size and behavior, and that a number of them overcame their growths without manifesting the antibody at any of repeated bleedings,—whence it seems plain that regression of the growth, at least as it occurs in some instances, is probably not due to the specific antibody. The question was not investigated whether regression of the tumor in these cases might have been due to genetically determined isoantigenic differences between the cells of the tumor and those of the hosts, as has proved true with other cancers (9), though more has been said elsewhere about the specific antibody as influencing the proliferation of Brown-Pearce tumor cells (10).

Genetic or Constitutional Factors as Influencing Development of the Specific Brown-Pearce Antibody

The findings with the various breeds of rabbits make it plain that genetic or constitutional factors influence notably the development of the specific anti-

[2] Similar findings were got in other experiments, not here described, in which multiple implantations of the Brown-Pearce tumor were made in hybrid rabbits of New Zealand and Dutch breeds.

body, but the nature of these factors remains obscure except in one respect. It seemed possible that the specific Brown-Pearce antibody might be an isoantibody of the sort encountered by Gorer and by Lumsden in mice and rats with induced resistance to certain transplanted cancers (9). In their experiments, genetically determined antigens were identified in tumor cells which were presumably the same or nearly the same as those of the normal cells of the original host and of related animals. When the growths were transplanted to hosts with tissues lacking the antigens mentioned, isoantibodies were stimulated which proved capable of agglutinating the erythrocytes of antigen-carrying animals, and the growths regressed as a rule in such hosts while enlarging progressively in animals with tissues having the antigens and not developing isoantibodies. The facts are wholly different in the case of the Brown-Pearce tumor and the specific antibody. For while the antibody reacts regularly with extracts of the Brown-Pearce tumor, as already several times mentioned, many sera containing high titers of it have consistently failed to react with similar extracts of various normal tissues, these procured from more than fifty rabbits in all. Also sera containing much of the specific antibody have failed to react with extracts of the normal tissues either of rabbits in which the Brown-Pearce tumor had grown progressively and metastasized or of those in which the tumor regressed, with or without the development of the specific antibody. Furthermore the specific antibody has not lysed or agglutinated the erythrocytes of any one of more than thirty rabbits, including tumor-bearing and tumor-regressed hosts as well as normal animals. From the findings as a whole, it seems plain that the specific Brown-Pearce antibody is probably not an isoantibody of the type mentioned.

RECAPITULATION AND DISCUSSION

Rabbits carrying transplanted cancers of various types frequently develop what have been termed induced tissue antibodies, which will fix complement in mixture with saline extracts of various normal and neoplastic rabbit tissues, as an associated paper has shown (3). In the present work, a detailed study has been made of the incidence and specificity of another type of antibody, which will fix complement in mixture with a distinctive constituent of the Brown-Pearce carcinoma. The findings have special interest in relation to the work of Cheever (4), who had only limited success in attempts to procure antibodies that would react specifically with extracts of the Brown-Pearce tumor, and to that of Jacobs and Houghton, whose results were uncertain in a similar undertaking (5).

The Brown-Pearce antibody has not been detectable in the blood of normal rabbits or in that of rabbits carrying tumors of other kinds (2, 3). It often reached high titer, however, and usually without the concomitant development of induced tissue antibodies, in certain rabbits of a special breed—"blue-cross" hybrids of The Rockefeller Institute strain,—following intramuscular implanta-

tion with the Brown-Pearce tumor. Market-bought agouti and chinchilla hybrids frequently developed the induced tissue antibodies, and perhaps the specific Brown-Pearce antibody also, following growth of the tumor (Tables I and II).

The substance with which the specific Brown-Pearce antibody reacts is regularly present in large amounts in watery or saline extracts of that tumor (2). It was not detected in similar extracts of various normal rabbit tissues, in extracts of other rabbit neoplasms, or in extracts of rabbit pus (Table III). Absorption experiments provided further evidence of the distinction between the specific Brown-Pearce antibody and the induced tissue antibodies. For the Brown-Pearce antibody was specifically absorbed by extracts of that growth though not by extracts of normal rabbit tissues, whereas the induced antibodies were absorbed by extracts of normal tissues (Table IV).

Cell-free, watery extracts of the Brown-Pearce tumor, injected intraperitoneally, stimulated the development of the specific antibody in sixteen of a total of forty-four "blue-cross" rabbits, though they failed to do so when injected in the same way into more than fifty agouti, chinchilla, and Dutch hybrid rabbits (Tables V and VI). Extracts of the tumor likewise elicited anamnestic responses when injected intraperitoneally into rabbits previously manifesting the specific antibody. Similar extracts of normal rabbit tissues failed to elicit the anamnestic response, although they, and the tumor extracts as well, often raised the titer of induced antibodies in rabbits previously manifesting these as result of tumor growth.

The findings with the various breeds of rabbits would seem to indicate that constitutional factors influence the development of the specific antibody, but the nature of these factors remains obscure. The observations tend to show, however, that the specific antibody is probably not an isoantibody of the type encountered by Gorer and by Lumsden in animals manifesting induced resistance to tumors (9). For, unlike the isoantibodies just mentioned, the specific Brown-Pearce antibody has regularly failed to react with the erythrocytes or with extracts of the normal tissues of many animals, including tumor-susceptible and tumor-resistant hosts as well as normal animals.

An analysis of the development of the Brown-Pearce antibody in relation to the course of implanted tumors brought out the fact that a number of the "blue-cross" rabbits overcame their growths in the absence of detectable amounts of the antibody (Tables I and II). Hence it would appear that regression of the growth, at least as it occurs in many instances, is probably not brought about by the specific antibody.[3] Further studies have indicated, however, that the specific antibody may exert an effect on the living Brown-Pearce tumor cells (10).

[3] Much work indicates that the regression of transplanted cancers usually depends upon genetically determined antigenic differences between the cells of the tumor and those of the host (9), and this may hold true in the case of the Brown-Pearce carcinoma also.

The results of previous experiments have suggested that the distinctive constituent of Brown-Pearce tumor cells may be a protein (2). Recent findings, not yet reported in detail, have shown that it is acted upon *in vitro* by purified proteolytic enzymes (chymotrypsin and trypsin), these rapidly rendering it unable to react with its specific antibody. Further experiments in collaboration with Dr. Albert Claude have shown that watery extracts of the Brown-Pearce tumor, purified by repeated differential centrifugations, are made up of small spheroid particles in suspension which prove visible under the darkfield microscope and seem fairly homogeneous as to size. These are composed of about 50 per cent of alcohol- and ether-extractable lipids and a non-lipid fraction having about 14 per cent nitrogen, 50 per cent carbon, and 1 per cent phosphorus, and they give strongly positive tests for ribonucleic acid, being similar in these respects to the "small particles" or cytoplasmic "microsomes" which have been found in many normal tissue cells (11). The purified suspensions prepared as described from the Brown-Pearce tumor, and those procured in the same way from normal rabbit tissues, have regularly fixed complement *in vitro* in mixture with sera containing the natural and induced tissue antibodies (3), whereas the Brown-Pearce suspensions alone have given reactions with sera containing only the specific Brown-Pearce antibody. From these various observations it seems probable that the distinctive constituent may be associated with the small particles or microsomes of the Brown-Pearce carcinoma cell, the latter having a chemical constitution and serologically identifiable constituents in common with the microsomes of many normal tissue cells. The possible association of the Brown-Pearce constituent with the microsomes has the greater interest since the filtrable agent responsible for Chicken Tumor I seems to be associated with the microsomes of the fowl sarcoma cells (11).

SUMMARY

A detailed study has been made of an antibody which appears in the blood of certain rabbits implanted with the Brown-Pearce carcinoma or injected with extracts of it and which reacts specifically *in vitro* in mixture with a distinctive sedimentable constituent of the Brown-Pearce tumor cell. The observations as a whole seem to indicate that this constituent of the Brown-Pearce tumor differs notably from certain other sedimentable substances which can be extracted from various rabbit tissues and identified by serological means. The implications of the findings are discussed.

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