SUPPRESSION OF GROWTH OF BROWN-PEARCE TUMOR CELLS BY A SPECIFIC ANTIBODY*

WITH A CONSIDERATION OF THE NATURE OF THE REACTING CELL CONSTITUENT

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An antibody which appears in the blood of certain rabbits implanted with the Brown-Pearce carcinoma and which reacts specifically *in vitro* with a distinctive sedimentable constituent of the tumor cells has been described in previous reports (1). Its ability to suppress the growth of living Brown-Pearce tumor cells will now be recorded (2). The findings provide evidence that the cell constituent with which the antibody reacts may be responsible for the proliferation of the tumor cells, and they will be discussed in this relation.

Methods

Two types of experiments were employed. *In vivo* tests were made by studying the outcome of implantations of the tumor cells into various rabbits, some of which had the specific antibody in their blood as result of previous intraperitoneal injections of cell-free saline extracts of the growth (3). For *in vitro* tests, suspensions of the tumor cells were mixed and incubated 2 to 3 hours at 37°C. with rabbit sera containing the specific antibody and with various control sera; the mixtures were then implanted intramuscularly into normal, susceptible hosts, with later charting of the results.

A standardized complement fixation test was employed as in previous studies (1) to detect the specific antibody. In the charts that follow, the titer of the antibody is expressed numerically (1:4, 1:128, etc.) as the highest dilution of serum in saline that gave +++ or better fixation of 2 units of complement in mixture with a 1:40 saline extract of frozen tumor tissue as antigen. When a serum specimen failed to react at all in the test in any of the dilutions from 1:2 (the lowest feasible dilution) to 1:128, the titer has been recorded as zero.

Suspensions of living tumor cells were procured from market-bought hybrid hosts by harvesting vigorously proliferating growths in testicle or muscle with precautions for asepsis, and pressing carefully selected portions of "healthy" tumor tissue through a 40 mesh monel metal sieve into Locke's solution, pH 7.3 to 7.4. The suspensions were put into tall cylinders for 5 to 15 minutes and then pipetted off from the debris that had settled out. They contained about 0.05 gm. of tissue per cc., and under the microscope showed from 5 to 20 or more individually suspended tumor cells per high power field (× 400), along with a few clumps

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and aggregates of tumor cells and occasional erythrocytes and leukocytes. In routine transfers, 1.0 cc. amounts of such suspensions were usually injected into the testicles or into the foreleg and anterior and posterior thigh muscles of normal rabbits.

In tests for immunity to the tumor cells *in vivo*, use was made of young adult "blue cross" hybrid rabbits of the Rockefeller Institute strain. A considerable proportion of these animals develop the specific Brown-Pearce antibody following intraperitoneal injections of cell-free, saline extracts of the tumor, as described in a preceding paper (3). The saline extracts used for immunization purposes were made by grinding with sand tumor tissue that had been stored for periods from one to several months at −22°C., and suspending the ground tissue in 9 volumes of 0.9 per cent NaCl. The suspensions were then centrifuged twice at 4400 R.P.M., first for 5 minutes with removal of the supernatant liquid, which was spun again for 15 minutes and carefully taken off. Extracts prepared in this way, though moderately opalescent, have been without exception free from intact cells as the microscope showed, and in every experiment control tests proved that they did not give rise to tumors when injected into the testicles of normal susceptible rabbits. For immunization, three or four injections were given intraperitoneally at 4 day intervals of 10 cc. of the 1:10 saline extracts, prepared fresh each time from the frozen stock tissue. Seven or 8 days after the last intraperitoneal injection the rabbits were bled for serum; complement fixation tests showed that, as a rule, only about half or less of the injected rabbits had developed the specific antibody in detectable titer. 1.0 cc. of a suspension of living tumor cells, prepared as described above, was injected into two or more leg muscle situations in all of the injected rabbits, usually on the same day the animals were bled, occasionally on the following day. The size of the resulting tumors was determined by palpation at intervals thereafter.

For the *in vitro* tests, serum specimens were procured on the day of the experiment from "blue cross" rabbits known to provide the specific antibody in high titer, as also from normal controls of the same stock. The sera were mixed in equal parts with fresh suspensions of living tumor cells, prepared as already described, in small flasks coated inside with a thin film of paraffin. The mixtures were then incubated at 37°C. for 2 to 3 hours, with occasional gentle shaking. To make certain that the control and experimental mixtures were comparable as to pH, this was determined with the glass electrode immediately after the incubation in each experiment, at which time they gave values ranging between pH 7.45 and 8.08, those containing the specific antibody deviating no more from pH 7.4 than did the controls. 1.0 cc. of each mixture was implanted with syringe and 23 gauge needle into the leg muscles of three or four normal agouti or chinchilla rabbits, the sites being systematically varied from animal to animal and care being taken to inject control and experimental mixtures into corresponding situations in left and right legs. (In some experiments, 0.5 cc. amounts of the various mixtures were injected into several situations in the skin of the flanks.) The resulting growths were examined by palpation at intervals of 2 to 4 days beginning about the 10th day, and charted in silhouette. At the end of each experiment the test animals were killed; their growths were then excised, trimmed free from surrounding muscle, and accurately charted to size on cellophane. To save space, only a few of the various tracings are recorded in the charts, but these are in every case representative of the findings as a whole.

Results of Implantation of Brown-Pearce Tumor Cells into Immunized Rabbits

Chart 1 shows the findings in ten "blue cross" rabbits that had first received four intraperitoneal injections of a 1:10 saline extract of frozen Brown-Pearce tumors, and 7 days after the last were implanted with 1.0 cc. of a suspension of Brown-Pearce tumor cells in both anterior thigh muscles. When the rabbits were bled on the 20th day, only one of them (10-70) provided serum that con-

tained the specific Brown-Pearce antibody as demonstrated by the standard tests, and this animal alone proved wholly resistant to the implants, manifesting no palpable growths at any of numerous examinations. The growths in the rest of the animals varied widely in size and course. In rabbit 10-68, for example, the tumors enlarged progressively, bringing about the death of the host with metastases on the 29th day; and in rabbit 10-64 the tumors, while slow in starting, by the 34th day had attained huge size, and they caused death on the 46th day. The majority of the rabbits (10-62, 10-63, 10-66, 10-67,10-69

Results of Implantation of Brown-Pearce Tumor Cells into Immunized Rabbits

Treatment	Rabbit No.	Titer of specific Brown-Pearce antibody (20th day)	Outcome of implantations with Brown-Pearce tumor cells: 2 intramuscular situations							
			9 days after implantation		13 days		20 days		34 days	
			LAT	RAT	LAT	RAT	LAT	RAT	LAT	RAT
All rabbits injected intraperitoneally with 10cc. of a 1:10 saline extract of Brown-Pearce tumors on the 1st, 5th, 9th, and 13th days; tumor cells implanted on the 20th day	10-62	0	●	●	●	●	n	n	n	n
	10-63	0	●	●	●	●	●	●	n	n
	10-64	0	•	n	●	●	⬮	⬮	⬮	⬮
	10-66	0	●	●	●	●	⬮	⬮	•	n
	10-67	0	●	●	●	•	n	n	n	n
	10-68	0	●	●	●	●	⬮	⬮	† with metas 29th day	
	10-69	0	●	●	⬮	⬮	●	•	n	n
	10-70	1:32	n	n	n	n	n	n	n	n
	10-71	0	●	●	⬮	⬮	n	n	n	n

0 — 2cm.

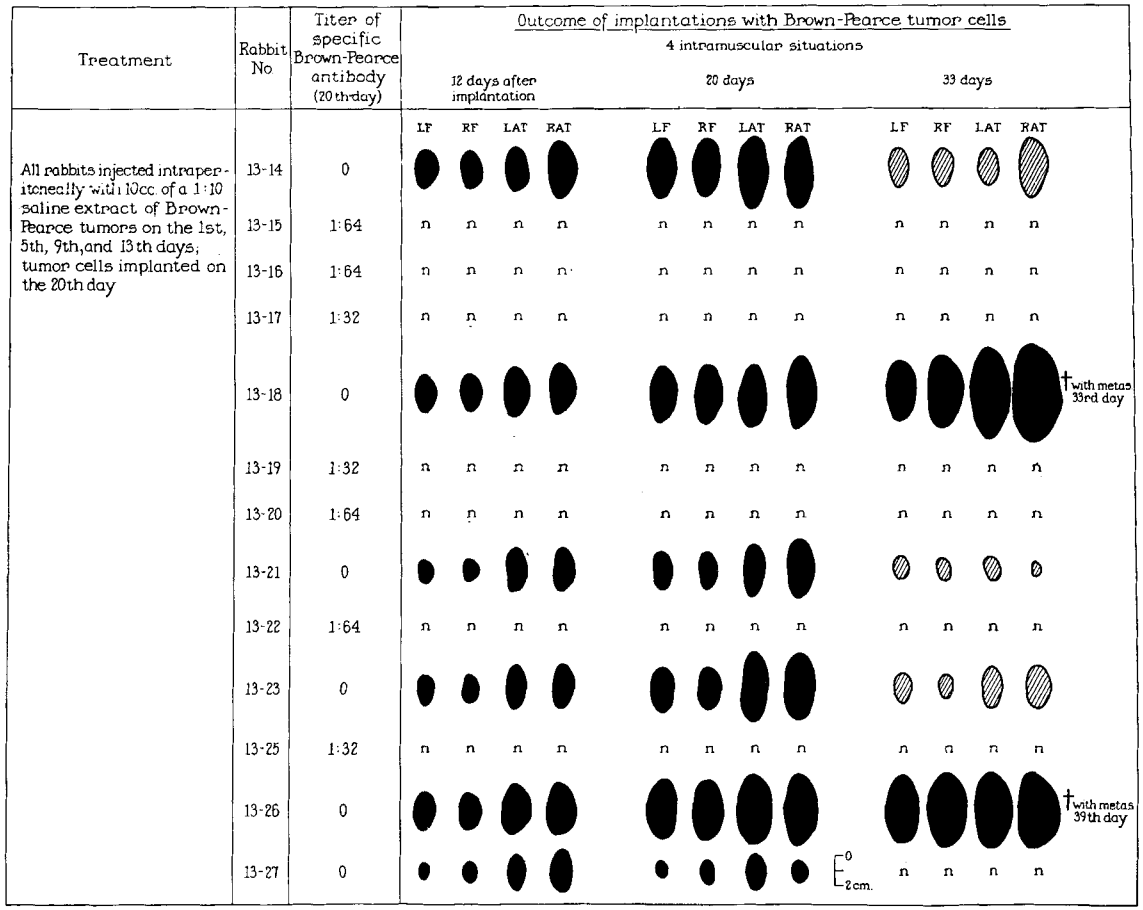
n=nil; LAT, RAT = left and right anterior thigh muscles, respectively
The saline extracts employed for immunization were free from viable tumor cells as control tests showed.
The same holds true in the experiments that follow.

CHART 1

10-71) developed good sized tumors that later regressed, this happening abruptly as a rule between the 13th and 34th days. It is noteworthy that progression as well as regression of the tumors took place in rabbits that had failed to develop the specific Brown-Pearce antibody in demonstrable quantity after the immunizing injections,—a commonplace finding, as will become apparent from the later charts.

In a second experiment of similar sort (Chart 2), cell-free saline extracts of the Brown-Pearce tumor were injected intraperitoneally into fourteen of the inbred "blue cross" rabbits, of which one died before the 20th day and was discarded. Seven of the animals had developed the specific Brown-Pearce anti-

Results of Implantation of Brown-Pearce Tumor Cells into Immunized Rabbits



n = nil; LF, RF, LAT, RAT = left and right forelegs and left and right anterior thigh muscles, respectively.
The hatched tumors later disappeared.

CHART 2

body when all were bled on the 20th day, all providing sera that gave +++ complement fixation or better in the standard test in dilutions of 1:32 or 1:64. These seven animals (13-15, 13-16, 13-17, 13-19, 13-20, 13-22, 13-25) all proved resistant to implantation with living Brown-Pearce tumor cells in four intramuscular situations, whereas the rabbits which had failed to develop the specific antibody as judged by the serum tests (13-14, 13-18, 13-21, 13-23, 13-26, 13-27) proved susceptible, the implantations resulting in growths at every situation in each of the animals. As in the experiment of Chart 1, the tumors grew progressively in some of the rabbits (13–18, 13–26) and underwent secondary retrogression in others (13–14, 13–21, 13–23, 13–27) in which the specific Brown-Pearce antibody was absent at the time of implantation.

Chart 3 records the results of a third experiment in which living Brown-Pearce tumor cells were implanted into six intramuscular situations in twenty "blue cross" rabbits, sixteen of which had been immunized with intraperitoneal injections of saline extracts of the Brown-Pearce tumor as in the two preceding experiments, the remainder being normal controls. Four of the immunized rabbits (6-56, 6-57, 6-62, 6-98) had sera that contained the specific Brown-Pearce antibody in titers of 1:16 to 1:64 at the time of the implantations, and these rabbits failed to develop palpable tumors. A fifth rabbit (6-99) had serum with a relatively low titer of the antibody (1:2); and in this animal good sized palpable tumors were present at all of the implantation sites on the 11th and 14th days, but they had begun to dwindle at the examination on the 17th day and had disappeared by the 21st day. The other rabbits of the treated group failed to develop the specific antibody; some of them manifested tumors that enlarged progressively (rabbits 6-54, 6-64), while others had palpable growths that sooner or later regressed (rabbits 6-53, 6-55, 6-58, 6-59, 6-61, 7-00, 7-02), as was the case in the rabbits of the control group also.

Specificity of the Induced Resistance

In the experiments already described, rabbits that had developed the specific antibody as result of immunization with cell-free extracts of the Brown-Pearce tumor proved resistant to implantations with the living cells of that growth. It seemed important to learn next whether the resistance, like the antibody, is specific. For this purpose, a number of "blue cross" hybrid rabbits were immunized in the usual way and then, along with normal animals of the same stock, were implanted intramuscularly with suspensions of the living cells of three transplanted rabbit tumors: (*a*) the Brown-Pearce tumor, (*b*) the V2 carcinoma (4), and (*c*) the Rabbit Sarcoma I of Andrewes and Ahlström (5).

The findings are set down in Chart 4. With the aim of disclosing any differences in sharp contrast, a very dense suspension of Brown-Pearce tumor cells had been implanted, and thinner ones of the other two tumors. But this result was not attained; for while large Brown-Pearce tumors had appeared by

Results of Implantation of Brown-Pearce Tumor Cells into Immunized Rabbits

Treatment	Rabbit No.	Titer of specific Brown-Pearce antibody (17th day)	Outcome of implantations with Brown-Pearce tumor cells in 6 intramuscular situations: 11 days after implantation	21 days	35 days
			LF RF LAT RAT LPT RPT	LF RF LAT RAT LPT RPT	LF RF LAT RAT LPT RPT
a) Rabbits injected intraperitoneally with 10 cc. of a 1:10 saline extract of Brown-Pearce tumors on the 1st, 5th, and 9th days: tumor cells implanted on the 17th day	6-53	0	● ● ● ● ● ●	● ● ● ● ● ●	n ● n ● n n
	6-54	0	● ● ● ● * *	● ● ● ● * *	† Miliary metastases, 26 th day
	6-55	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● n n ● ●
	6-56	1:64	n n n n n n	n n n n n n	n n n n n n
	6-57	1:16	n n n n n n	n n n n n n	n n n n n n
	6-58	0	● ● ● ● ● ●	● ● ● ● ● ●	n ● n ● ● ●
	6-59	0	● ● ● ● ● n	● ● n ● n n	n ● n n n n
	6-61	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● ● ● ● ●
	6-62	1:32	n n n n n n	n n n n n n	n n n n n n
	6-63	0	● ● ● ● ● ●	n n n n n n	n n n n n n
	6-64	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● ● ● ● ●
	6-98	1:32	n n n n n n	n n n n n n	n n n n n n
	6-99	1:2	● ● ● ● ● ●	n n n n n n	n n n n n n
	7-00	0	● ● ● ● ● n	● n n n n n	n n n n n n
	7-01	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● ● ● ● ●
	7-02	0	n n ● ● ● ●	n n n n n n	n n n n n n
				0 3cm.	
b) Control rabbits, not immunized but implanted as above.	7-03	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● ● ● ● ●
	7-04	0	● ● ● ● ● ●	● ● ● ● ● ●	● ● n n ● ●
	7-05	0	● ● ● ● ● ●	● ● ● ● ● ●	n ● n n n n
	7-06	0	● ● ● ● ● ●	● ● n n n n	n n n n n n

n = nil; LF = left foreleg, RAT = right anterior thigh; RPT = right posterior thigh; etc.
* = not implanted.

CHART 3

Selective Effect of the Specific Antibody on Brown-Pearce Tumor Cells

Treatment	Rabbit No.	Titer of specific Brown-Pearce antibody (17th day)	Outcome of implantations: with Brown-Pearce cells, 7 days after implantation	with Brown-Pearce cells, 14 days after implantation	with Brown-Pearce cells, 22 days after implantation	with Brown-Pearce cells, 30 days after implantation	with V2 carcinoma cells, 14 days after implantation	with V2 carcinoma cells, 22 days after implantation	with V2 carcinoma cells, 30 days after implantation	with Sarcoma I cells, 14 days after implantation	with Sarcoma I cells, 22 days after implantation
			LAT RAT	LAT RAT	LAT RAT	LAT RAT	LF RF	LF RF	LF RF	LPT RPT	LPT RPT
a) Rabbits injected intraperitoneally with 10cc of a 1:10 saline extract of Brown-Pearce tumors on the 1st, 5th and 9th days; implantation of Brown-Pearce cells, V2 carcinoma cells and Sarcoma I cells on the 17th day	8-56	1:64	n n	n n	n n	n n		n n	n n		
	8-54	1:32	n	n	n	n n		n n	n n		
	8-55	1:32				n n					
	8-49	1:32	n			n n	n				
	8-60	1:16	n	n							
	8-45	1:32									
	8-58	1:32	n	n		†					
	8-51	1:16									
	8-53	1:4				†					
	8-46	0			†			†			
	8-48	0									
	8-50	0			†			†			
	8-52	0			†			†			
	8-57	0									
	8-59	0			†			†			
							E 0 3 cm.				
b) Control rabbits, not immunized, but implanted as above	8-61	0									
	8-62	0									
	8-63	0			n	n					
	8-64	0									
	8-65	0									
	8-66	0									

n=nil; LAT=left anterior thigh; LF=left foreleg; RPT=right posterior thigh; etc.

The dose of Brown-Pearce tumor cells was very large, as witness the size of the growths in the control animals seven days after the implantations.

CHART 4

the 7th day in the control rabbits and in the treated ones that had failed to develop the specific antibody (8-46, 8-48, 8-50, 8-52, 8-57, 8-59), the amount of tumor tissue introduced proved overwhelming to all except one of the rabbits that had developed the antibody, these animals (8-54, 8-55, 8-49, 8-60, 8-45, 8-58, 8-51, 8-53) all manifesting palpable Brown-Pearce tumors, which were, however, much smaller than those in the controls and in the antibody-free animals. The one rabbit with antibody titer as high as 1:64 (8-56) proved wholly resistant to implantations with the Brown-Pearce tumor cells. The Sarcoma I grew progressively in all of the rabbits throughout the period of observation (the growths had attained such size on the 30th day that their silhouettes had to be omitted from the chart, as rendering the latter unwieldy), and the V2 carcinoma did so in all except two (8-56, 8-54), in which palpable growths appeared early but promptly regressed. It is plain that the rabbits having the specific Brown-Pearce antibody offered no primary resistance to the rather small implantations of V2 carcinoma and Sarcoma I cells, tumors resulting from these implantations which were initially quite as large as were those in the antibody-free and control groups. All of the rabbits died as result of one or another of the tumors, some of them as early as the 22nd day. It is interesting to note in passing that Brown-Pearce tumors regressed in three of the control rabbits (8-55, 8-63, 8-65) in which the V2 carcinoma and Rabbit Sarcoma I enlarged progressively; and that in rabbits 8-56 and 8-54 (the two animals with highest titers of the specific Brown-Pearce antibody) the V2 carcinoma regressed, while the sarcomas continued to enlarge.

The results of further observations are presented in Chart 5. A number of rabbits in which Brown-Pearce tumors had previously regressed (see Charts 1 and 3) were given three intraperitoneal injections at 4 day intervals of 10 cc. of 1:10 cell-free, saline extracts of frozen Brown-Pearce tumor tissue, to raise to a high level the titer of specific Brown-Pearce antibody by means of the anamnestic response previously observed (3). After a further interval of 8 days, serum was procured and tested for the specific antibody, and the rabbits were implanted as before with suspensions of Brown-Pearce, V2 carcinoma, and Rabbit Sarcoma I cells. As might have been anticipated, the rabbits in which Brown-Pearce tumors had previously failed to grow or had regressed (see Charts 1 and 3) all proved resistant on reimplantation with Brown-Pearce tumor cells, though growths resulted from implantations of the same cell suspension in all of the control animals. Yet as a group the resistant rabbits proved as susceptible as the controls to the V2 carcinoma and Rabbit Sarcoma I cells. It is especially noteworthy that both the V2 carcinoma and the Rabbit Sarcoma I grew progressively in the three animals that possessed the specific antibody (6-56, 6-57, 10-70), though this was present in high titer at the time of the implantations, owing to purposeful stimulation. It is also interesting to note that the specific Brown-Pearce antibody had been absent at all of the

Results of Implantation of Various Tumors into Rabbits Resistant to the Brown-Pearce Carcinoma

Treatment	Rabbit No	Days since previous implantation with Brown-Pearce tumor cells	Titer of specific Brown-Pearce antibody	Outcome of implantations: with Brown-Pearce cells, 14 days after implantation	with Brown-Pearce cells, 22 days after implantation	with Brown-Pearce cells, 30 days after implantation	with V2 carcinoma cells, 14 days	with V2 carcinoma cells, 22 days	with V2 carcinoma cells, 30 days	with Sarcoma I cells, 14 days	with Sarcoma I cells, 22 days	with Sarcoma I cells, 30 days
				LAT RAT	LAT RAT	LAT RAT	LF RF	LF RF	LF RF	LPT RPT	LPT RPT	LPT RPT
a) Rabbits in which Brown-Pearce tumors had previously regressed: saline suspensions of Brown-Pearce tumor injected intraperitoneally to increase titer of specific antibody	6-56	328	1:128	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
	6-57	"	1:32	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
	6-58	"	0	n n	n n	n n	n ●	● ●	● ●	● ●	● ●	● ●
	6-63	"	0	n n	n n	n n	n n	● ●	● ●	● n	● ●	● ●
	7-01	"	0	n n	n n	n n	n n	n n	n n	● n	● n	n
	7-00	"	0	n n	n n	n n	n n	n n	n n	● ●	● ●	● ●
	10-62	50	0	n n	n n	n n	n n	n ●	n n	● n	● ●	● ●
	10-63	"	0	n n	n n	n n	n n	● n	● n	● ●	● ●	● ●
	10-66	"	0	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
	10-67	"	0	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
	10-69	"	0	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
	10-70	"	1:64	n n	n n	n n	● ●	● ●	● ●	● ●	● ●	● ●
								0 3cm.				
b) Normal control rabbits implanted as above	12-76		0	● ●	● ●	0 n	● n	● ●	● ●	● ●	● ●	● ●
	12-77		0	● ●	● ●	● ●	n n	n n	n n	● ●	● ●	● ●
	12-78		0	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●	● ●
	12-79		0	● ●	● ●	n n	n n	n n	n n	● ●	● ●	● ●
	12-80		0	● ●	● ●	0 0	● ●	● ●	● ●	● ●	● ●	● ●
	12-81		0	● ●	● ●	● ●	n n	n ●	n ●	● ●	● ●	● ●

n = *nil*; LAT = left anterior thigh; LF = left foreleg; RPT = right posterior thigh; etc.

Some of the rabbits had been used in previous experiments – see Charts 1 and 3.

CHART 5

repeated bleedings of the rabbits in which Brown-Pearce tumors had previously grown for a time and then regressed (animals 6-58, 6-63, 7-01, 7-00 of Chart 3 and 10-62, 10-63, 10-66, 10-67, 10-69 of Chart 1), and that in the present test it was likewise absent following potent immunizing injections. Even so the animals proved resistant on implantation with the Brown-Pearce tumor cells, as Chart 5 shows.

Effect of the Specific Antibody on the Course of Established Brown-Pearce Tumors

The preceding experiments have shown that rabbits whose serum contained the Brown-Pearce antibody in significant titer as result of repeated intraperitoneal injections of cell-free, saline extracts of the growth were resistant to implanted Brown-Pearce tumor cells, whereas normal rabbits and those that failed to develop the specific antibody following the injections proved primarily susceptible[1]. In the resistant animals just referred to the specific antibody was present at the time the implantations were made. What can be said about the effect of the antibody on tumors already growing at the time of its appearance? Does secondary retrogression take place in such instances? As bearing on these questions, the observation was made in preceding experiments (3) that rabbits which failed to manifest the specific antibody at all of repeated bleedings often overcame their Brown-Pearce tumors nevertheless, whence it was concluded that "regression of the growth, at least as it occurs in some instances, is probably not due to the specific antibody" (3).

In a subsequent detailed analysis of the outcome of implantations of the Brown-Pearce tumor in relation to the development of the antibody in forty-three rabbits of various breeds, thirteen cases were encountered in which Brown-Pearce tumors that had reached 1.0 to 4.0 cm. in diameter during the first 10 to 20 days after implantation regressed more or less abruptly during the ensuing weeks, although the specific antibody could not be detected in their blood serum at any of repeated tests between the 16th and 50th days. The course of the tumors in the thirteen animals just mentioned was practically identical with that in eight other rabbits in which abrupt regression likewise occurred during the 3rd, 4th, or 5th weeks, though in the latter instances the specific antibody appeared in high titer, usually before or during the 3rd week after implantation.

In sum, past findings show that regression of established Brown-Pearce carcinomas has been observed more frequently in the absence of the specific antibody than in its presence; and along with certain findings of the present

[1] This constitutes perhaps the second instance in which resistance to transplanted tumor cells has been elicited by means of sedimentable cell constituents, as distinct from intact living cells. For in two experiments not reported in detail, the observation was previously made that 5 per cent and 35 per cent respectively of C58 mice injected intraperitoneally with sedimented materials procured from Line I leukemia cells later survived implantations of the tumor cells that overcame all of the uninjected control animals (MacDowell, E. C., Claude, A., *et al., Carnegie Institution of Washington Year Book No. 40*, 1940–41, 248).

paper (see especially Charts 1, 5, and 9) they make it plain that factors other than the specific antibody are probably responsible for regression of the growth in the majority of cases. That the antibody may have some influence on the course of the tumors, however, is indicated by a fact already mentioned, namely, that abrupt regression of the tumors took place in all of the rabbits which developed it in high titer (see also the examples of Charts 3 and 4), while on the other hand scrutiny of the records of twenty-two rabbits with progressively enlarging tumors shows that eighteen of these had no detectable antibody in their blood at any of several bleedings, while three others showed small or doubtful amounts of it. Yet in one rabbit there was fulminant growth of the tumor, with widespread metastases and death on the 34th day after implantation, in spite of the presence of an increasing titer of the antibody (1:2 on the 18th day and 1:64 on the 28th day). Further implications of these observations will be considered in the Discussion.

Selective Effect of the Specific Antibody on Brown-Pearce Tumor Cells in Vitro

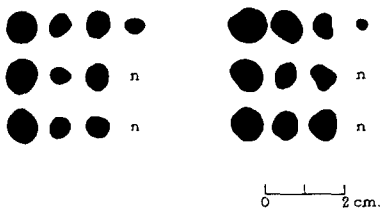
In a first test to learn whether the Brown-Pearce antibody has an effect on the living tumor cells *in vitro*, use was made of fresh serum specimens from a normal "blue cross" rabbit and from another such animal known from previous tests to provide the specific antibody in high titer. These sera were mixed in equal parts in paraffin-lined flasks with a fresh suspension of living Brown-Pearce tumor cells, prepared as already described. After a sojourn of 2 hours in a water bath at 37°C., 0.5 cc. of each mixture was implanted intradermally in three situations on the flanks of four normal agouti test rabbits. Tracings were made on cellophane of the resulting growths on the 8th, 10th, and 12th days. These are reproduced in Chart 6, from which it will be seen that the mixture of Brown-Pearce tumor cells plus normal serum gave rise to growths, whereas that containing tumor cells plus the antibody-containing serum did not.

Chart 7 shows the results of a similar experiment in which various mixtures were implanted intramuscularly in nine normal rabbits. In the first three test rabbits, implantations of mixture (*a*), which contained the Brown-Pearce tumor cells plus Locke's solution in equal parts, resulted in large growths, and so too did implantations of mixture (*b*), which contained the tumor cells plus fresh serum from normal control rabbit 7-89. Mixture (*c*), however, made of the tumor cell suspension plus a serum containing the specific antibody in a titer of 1:128, proved innocuous when implanted in precisely the same way in corresponding muscle situations in the three test animals. Implantations of mixture (*d*), which contained the tumor cell suspension plus the serum from V2 carcinoma rabbit 21-50, resulted in tumors about like those resulting from the mixture with Locke's solution and with normal control serum. So too in the other six test rabbits, mixtures containing Brown-Pearce cells plus sera having high titers of the Brown-Pearce antibody (*f*, *j*, *k*) failed to produce

tumors or gave rise to comparatively small ones, whereas the mixture containing Locke's solution (*e*) and that with serum from a second normal rabbit (*i*) gave rise to large tumors, as did also the mixtures containing sera from rabbits with Sarcoma I (*h*, *l*), and that containing the serum of another V2 carcinoma rabbit (*g*).

The findings with the sera of the V2 carcinoma and Sarcoma I rabbits have additional interest because, as Table I shows, these specimens contained antibodies which react with constituents of various normal and neoplastic rabbit tissues—the induced tissue antibodies described in a preceding paper (6).

Effect of Specific Antibody on Brown-Pearce Tumor Cells *in Vitro*

In vitro mixtures	Outcome of intradermal implantations		
Brown-Pearce tumor cells plus	8th day	10th day	12th day
	Test rabbits 1 2 3 4	1 2 3 4	1 2 3 4
a) Normal control rabbit serum 7-91			
b) Rabbit serum 5-47, which contained the specific Brown-Pearce antibody in a titer of 1:128	n n n n	n n n n	n n n n
	n n n n	n n n n	n n n n
	n n n n	n n n n	n n n n

Mixtures kept at 37°C. for 2 hours prior to implantation.
n = nil

CHART 6

The serum of rabbit 20-52, which carried large cystic V2 carcinomas of many weeks' duration, fixed complement in considerable titer with all of a variety of normal and neoplastic rabbit tissue antigens, including that made from the Brown-Pearce tumor; and much the same was true of the serum of rabbit 21-50 which also had large cystic V2 carcinomas. The sera of Sarcoma I rabbits 9-40 and 9-41 fixed complement moderately well in mixture with the normal rabbit spleen antigen, and one of them also reacted slightly with the Brown-Pearce and Sarcoma I antigens. By contrast, the sera of the two normal control rabbits (7-89 and 7-90) failed to react with any of the antigens. Rabbits 5-47, 5-51, 5-52, and 5-53 were animals in which Brown-Pearce tumors had regressed several months previously; the specific antibody in their blood had been

raised to the levels shown in the table by intraperitoneal injections of 10 cc. of 1:10 saline extracts of the growth 12 and 8 days prior to the bleedings. The serum of rabbit 5-47 contained only the specific Brown-Pearce antibody, and that of rabbit 5-51 had only slight ability to fix complement in mixture with the normal spleen antigen in addition to its ability to react with the Brown-Pearce tumor material. The sera of rabbits 5-52 and 5-53, however, contained induced tissue antibodies (6) in addition to the specific Brown-Pearce antibody,

Effect of Specific Antibody on Brown-Pearce Tumor Cells

Materials implanted intramuscularly Brown-Pearce tumor cells plus	Titer of specific Brown-Pearce antibody	Outcome of implantations Tumors excised at necropsy on the 15th day
		Test rabbits 1 2 3
a) Locke's solution		
b) Serum from normal control rabbit 7-89	0	
c) " " Brown-Pearce tumor rabbit 5-47	1:128	nil nil nil
d) " " V2 carcinoma rabbit 21-50	0	
		4 5 6
e) Locke's solution		
f) Serum from Brown-Pearce tumor rabbit 5-53	1:256	nil nil nil
g) " " V2 carcinoma rabbit 20-52	0	0 3 cm
h) " " Sarcoma I rabbit 9-41	0	
		7 8 9
i) Serum from normal control rabbit 7-90	0	
j) " " Brown-Pearce tumor rabbit 5-51	1:256	nil nil
k) " " " " " " 5-52	1:256	nil
l) " " Sarcoma I rabbit 9-40	0	

The mixtures were kept at 37°C for 2 hours prior to implantation

CHART 7

as was shown by their ability to react with all of the normal and neoplastic tissue antigens employed.

From the findings of Chart 7 and Table I it would seem that the specific Brown-Pearce antibody is responsible for the antiproliferative effects of the sera of rabbits 5-47, 5-51, 5-52, and 5-53 on the Brown-Pearce tumor cells, the induced tissue antibodies having no discernible effect. For the sera of rabbits 5-47 and 5-51, which contained much of the specific antibody though little or none of the induced antibodies, were quite as effective in preventing growth of

the Brown-Pearce cells as were the sera of rabbits 5-52 and 5-53, which contained considerable titers of the induced antibodies in addition to the specific Brown-Pearce antibody; while furthermore, the sera of the V2 carcinoma rabbits (21-50 and 20-52), which contained higher titers of the induced anti-

TABL

Complement Fixation Tests

Source of sera	Brown-Pearce tumor, 1:40								Normal rabbit spleen, 1:40					
	Serum dilution								Serum dilution					
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:2	1:4	1:8	1:16	1:32	1:64
Normal control rab. 7-89	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normal control rab. 7-90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brown-Pearce rab. 5-47	++++	++++	++++	++++	++++	++++	+++±	±	0	0	0	0	0	0
Brown-Pearce rab. 5-51	++++	++++	++++	++++	++++	++++	+++±	++++	++	++	±	0	0	0
Brown-Pearce rab. 5-52	++++	++++	++++	++++	++++	++++	++++	+++	++++	+++±	+++	0	0	0
Brown-Pearce rab. 5-53	++++	++++	++++	++++	++++	++++	+++	+	+++±	+++±	++	0	0	0
V2 carcinoma rab. 21-50	++±	++±	++	+±	0	0	0	0	++++	++++	++++	+++	±	0
V2 carcinoma rab. 20-52	++++	++++	++++	++±	±	0	0	0	++++	++++	++++	+++	++++	++±
Sarcoma I rab. 9-40	0	0	0	0	0	0	0	0	+++±	+++±	+++	++	±	0
Sarcoma I rab. 9-41	+	+±	±	0	0	0	0	0	+++±	+++	++±	++	+±	+

The tests were made as in previous studies (1, 3, 6) with serum specimens that had been heated at 65°C. immediately before use.

bodies than any of the Brown-Pearce antisera, did not hinder the growth of the tumor cells.

In the experiment of Chart 8, fresh specimens of serum from Brown-Pearce tumor rabbits 5-47, 5-51, and 5-52, which as before contained the specific antibody in high titer, again inhibited growth of Brown-Pearce tumor cells, though not completely, whereas they had no effect on the cells of Rabbit Sarcoma I. The sera of rabbits 21-66, 9-98, and 10-00, which contained high titers

of induced tissue antibodies, as subsidiary tests showed, had no influence on the cells of either growth, nor had the sera of two normal rabbits (7-91 and 7-92).

To learn more about the effects of the antibody *in vitro* an experiment was made in which sera from normal rabbits and those carrying the Brown-Pearce

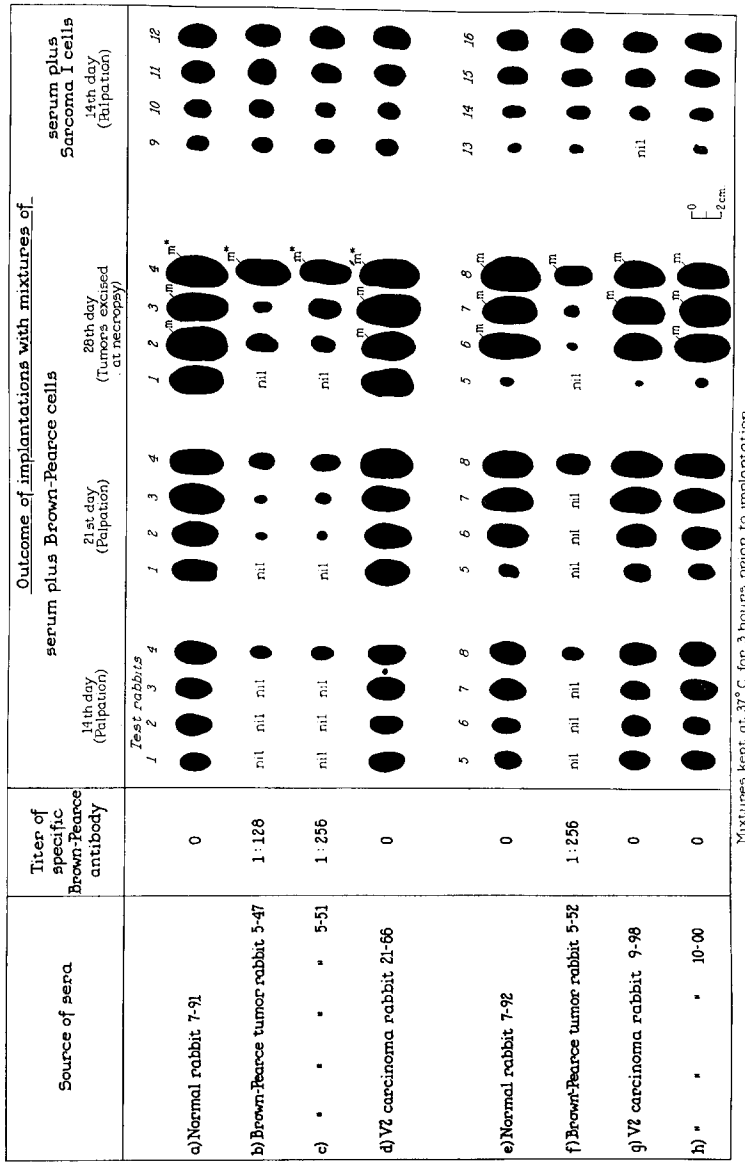
Σ I

with the Sera of Chart 7

ANTIGENS																	
Normal rabbit kidney, 1:40						V2 carcinoma, 1:40						Sarcoma I, 1:40					
Serum dilution						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	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

tumor were mixed with Brown-Pearce, Sarcoma I, and V2 carcinoma cells, respectively. Those that contained the specific antibody (specimens from rabbits 6-56, 6-57) inhibited the growth of Brown-Pearce tumor cells but had no influence on the growth of Sarcoma I and V2 carcinoma cells (Chart 9). The rest of the Brown-Pearce sera, though coming from rabbits in which the Brown-Pearce tumor had previously regressed (see Chart 5) and which were resistant to reimplantation with the cells of that tumor, as subsidiary tests

Selective Effect of the Specific Antibody on Brown-Pearce Tumor Cells



Mixtures kept at 37° C for 3 hours prior to implantation.
m = metastases in regional lymph nodes
m* = " " " " and widespread in viscera also.

CHART 8

showed, contained none of the specific antibody and had no influence on Brown-Pearce tumor cells *in vitro*, nor any on the cells of the two other transplanted growths,—a finding which conforms to previous observations with other tumors, namely that the blood of animals in which transplanted tumors have regressed does not usually affect the tumor cells *in vitro* (7).

Selective Effect of the Specific Antibody on Brown-Pearce Tumor Cells

Source of sera	Titer of specific Brown-Pearce antibody	Outcome of implantations with mixtures of: serum plus Brown-Pearce cells, 15th day	serum plus Sarcoma I cells, 15th day	serum plus V2 carcinoma cells, 25th day
		Test rabbits 1 2 3 4	9 10 11 12	17 18 19 20
a) Normal rabbit 6-50	0	● ● ● ●	● ● ● ●	● ● nil nil
b) Brown-Pearce tumor rabbit 6-56	1:256	nil nil nil nil	● ● ● ●	● ● nil nil
c) " " " " 6-58*	0	● ● ● ●	● ● ● ●	● ● nil nil
d) " " " " 6-63*	0	● ● ● ●	● ● ● ●	● ● nil nil
		5 6 7 8	13 14 15 16	21 22 23 24
e) Normal rabbit 6-51	0	● ● ● †	● ● ● ●	● ● ● ●
f) Brown-Pearce tumor rabbit 6-57	1:64	nil nil nil	● ● ● ●	● ● ● ●
g) " " " " 7-00*	0	● ● ●	● ● ● ●	● ● ● ●
h) " " " " 7-01*	0	● ● ●	● ● ● ●	● ● ● ●

0 — 2 cm.

Mixtures kept at 37°C. for 3 hours prior to implantation.
*Tumors had regressed yet no specific Brown-Pearce antibody had developed; rabbit resistant to re-implantation with Brown-Pearce tumor cells, as subsequent tests showed.

CHART 9

In Vitro Tests with Complement-Free Antisera

In all of the *in vitro* experiments described heretofore rabbit sera procured on the same or the preceding day were used unheated in mixture with unwashed tumor cells. To learn whether the antisera were effective after inactivation of their complement, three serum specimens which had stood 12 to 16 weeks in the refrigerator and which were known from trial complement fixation tests to contain the specific antibody in titers of 1:128, 1:256, and 1:256 respectively, were heated, along with serum specimens from three normal rabbits, at 56°C. for 30 minutes; they were free from demonstrable amounts of complement as subsidiary tests showed. The heated specimens were cooled in running water and mixed with a fresh suspension of Brown-Pearce tumor cells which

had been centrifuged three times and resuspended in successive changes of Locke's solution. The mixtures were kept 3 hours at 37°C. and then implanted as usual into four test rabbits. On the 9th and 14th days, tumors from 2.5 to 6.0 cm. in diameter were present at the sites where the mixtures with normal serum had been implanted, while those at which antibody-containing mixtures had been implanted remained negative with two exceptions, growths 0.8 and 1.4 cm. in diameter respectively arising in these. The findings left no doubt that antisera devoid of complement were capable of exerting their antiproliferative effect, though it is of course conceivable that the test animals may have supplied this to the implanted mixtures.

Cytological Observations

To learn whether the specific antibody causes any visible change in Brown-Pearce tumor cells exposed *in vitro* to its action, practically all of the mixtures in the experiments just described were examined under the microscope immediately after the implantations. The findings were similar in every experiment: The mixtures that contained the specific antisera had intact and for the most part individually suspended tumor cells which were as numerous as were the cells in the normal serum mixtures and which differed from the latter no whit in appearance and distribution. When trypan blue in saline solution was added to the various mixtures in final dye concentrations of 1:300 to 1:1000, the number of cells having stained nuclei,—and which were presumably dead (8),—was usually less than 30 per cent; and in the various experiments there was never a noteworthy difference in the proportions of stained and unstained cells in the mixtures containing the immune sera (procured from nine donors in all) and normal sera from control rabbits of the same stock. In one test, fresh guinea pig serum containing more than 4 units of complement was added to immune and normal serum mixtures and these were incubated at 37°C. for periods up to 18 hours; microscopic examinations at intervals throughout this period again failed to reveal significant diffierences in the number and appearance of the tumor cells in the experimental and control mixtures, although the proportion of cells with stainable nuclei in both types of mixtures increased markedly as the incubation proceeded; it seemed especially noteworthy that there was no lysis or agglutination of the cells in the mixtures with immune serum even after the prolonged incubation.

In a number of experiments samples of the various mixtures were spun lightly in the centrifuge and the pellets of sedimented cells were fixed in either 4 per cent formol-saline, Zenker formol, or Susa's fixative. Microscopic examination of sections stained with eosin and hematoxylin, Giemsa, Masson's trichrome, and Altmann's fuchsin stains again failed to reveal differences in the cells exposed to the immune and normal sera, the majority in all of the mixtures having nuclei that stained well.

DISCUSSION

The principal fact of the experiments here reported would seem to be that an antibody which reacts specifically with a distinctive sedimentable constituent of Brown-Pearce tumor cells is able to suppress their growth under various experimental conditions. Before considering the implications of the findings it may prove enlightening to review briefly what is known about some of the factors that influence the behavior of transplanted tumor cells, with particular reference to the effects of antibodies on them.

Numerous observations have shown that the fate of transplanted tissue cells both normal and neoplastic is largely influenced by genetically determined factors (9). Yet the nature of these factors remains obscure, though it is known that they are often multiple (10) and that sometimes their number may diminish during the course of successive transplantations (11). Furthermore, the mechanism whereby transplanted tissues are overcome in resistant hosts is still undisclosed; in particular, the possible functions of phagocytic and lymphoid cells remain ambiguous in spite of many attempts to discern them (12), and much the same would seem to be true of the part played by humoral influences, as the following citations attest:—

Many years ago Lambert and Hanes showed that the cells of a rat sarcoma would grow quite as vigorously in plasma from tumor-immune rats as in that from normal or tumor-bearing ones (7), and Mottram and Russ (13) and Leitch (14) found that tumor cells incubated for periods of several hours at 37°C. with the serum of tumor-immune hosts were not demonstrably affected thereby; and it is now well known that attempts to transfer tumor immunity passively with the serum of animals in which tumors had regressed have almost regularly failed (12). In an exhaustive review of the literature up to 1929, Woglom (12) concluded that "... except for a few isolated observations which run contrary to the general evidence, no sign of the existence of agents similar to the antibodies so easily demonstrated in the domain of bacteriology has yet been discovered in connection with cancer."

Despite all this, the more recent experiments of Lumsden (15) and Gorer (16) have shown that the fate of certain transplanted cancers of mice and rats can be correlated to a considerable extent with the presence or absence of isoantigens in the cells of the recipients, the tumors in general growing progressively in hosts whose cells possess antigens in common with those of the tumor cells, and regressing in animals with cells devoid of such antigens. Both observers found that isohemagglutinins appeared transiently in the blood of host animals during or immediately after the regression of transplanted tumors, as also in that of normal animals injected repeatedly with blood or minced tissues from suitable individuals of the same species, either selected hybrids or members of a different inbred line. In addition, Lumsden noted that the titer of isohemagglutinins in the sera of tumor-regressed animals ran roughly parallel with the ability of the sera to dissolve homologous macrophages and sarcoma cells in tissue culture, and Gorer observed that antisera prepared by injecting leukemic cells into mice naturally resistant to them were capable of retarding the growth of the leukemic cells under appropriate conditions.

While these observations would seem to imply that isoantibodies may be directly

responsible for the regression of transplanted tumors, it should be pointed out that the findings as a whole are far from conclusive in this respect. For Gorer observed qualitative differences in the hemagglutinin and the protective antibody that appeared in the blood of his immunized mice, and he concluded that the former probably had little importance in the defensive reactions (16); while Lumsden likewise observed noteworthy differences in the isoantibodies elicited by nucleated and non-nucleated cells, and differences also in the antisera of rats immunized against homologous malignant (sarcoma) and non-malignant (spleen, testis) tissues (15). Furthermore the isoantibodies that appeared in the blood of the tumor-regressed hosts in the experiments of Lumsden and Gorer were of low titer and transitory, and it is conceivable that they represented mere parallel, or perhaps adjuvant, reactions. In this relation it is noteworthy that the resistance to tumors which can be elicited by the injection of minced tissues into normal animals is likewise transitory and easily overcome as a rule (9), while the immunity that results when a transplanted tumor regresses is enduring, practically absolute, and independent, as already mentioned, of readily demonstrable and persisting humoral factors.

As bearing further on the points under discussion, it should be noted that tumor cells often grow progressively in hosts having cells with isoantigenic constituents presumably different from their own (9, 16), and sometimes, under special circumstances, in hosts of alien species (18); while furthermore, in certain exceptional instances the pattern of transplantability of neoplastic cells arising in inbred stocks of mice and their hybrids has been found to differ from that of normal tissue cells of identical provenance (17), and several investigations have indicated that resistance to various tumors may be stimulated experimentally in hosts of the inbred lines of animals in which they originated (19), though it seems doubtful that the animals of the various lines were actually homozygous.

From the findings just set forth it becomes plain that further observations are necessary to determine the precise part played by isoantibodies and perhaps by other genetically determined influences in bringing about the regression of transplanted tumor cells. In the case of the Brown-Pearce carcinoma, the fact that the tumor arose in a hybrid host and has been transplanted in mixed stocks during more than two decades will render difficult if not impossible a precise analysis of any genetic factors which may be concerned in its frequent regression. Yet there is no reason to suppose that the processes involved therein differ significantly from those concerned in the regression of other cancers transplanted in hybrid hosts.

Whatever may cause regression of the Brown-Pearce carcinoma, it is clear that some factor (or factors) other than the specific antibody of the present work is responsible for this in most instances; for regression of the growth (and resistance to reimplantation with it) has been observed more frequently in the absence of the specific antibody than in its presence, as the present and preceding studies show (1, 3). Indeed there is no certainty that the specific antibody ever operates solely to bring about regression, though an observation already cited, namely that the growth regressed abruptly in most of the

hosts that developed antibody in high titer while frequently it grew progressively in hosts that failed to do so, suggests the possibility, among others, that the antibody may have influenced the outcome. As distinguishing further between the effects of the specific antibody and that of the unknown factor (or factors) responsible for regression, the fact deserves mention that the sera of hosts in which the tumor had regressed did not suppress the growth of the tumor cells *in vitro* unless they contained the specific antibody (Charts 5 and 9). In addition it may be pointed out that the specific Brown-Pearce antibody is by definition not an isoantibody, since detailed studies have shown that while it has always reacted with extracts of the Brown-Pearce carcinoma it has regularly failed to react with extracts of a variety of other rabbit tissues, either normal or neoplastic, whether procured from normal animals or from hosts in which the Brown-Pearce tumor had grown progressively and metastasized or from others in which it had regressed, and it has not agglutinated the erythrocytes of rabbits in any of the categories just mentioned (3).

What further can be said about the effects of the specific antibody on the Brown-Pearce tumor cell? The cytological observations indicate that the antibody does not bring about gross alterations in the appearance of the tumor cells when incubated *in vitro* with them, although it suppresses their ability to grow. In this relation it should be noted that the possibility has long been perceived that antibodies may perhaps render cells unable to proliferate without altering notably their forms or other functions, in this respect resembling the more ideal chemotherapeutic agents (20). As exemplifying this possibility, the work of Ascoli and of Dochez and Avery on the "antiblastic" effects of certain antibacterial antibodies may be cited (21), and also the observations of Taliaferro, who has described "ablastic" antibodies that suppress the reproduction of *Trypanosoma lewisi* in the rat and *T. duttoni* in the mouse, the antibodies accomplishing this suppression moreover without visible or permanent injury to the parasites, which remain alive, motile, and capable of infecting new hosts after a sojourn of months in the blood of animals having effective antibody titers (22).[2] It is interesting and perhaps significant that the Brown-Pearce tumor cells do not seem to "protect" their distinctive constituent from the action of the specific antibody, whereas many living cells and notably those of certain neoplasms provide such protection for viruses (23).

Much effort has been made to learn the nature of the distinctive sedimentable constituent of the Brown-Pearce carcinoma cell. It has a remarkable tissue specificity, being regularly present in extracts of the Brown-Pearce carcinoma

[2] For another possible explanation of the findings, see the paper of D. L. Augustine (*Proc. Am. Acad. Arts and Sciences*, 1943, **75**, 85). In this relation it is interesting to note Medawar's hypothesis that the destruction of homologous skin grafts in rabbits is due to the action of antibodies that prevent nuclear division in the cells of the grafted tissue (Medawar, P. B., *J. Anat.*, 1945, **79**, 157).

but not detectable in extracts of other rabbit tissues, either normal or neoplastic (1, 3). Its chemical properties suggest that it may be a protein, perhaps a nucleoprotein; filtration and centrifugation experiments have shown that it is always associated with particles having a size comparable to that of the larger viruses; while serological, chemical, and morphological observations considered together have indicated that the distinctive substance may be associated with the "microsomes" of the Brown-Pearce tumor cells (1, 3), perhaps in somewhat the way that the filtrable agent responsible for Chicken Tumor I seems to be associated with the microsomes of the fowl sarcoma cells (24). Unlike the filtrable tumor-producing agents as a class, the distinctive constituent of the Brown-Pearce tumor cell seems to lack the ability to parasitize other tissue cells—at any rate it has thus far failed to accomplish this under a variety of experimental conditions (1), the negative results conforming to those of attempts to extract pathogenic agents from the generality of mammalian tumors. Whatever its chemical and physical nature may be, the distinctive constituent may conceivably play a crucial part in the proliferative activities of the Brown-Pearce tumor cells, inasmuch as their growth is suppressed by the antibody that reacts specifically with it. Indeed it may be their ". . . actuating cause—a self-reproducing substance that maintains the tumor cells as such . . ." in Rous' words (25), perhaps an "autokatalytic growth substance" such as Leo Loeb has tentatively postulated as effecting the cancerous state within cells (26), or a distinctive cytoplasmic constituent of the sort that cytologists have long contemplated as possible determinants of cellular differentiation (27) and as such responsible at least in part for the distinctive traits and neoplastic activities of the Brown-Pearce tumor cells. That it may not be unique is indicated by observations on the V2 rabbit carcinoma, the cells of which have been found to yield another sedimentable substance, identifiable by serological means, which has not been detectable in extracts of normal rabbit tissues or in those of other rabbit neoplasms, including virus papillomas of the type from which the V2 carcinoma originally derived (28).

SUMMARY

Experiments are reported in detail which show that an antibody which appears in the blood of certain rabbits implanted with the Brown-Pearce tumor or injected with cell-free extracts of it is capable of suppressing the growth of the tumor cells under a variety of experimental conditions, the effects of the antibody being wholly distinct from those of unknown factors that frequently bring about regression of the growth. The implications of the findings are discussed with particular reference to facts indicating that the distinctive cell constituent with which the antibody reacts may play a significant part in the proliferative activities of the Brown-Pearce tumor cell.

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