

# ON THE EFFECT OF SUBCUTANEOUS ADMINISTRATION OF ANTITUMORAL SERUM ON THE DEVELOPMENT AND GROWTH OF THE TRANSPLANTED BROWN-PEARCE TUMOR

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Specific immune sera have been used in the last few years to determine the characteristics of the antigenic properties of tumors. With the help of these sera a number of authors [4, 5 and others] established that a single antigen specific for all cancerous tumors of the human does not exist. In the organs and tissues of a cancer patient that are not affected by tumor the "cancer antigen" is absent [6], and it is contained only in the tumor elements. By its chemical nature the tumor antigen presents itself as an intricate complex of amino acids [3]. The acquisition of monospecific sera in connection with the establishment of a "cancer antigen" [7, 8 and others] and their use for the treatment of a tumor process is, essentially speaking, only beginning [2, 9, 10], and would seem to open new possibilities in the treatment of cancer. This was well expressed by V. S. Gostev [1] in the following words: "The acquisition of monospecific antisera and monospecific antibodies will set the stage for a successful solution to the problem of cytotoxino-therapy, in particular to the problem of the action of antibodies on the malignant growth of tissues in the cancer afflictions." In our investigations we did not begin from the idea of obtaining sera which would contain only antibodies specific for tumor antigen. The prevalent idea of the necessity, as much as possible, of enriching the obtained sera with specific antibodies and getting rid of "ballast" substances scarcely reflects the complex immunological mechanisms determining the resistant properties of the organism to the disease in general, and, in particular, to the tumor process. The fact is that the biological characteristics of tumors are related, in a close manner, not only to the species specificities, and reflect not only these, but also to the properties obtaining in animals of other species. Thus, in the tumor process to a greater or lesser degree there appear mechanisms departing from their roots deep in the evolutionary development and establishment of the organism. These peculiarities also determine the unique reactive properties of the organism as a whole, and are expressed in the formation of characteristics of the individual tumor

process. The viewpoint of V. S. Gostev noted above, reflecting the contemporary direction in cytotoxino-therapy, is one-sided, and the expected therapeutic effect from such sera is related to their selective action on the tumor process.

Preparing the antitumoral sera, we first took into account the role of the nervous system in the phenomena of immunity, and especially its receptor link, inasmuch as this dictated the necessity of including in the immunobiological reactions the basic barriers of the organism from the aspect of its evolutionary development and establishment. Secondly, we directed our attention to the structure and function, comparable to a mosaic, of tumor tissue (the presence of different antigens in the same tumor) and the possibility of differentiating the tumor elements. All these gave us a basis for speaking of the necessity of all the "specific" and "nonspecific" antibodies being present in the obtained sera, having a wider range of activity in the fundamental mechanisms giving rise to, and elevating, the resistant properties of the organism.

## EXPERIMENTAL METHOD

We obtained sera from the blood of dogs which were immunized with a suspension of the Brown-Pearce tumor in physiological saline. The sera were assayed in biological tests, whereby the action of the sera in these tests was accompanied by inhibition of cell division and symptoms of hyperplasia of organs rich in reticuloendothelium. Investigations were carried out on the transplantable strain of Brown-Pearce rabbit tumor. Fifty-six male rabbits were used for the experiments (35 experimental and 21 control). The transplantation was performed in the left gonad by means of introducing 0.5 ml of a tumor suspension in physiological saline. After 18 days from the moment of this inoculation we began to inject the experimental group 2-3 times per week with the antitumoral serum on the basis of 0.3 ml per kilogram of body weight. In the rabbits at that time it was possible to see an increase in the size and density

of the afflicted gland, and a random mortality of the animals (Table 1) in the first 2-3 weeks passing after the moment of inoculation testified to the magnitude of injury of the internal organs. In Tables 1 and 2 there is included only a part of the material, since changes of analogous presentation were observed in the remaining animals.

#### EXPERIMENTAL RESULTS

In the control group (see Table 1) we observed no cases of spontaneous resorption of the tumors. The animals in this group lost weight, the process clearly progressed (a marked increase was noted in the size of the sex gland, it was possible to feel rod-like extensions emanating from appendages in the pelvic region, clusters of nodes in the peritoneal cavity, etc.), and at autopsy this was confirmed on occasion by marked metastatic involvement of almost all the internal organs. In the experimental group we attempted to treat the tumor-bearing animals to the point of clinical recovery (normalization of the blood picture, increase in weight, absence of metastases in a randomly sacrificed animal, etc.).

Using microscopic investigation it was discovered that the tumor consisted of epithelial elements, circular in form, lying very close together and not possessing a natural stroma. Not infrequently there were encountered foci of hemorrhages and here and there, vessels of the capillary type. The tumor clusters tended toward a central necrosis. In the area of necrotic foci there was observed karyorrhexis, pyknosis, and occasionally, vacuolization of the cell nucleus. The tumor cells grew destructively and in an infiltrating fashion. Along with the progression of the tumor process a leukocytosis developed (attaining up to 18,000 to 20,000 at the end of the animal's life) involving the neutrophilic forms and with a concomitant decrease in the lymphoid elements. In the course of the rabbits' recovery their weight usually increased, and only in those cases where the process continued, although involvement was limited to only the surrounding tissues, did the weight not only not increase, but significantly fall (Table 2).

Along with the administration of the serum, the duration of the animals' life increased, and on autopsy it was possible to note a complete absence of visible metastases and a resorption of the original focus. However, the fact is noteworthy that in those cases where the original focus existed for a prolonged period of time, metastases in the lungs, diaphragm, and kidneys were persistently retained as well. Apparently, it makes sense at a given stage of treatment to remove the primary focus and secure the liquidation of metastases as fast as possible. As the amount of administered serum was increased at times, the involvement of the organs progressively decreased, especially those which are most quickly involved by metastases (see Table 2). In the

process of resorption of the metastases it was possible to observe how the metastases, in the form of succulent whitish knots jutting out on the surface of the organ, then acquired a faded white, and subsequently yellowish, color. At that point they did not protrude on the surface of the organ or tissue and they were slightly deformed.

Upon microscopic investigation during the process of resorption both the primary focus and the metastases drew attention to a phenomenon which was described by us earlier in our study of the role of the nervous system in the tumor process. A reaction of the mesenchymal elements, the microphages (macrophages) was absent, growth of the connective tissue elements was noted, etc., and in the meantime, amidst the mass of tumor cells, fields of lysed tumor elements were encountered, in which were disseminated remnants of nuclear substance, separate preserved nuclei or their shadows. Here and there parts were found, presenting themselves as conglomerates of swollen tumor cells with coarse clumps of nuclear substance, staining intensely with hematoxylin, creating the impression of a peculiar "condensation" of nuclear material inside the cell protoplasm.

We postulate that in the internal milieu of the organism there appear oncolytic factors which are not only capable of causing lysis of the tumor cells but also of the unique process of "condensation" of the nuclear material; as a consequence of this the vital properties of the tumor are markedly disrupted and the action of enzymatic origin is facilitated, guaranteeing the further molecular disintegration of the living material in the tumor cells and their resorption.

The resultant reactive shifts in the organism appear in the blood as well. If, before the administration of the serum, there existed a tendency toward the upgrowth of a leukocytosis involving the neutrophilic forms and a considerable decrease in the lymphoid forms, then with regular administration of the serum the leukocytosis subsided and the relative composition of neutrophilic forms gradually fell; in conjunction with this the lymphocytic series rose, and even isolated lymphohistiocytic cells appeared. Thus, the blood picture gradually returned to normal.

#### SUMMARY

Brown-Pearce tumor was inoculated to experimental rabbits. Serum obtained from dogs immunized with suspension of Brown-Pearce tumor in physiological saline was injected to 35 experimental rabbits (2-3 times a week, 0.3 ml per kg of body weight). No serum was administered to 21 control animals inoculated with Brown-Pearce tumor. Individual rabbits of experimental groups were sacrificed during the process of treatment; the surviving experimental animals recovered in time as a result of serum treatment. Individual control animals were sacrificed too, whereas the rest of the rabbits of this

TABLE 1. Experimental Data (control group)\*

Color of the rabbit	Weight of the rabbit(g)		Duration of life (in days)	Sites of metastatic involvement												Comments			
	before inoculation	at time of death		lungs	diaphragm	epiploon	gastric serosa	spleen	liver	small intest. serosa	lge. intest. serosa	mesentery	adrenal	kidney	peritoneum		urinary bladder	gonad	
Gray	1 770	1 620	17	++	—	++	++	++	++	++	++	++	++	++	++	++	++	++	—
»	2 300	2 200	19	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
»	2 300	1 700	27	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
White	1 910	1 780	17	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
»	2 300	2 110	30	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
»	1 710	1 600	25	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
Gray	1 930	1 580	23	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
Black	2 730	2 630	18	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	—
White	1 920	1 820	16	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Sacrificed (16 days)
»	1 950	1 930	30	—	+	++	++	++	++	++	++	++	++	++	++	++	++	++	—
»	2 700	2 460	45	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Sacrificed after 20 days
Gray	2 400	1 950	20	—	+	++	++	++	++	++	++	++	++	++	++	++	++	++	—
White	2 150	1 900	12	—	++	++	++	++	++	++	++	++	++	++	++	++	++	++	Sacrificed after 12 days

\* Arbitrarily, for the expression of the degree of involvement of the organs or tissues by tumor, we adopted the following symbols: Isolated focus +, a group of foci ++, involvement of 3/4 +++, involvement of the entire organ +++++, and lack of involvement — (minus).

TABLE 2. Experimental Data (experimental group)

Color of the rabbit	Weight of the rabbit (in g)	Initiation of treatment (in the numerator), number of administrations of antitumoral serum (in the denominator)	Sites of metastatic involvement												Duration of life (in days) at the time of sacrifice				
	before inoculation		at time of death	heart	lungs	diaphragm	epiploon	gastric serosa	spleen	liver	small intest. serosa	lge. intest. serosa	mesentery	adrenal		kidney	peritoneum	urinary bladder	gonad
White	2 650	1 850	18th day/4	-	+	++++	-	-	-	-	-	-	++++	-	+	++	-	++++	27
Gray	1 900	2 050	18th day/5	-	+	++++	++++	-	-	++	+	+	+	-	++	-	-	++++	34
	2 150	2 470	18th day/18	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	195
White	2 300	2 530	18th day/13	-	-	-	+	-	-	-	-	+	+	-	++	+	-	++	62
	2 400	2 410	18th day/16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	77
"	2 330	1 900	18th day/3	-	+	+	++++	-	+	-	-	-	+	-	+	+	-	++++	27
"	1 830	2 130	18th day/7	-	-	-	-	-	-	-	-	-	-	++	-	-	-	++	175
"	1 600	1 730	18th day/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91
"	2 300	2 000	18th day/7	-	+	+	+	-	-	-	-	-	+	-	++++	-	-	++++	39
"	2 050	1 900	18th day/13	-	+	-	-	-	-	-	-	-	-	-	+	-	-	++	69
"	1 830	2 050	18th day/17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	196
"	2 030	1 620	18th day/15	-	-	-	-	-	-	-	-	-	-	-	++++	-	-	++	101
"	1 900	1 330	21st day/7	-	-	+	++++	-	-	-	-	-	+	-	++	+	-	++++	41
Gray	1 700	1 580	21st day/2	-	+	++++	++++	-	-	-	-	+	++	-	+	+	-	++++	28

group perished 17 to 45 days after the inoculation of the tumor. Resorption of the primary focus and metastases was manifested macroscopically in the acquisition of faded yellow color by the tumor foci, their flattening and tendency to become deformed. Microscopically the primary foci and metastases exhibited the presence of the fields of tumor cell lysis, alternating with the areas of tumor cells in conditions of swelling and "condensation" of the nuclear substance, exteriorly to the cellular protoplasm.

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\* Original Russian pagination. See C.B. translation.