STUDY OF THE CHEMICAL NATURE OF THE SPECIFIC ANTIGEN SUBSTANCE OF TUMORS

V. S. Korosteleva, M. I. Karlina, N. V. Cherburkina

From the Laboratory of Immunology (Director: Prof. P. N. Kosyakov) and the Laboratory of Virus Biochemistry (Director: V. I. Tovarnitsky), D. I. Ivanov Institute of Virology of the Academy of Medical Sciences, Moscow

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The question of the chemical nature of the antigenic substance of cancerous tumors in man has been the subject of study by many scientists. A number of investigators [9, 10, 12, 13, 15, 16] have related the specific antigenic properties of tumors to lipoid substances. However, these findings have not been confirmed by subsequent experimental verification [1, 4, 11, 14]. The contradictory nature of results obtained may be explained by the diversity, and in a number of cases incompleteness, of the methods by means of which the investigators have sought to reveal the carcinoma antigens. Therefore, the questions of the presence in tumors of a specific antigen, and even more so the question of its nature, has remained an open one.

The method of anaphylaxis with desensitization worked out by L. A. Zilber et al., made it possible for the first time to establish that a specific tumorous component is contained in the nucleoproteid fraction of the tumorous tissue [2].

A. K. Saakov [8] obtained with the methods of dermatol agglutination and of complement fixation, data on the specific activity of the globulin fraction from human tumors.

Subsequent investigations of tumors in animals, carried out by L. A. Zilber et al., [3] provide evidence that the carriers of the specific serological activity are to be found in the albumin from mitochondria, both the microsome and the first globulin fraction.

V. S. Korosteleva [5] found that treating the tumorous tissue with a 5% solution of formalin and subsequent extraction by physiological solution make possible the separation of the specific tumorous antigen from the antigenic substances common to the tumorous and normal human tissues. Thanks to this, one has succeeded in furnishing fresh proof of the presence of a specific tumorous antigen by means of the complement fixation and absorption reactions. These experiments have served as a starting point for obtaining specific anti-tumorous sera [6, 7].

The purpose of the present work was to separate and further purify the tumorous antigen from the inert substances, and also to study its chemical composition.

EXPERIMENTAL METHODS

For the purpose of isolating the tumorous antigen, saline extracts (1:20) were prepared from the metastasis of the cancer in the liver, extracts of the liver of a healthy person serving as control, after preserving them in a 5% formalin. The testing of the original salt extracts in the complement-fixation reaction showed that the extract from the metastasis of the cancer in the liver contained, as can be seen from Table 1, a specific tumorous antigen. The extract, obtained by the same means from the normal hepatic tissue, treated with formalin, did not possess antigenic properties.

For the purpose of isolating and purifying the specific antigenic substance, various methods of treating the extract of the tumor were tested; fractional precipitation by ammonium sulfate, alcohol, and acetone, and also precipitation at the isoelectric points. The precipitates obtained and the supernatant liquids were tested in serological trials.

EXPERIMENTAL RESULTS

Preliminary examination of the properties of the specific tumorous antigen showed that it did not precipitate on analysis in distilled water; it was not precipitated by salts of bivalent metals, there was weak coagulation on boiling; and it was not extracted or inactivated by butanol.

The results of these experiments give grounds for considering that the tumorous antigen studied is not a simple albumin, but some kind of albumin complex.

Fractionation of the saline extract from the tumorous tissue by ammonium sulfate at various concentrations showed that the precipitates formed best at 30-50% ammonium sulfate concentrations. It should be noted that with the same treatment of the saline extract of healthy hepatic tissue, no precipitation occurred at any concentration of ammonium sulfate. This fact already allowed one to hope that the specific antigenic substance would be separated from the inert substances.

In an other series of tests, the saline extracts were treated with various amounts of ethyl alcohol. Precipitation occurred with all concentrations of the alcohol.

Precipitation of the antigenic substance from the salt extract was also effected by acetone at a concentration of 50%.

The precipitates obtained by these means were dissolved in an equal volume of 0.1 N sodium hydroxide, neutralized by acid and subjected to serological investigation.

Analysis established the presence of active fractions, and fractions not possessing specific tumorous activity. The most active was the acetone-precipitated fraction (Table 2).

Therefore, all the investigations to identify the chemical nature of the antigen of the tumorous tissue were conducted on the residues obtained by the method of precipitation by acetone. We regarded these residues as a raw impure antigenic material. The amount of antigen obtained in the various tests varied from 22 to 42 mg of dried substance per 100 ml of saline extract.

Total nitrogen content in these antigens varied from 7.0 to 9.2% (on dried substance). The phosphorous content amounted to 0.65 to 0.9%. The dried antigen residue was subjected to hydrolysis with 0.1 N acetic acid to break down the complex, according to the generally accepted method.

As a result of hydrolysis, three fractions were obtained: a residue of pure albumins, an aqueous layer in which sugars were assumed to be present, and a lipid layer. The albuminous residue was subjected to a detailed examination. Its nitrogen content was equal to 14.2%, which corresponds to the mean nitrogen content in albumins. The original dried residue of the antigen and the albuminous residue, after break down of the antigenic complement, was analyzed for amino acid content, by paper chromatography, for which hydrolysis of these preparations was carried out with hydrochloric acid (6 N) for 28 hours.

By means of arranging a series of one-dimensional chromatograms with a different collection of "witnesses" in the product of hydrolysis of the original antigen, we succeeded in identifying the amino acids: threonine, cystine, alanine, proline and leucine (5 spots).

The remaining spots (in all, 8 appeared) might have been a mixture of hexone bases (two spots) and mono- and dicarboxylic acids glycine and serine. The absence of tyrosine and phenylalanine has not as yet been proved.

For a more detailed analysis of the amino acid composition of the albumin fraction of the original antigen, the product of hydrolysis was in addition subjected to two-dimensional chromatography (Fig. 1).

13 spots appeared, and we identified 11 amino acids. Apart from cystine, threonine and alanine, the presence of all the hexone bases was established with precision: lysine, histidine, arginine, dicarboxylic acids (aspartic and glutamic), glycine, serine and valine. The presence of proline and leucine in the product of

hydrolysis was unquestionably confirmed with one-dimensional chromatograms.

Only one-dimensional chromatography was conducted on the product of hydrolysis of the albumin fraction of the antigen in which 10 identified spots appeared more distinctly (Fig.2). Thus, we succeeded in establishing that the amino acid composition of the albumin fraction of the antigen before and after break down of the complex, includes a uniform number of amino acids.

In one of the experiments, ether was added to the saline extract to remove lipids, and the saline extract was tested by the complement-fixation reaction. It was apparent that the extract (freed from fat) was serologically just as active as in the original extract. This justified us in considering that the free lipids do not have a role to play in its antigenic activity.

For the detailed serological investigations of the antigen in the complement fixation reaction, and in immunization of rabbits, a portion of dried powdered antigen (precipitated by the acetone and freed from fat), was prepared.

As in the earlier tests, the antigen powder was dissolved in a decinormal solution of sodium hydroxide, but 15 times more concentrated than the original extract, and the solution was neutralized with hydrochloric acid. Serological study of the preparation (referred to below as K-3), established that considered as an antigen, it gave a positive complement-fixation reaction with anti-tumorous sera, and did not react at all with sera against normal human organs: anti-hepatic and anti-splenic sera (see sera No. 49, 980, and 57 in Table 3), i. e., it possessed the properties of a specific tumorous antigen.

TABLE 1

Investigation by the Method of Complement Fixation of Saline Extracts From Human Tumorous and Hepatic Tissues, Treated with 5% Formalin Solution

Description of sera	Attenuation of sera	Antigen-native		Antigen-fixed		Sera control
		Tumor № 3	Liver № 42	Tumor № 3	Liver № 42	
Anti-tumorous No. 49	1:80	++++	++	++++	±	±
	1:160	+++	+	++++	—	—
	1:320	++	±	++++	—	—
	1:640	+	—	+++	—	—
Anti-hepatic No. 883	1:80	+++	++++	—	—	—
	1:160	++	++++	—	—	—
	1:320	±	++++	—	—	—
	1:640	—	+++	—	—	—
Controls	Antigens	—	—	—	—	
	Complement	—				
	Hemolytic system	++++				

In view of the fact that preparation K-3 was subject to purification and was 15 times more concentrated in comparison with the original saline extract, we sought to apply it in immunization of rabbits, in order to obtain specific anti-tumorous sera. For this purpose, three rabbits were immunized for four weeks with preparation K-3 (3 injections each daily). On the 8th day after the last injection of antigen, blood specimens were taken from the rabbits, and Nos. 377, 729, 672 obtained were investigated in complement-fixation reaction.

TABLE 2

Investigation of the Acetone Fraction by the Method of Complement Fixation

Description of sera	Attenuation of sera	Antigens from native tissues		Original extract from fixed Tumor No. 3	Acetone fraction	Control sera
		Tumor № 3	Liver № 1			
Anti-tumorous No. 49	1:40	++++	—	++++	++++	—
	1:80	++++	—	+++	++++	—
	1:160	++++	—	++	+++	—
	1:320	+++	—	±	++	—
Anti-hepatic No. 883	1:40	—	++++	—	—	—
	1:80	—	++++	—	—	—
	1:160	—	++++	—	—	—
	1:320	—	+++	—	—	—
Controls	Antigens	—	—	—	—	
	Complement	—				
	Hemolytic system	++++				

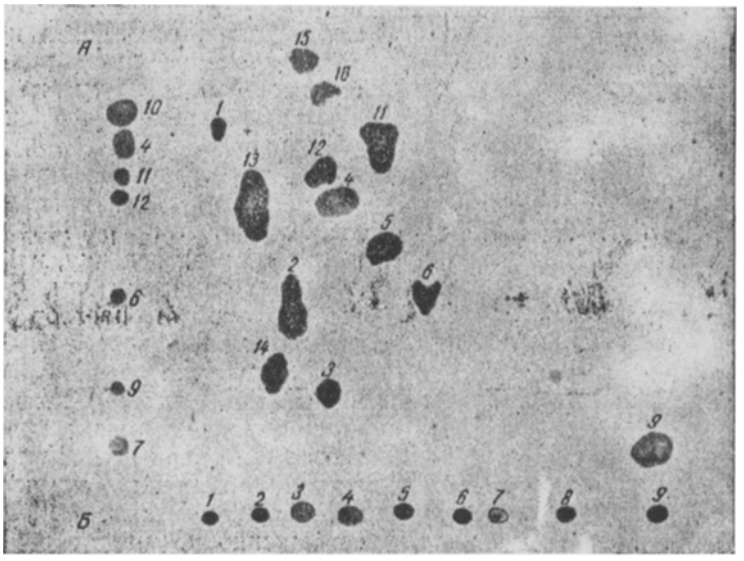

Fig. 1. Chromatogram.
1) Cystine, 2) lysine, 3) arginine, 4) glycine, 5) threonine, 6) alanine, 7) proline, 8) tyrosine, 9) methionine + valine, 10) aspartic acid, 11) glutamic acid, 12) serine, 13) histidine, 14) and 15) not identified; a. witnesses-phenol, b. witnesses-butanol

TABLE 3

Investigation of Sera of Rabbits Immunized with Preparation K-3

Sera	Attenuation of sera	Antigens from native tissues					Antigenic formalized							Control Sera
		Tumor No. 3 glycer.	Tumor No. 2	Tumor No. 31	Liver	Spleen	K-3	Liver	Spleen	Kidney	Cardiac musc.	Lung		
№ 377 to antigen K-3	1 : 20	++++	++	+++	±	—	++++	+	±	+	—	±		—
	1 : 40	++	+	+	—	—	++++	—	—	—	—	—		—
	1 : 80	±	±	—	—	—	++++	—	—	—	—	—		—
	1 : 160	—	—	—	—	—	-+++	—	—	—	—	—		—
№ 729 to antigen K-3	1 : 20	—	—	—	—	—	++++	—	—	—	—	—		—
	1 : 40	—	—	—	—	—	++++	—	—	—	—	—		—
	1 : 80	—	—	—	—	—	+++	—	—	—	—	—		—
	1 : 160	—	—	—	—	—	+	—	—	—	—	—		—
№ 672 to antigen K-3	1 : 20	—	—	—	—	—	++++	—	—	—	—	—		—
	1 : 40	—	—	—	—	—	++++	—	—	—	—	—		—
	1 : 80	—	—	—	—	—	+++	—	—	—	—	—		—
	1 : 160	—	—	—	—	—	+	—	—	—	—	—		—
№ 49 anti-tumorous	1 : 40	++++	+++	+++	++	++	++++	—	—	—	—	—		—
	1 : 80	++++	++	++	+	++	++++	—	—	—	—	—		—
	1 : 160	+++	—	++	—	—	+	—	—	—	—	—		—
	1 : 320	—	—	—	—	—	±	—	—	—	—	—		—
№ 980 anti-hepatic	1 : 80	—	++	++++	++++	++++	—	—	—	—	—	—		—
	1 : 160	—	±	+++	++++	±	—	—	—	—	—	—		—
	1 : 320	—	—	—	++++	—	—	—	—	—	—	—		—
	1 : 640	—	—	—	±	—	—	—	—	—	—	—		—
№ 57 anti-splenic	1 : 80	—	++++	++++	++++	++++	—	—	—	—	—	—		—
	1 : 160	—	++	+++	+++	++++	—	—	—	—	—	—		—
	1 : 320	—	—	—	±	++++	—	—	—	—	—	—		—
	1 : 640	—	—	—	—	++	—	—	—	—	—	—		—
Controls: Antigens		—	—	—	—	—	—							
Complement		—												
Hemolytic system		++++												

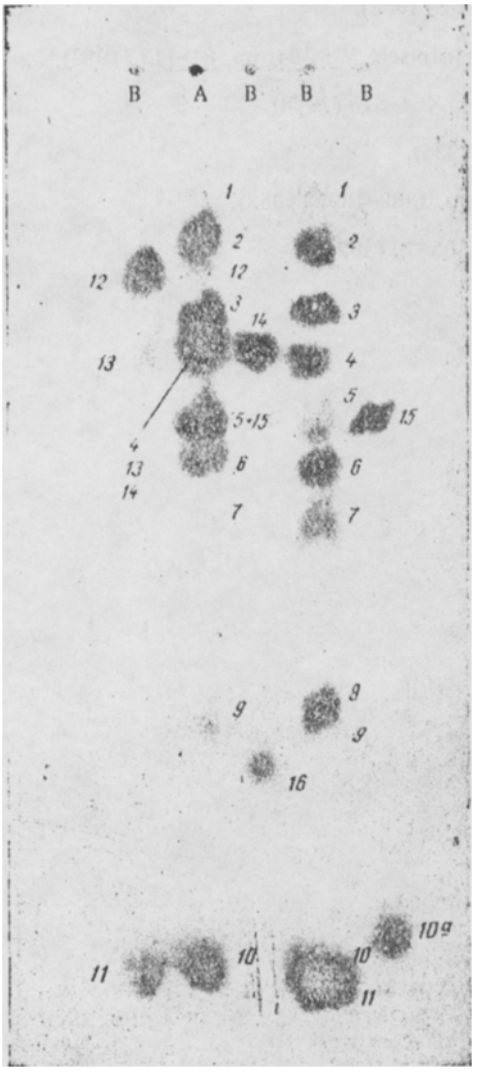

Fig. 2. Chromatogram.
1) Cystine, 2) lysine, 3) arginine, 4) glycine, 5) threonine, 6) alanine, 7) proline, 8) tyrosine, 9) valine, 10) leucine, 10a) iso-leucine, 11) phenylalanine, 12) histidine, 13) aspartic acid, 14) serine, 15) glutamic acid, 16) methionine; A – antigen, B – witnesses.

It is clear from Table 3 that all three sera contained antibodies to the antigen used for immunization.

Serum No. 377, in addition, gave a positive complement-fixation reaction, in dilutions of 1:20 and 1:40 and with antigens from the native tumorous tissues. The absence in the sera of antibodies to the antigens from normal human organs, both fresh and fixed, testifies to the fact that preparation K-3 is a more purified antigen than the original saline extract, and does not contain nonspecific substances common to tumorous and normal tissues.

Thus, from the cancerous tissues, after prolonged storage in a 5% solution of formalin, we succeeded in extracting by physiological solution an antigenic substance specific for human tumorous tissues, which was precipitated from the saline extract by acetone, and represented an intricate complex. The albumin of this antigenic substance presented 15 amino acids, of which 13 were identified: cystine, threonine, alanine, proline, leucine, lysine, histidine, arginine, aspartic acid, glutamic acid, serine, glycine and valine.

The free lipids contained in the saline extract did not possess antigenic activity. The isolated antigenic substance had the properties of a full-value antigen.

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