# THE ANTIGENIC STRUCTURE OF THE ROUS SARCOMA COMMUNICATION I. DETECTION OF THE SPECIFIC ROUS SARCOMA ANTIGEN BY THE PRECIPITATION TEST IN AGAR

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The precipitation test in agar has been used successfully in recent years in the study of the antigenic structure of multi-component systems [12], including tissues affected by viruses [9, 10, 14].

In the present research we attempted to use the method for the analysis of the antigenic structure of the Rous sarcoma—a tumor of fowls caused by a virus.

In the first stage of the investigation the antigenic composition of the Rous sarcoma was compared with the antigenic composition of the organs of healthy fowls. For purposes of comparison various organs and tissues of healthy fowls were used, as follows: liver, kidney, spleen, lung, ovaries, muscles, subcutaneous cellular tissue and blood serum, and in addition a transplanted fowl sarcoma initially induced by methylcholanthrene, and a culture of fibroblasts.

## METHOD

The work was undertaken with a strain of the Rous sarcoma virus obtained from Carr's laboratory. This strain of the virus has been transmitted for many years through fowls to the White Leghorn breed. The strain MKh-659 of methylcholanthrene sarcoma was obtained from A. M. Dyad'kova (Institute of Oncology, Leningrad) and also transmitted through fowls of the same breed.

Normal organs and tissues were taken from similar healthy fowls. Fibroblasts were obtained by I. S. Irlinym from 9-day chick embryos and subsequently grown in tissue culture.

As antigens we used extracts (pH = 7.0-7.2) of tissues ground with sand in physiological saline (1:5 - 1:8). Hyaluronidase was added to the Rous sarcoma extract in a proportion of 1:100. In order to remove coarse particles of tissue and sand, the extracts were centrifuged at the speed of 2500 rpm for 30 minutes.

The proteins were determined by the quantitative biuret reaction, and in some experiments by Kjeldahl's method. Theprotein content of theRous sarcoma extracts varied between 7 and 11 mg/kg, and that of the extracts of organs of healthy fowls and of sarcoma MKh-659 tissue from 12-15 to 20-30 mg/ml.

Antisera to tumor and normal fowl tissues were obtained by immunization of rabbits in accordance with the following scheme.

The first cycle of immunization consisted of three injections: the first a subcutaneous injection of 5 ml of extract with a lanolin depot, and the second and third intramuscular injections of 10 ml of extract. The interval between the first and second injections of the first cycle of immuniaztion was 14 days, and that between the second and third injections 7 days. The second cycle of immunization consisted of two intramuscular injections, each of 10 ml of extract, at an interval of 7 days. The interval between the first and second cycle of immunization was 30-40 days.

In accordance with this immunization scheme we obtained six rabbit sera against Rous sarcoma and two antisera against normal fowl liver, kidney, spleen, lung and muscle, normal fowl serum* and sarcoma MKh-659.

In the experiments both original and exhausted antisarcoma sera were used. Exhaustion was carried out fractionally, in several stages: At first, normal fowl serum was added, then a mixture of healthy fowl's organs (liver, kidney, spleen and muscle), and thirdly, an extract of sarcoma MKh-659. At each stage of the exhaustion procedure the mixture of antisarcoma serum with the exhausting antigens was incubated at 37° for 1-1.5 hours and at 4° for 2-4-16 hours. The precipitates formed on addition of antigens from normal tumor tissues were separated by centrifugation at 2000-2500 rpm for 5-10 minutes. As a rule the exhaustion of 1 ml of antiserum against Rous sarcoma required a total of 20-25 mg of protein of the exhausting antigens. The exhausted antisarcoma serum was investigated by means of the precipitation test in agar for completeness of exhaustion. If it continued to react with any of the antigens in a dose known to be greater than the dose of antigen used for exhaustion of the serum, further exhaustion in relation to this antigen was undertaken. Since the serum became diluted in the process of exhaustion, it was concentrated to

* During immunization with serum, 5 ml of normal fowl serum was given at each of the 5 injections.

the initial volume in a cellophane bag, cooled in a current of air from a fan. After concentration, the serum was dialyzed against physiological saline. The concentration and dialysis were carried out at a temperature of 6-8°.

The antigens and antisera were tested by the precipitation reaction in agar, in a micro-modification, and by the ring-precipitation test. The ring-precipitation test was performed in the usual manner. In the experiments we used 19 immune and 18 normal rabbit sera, 9 specimens of antigens from Rous sarcoma and 5 from sarcoma MKh-659, 10 different antigens each from the liver, kidney, spleen, lungs and muscles of healthy fowls and 10 normal fowl sera.

## RESULTS

A comparison between antigens from the Rous sarcoma and antigens from normal fowl's kidney is made in Fig. 1. The original antisarcoma serum gave several precipitation rings, characteristic of both Rous sarcoma and of normal fowl's kidney. Besides the general precipitation spectrum, an additional band of precipitation with antigen from Rous sarcoma was constantly observed. The same picture was observed with antigens from the liver, spleen, lungs, subcutaneous cellular tissue and ovaries of a healthy fowl, with normal fowl serum and with antigens from fibroblasts and from sarcoma MKh-659.

Only one precipitation band with antigen from Rous sarcoma appeared with the exhausted antisarcoma sera. This precipitation band was not observed with any antigen from healthy fowl's organs or from sarcoma MKh-659 (Fig. 2), despite the fact that the normal antigens and the antigens from sarcoma MKh-659 were present in the majority of experiments in a protein concentration two to three times greater than Rous sarcoma antigen.

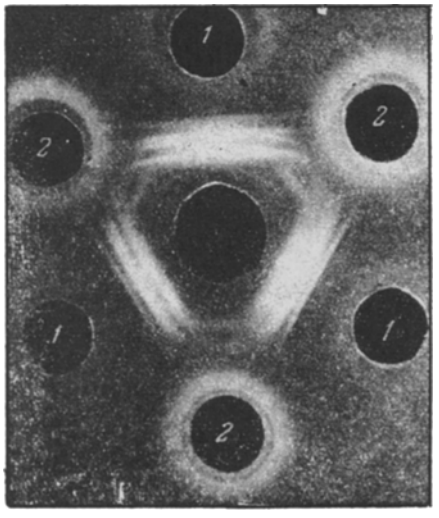


Fig. 1. Comparison of the antigenic composition of Rous sarcoma and of normal kidney. In the center) original serum No. 863 against Rous sarcoma. 1) antigens from Rous sarcoma tissue; 2) antigens from normal fowl's kidney tissue.

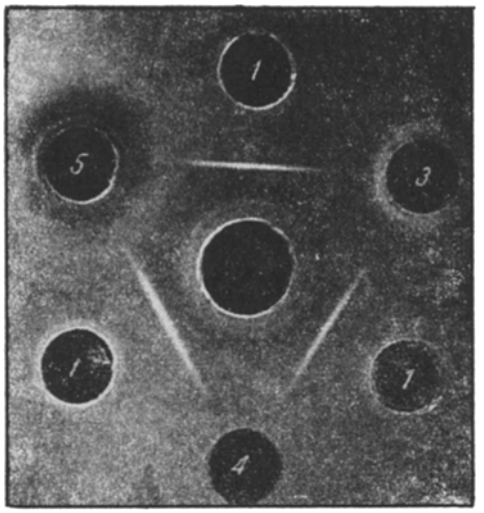


Fig. 2. Detection of specific Rous sarcoma antigen with exhausted antisarcoma serum. In the center) exhausted antiserum No. 863. Exhaustion carried out with normal fowl serum and a mixture of extracts of normal fowl's organs and of a methylcholanthrene tumor. 1) antigens from Rous sarcoma tissue; 3) antigens from tissue of tumor MKh-659; 4) normal serum; 5) antigens from healthy chick's muscle.

The specific Rous sarcoma antigen was also detected when the conditions of the test were even more exacting [1, 4].

One such experiment is illustrated in Fig. 3, from which it can be seen that the precipitation band formed by the specific Rous sarcoma antigen and by specific antibodies against this antigen rests with its ends in the hole containing a heterologous system.

This antigen, consequently, is absent from extracts of normal fowl tissues, and antibodies against it are present only in antiserum against Rous sarcoma.

We were then confronted with the problem: Is the specific Rous sarcoma antigen an isoantigen? In order to exclude this possibility, we induced the tumor not by a cell suspension but by a filtrate of a Rous sarcoma, and compared its antigenic composition with the antigens from the organs of the same fowl. In these experiments too, in which the possible participation of isoantigens was excluded, the specific Rous sarcoma antigen was detected just as clearly as in the preparations demonstrated above.

The precipitation test in agar thus revealed the presence of a specific Rous sarcoma antigen, which was not an isoantigen.

Specific Rous sarcoma antigen was detected in four of the six original sera and in five of the six exhausted antisarcoma sera (exhaustion facilitates the detection of the specific antigen).

Minimal Doses of Antigens in mg / ml of Protein[1] to Produce a Ring-Precipitation Reaction

| No. of rabbit | Antigens / Antisera | From liver tissue | From kidney tissue | From spleen tissue | From lung tissue | From muscle tissue | From normal fowl serum | From tissue of filterable Rous sarcoma | From tissue of methylcholanthrene tumor |
|---|---|---|---|---|---|---|---|---|---|
| 852 | Against liver tissue | 0,01 | 0,018 | 0,036 | 0,016 | 0,064 | 0,008 | 0,01 | 0,018 |
| 853 | | 0,01 | 0,018 | 0,036 | 0,016 | 0,128 | 0,008 | 0,02 | 0,018 |
| 854 | Against kidney tissue | 0,02 | 0,009 | 0,036 | 0,016 | 0,128 | 0,008 | 0,02 | 0,018 |
| 855 | | 0,01 | 0,009 | 0,036 | 0,008 | 0,128 | 0,004 | 0,01 | 0,035 |
| 856 | Against spleen tissue | — | — | — | — | — | — | — | — |
| 857 | | 0,04 | 0,018 | 0,036 | 0,008 | 0,064 | 0.004 | 0,02 | 0,035 |
| 858 | Against lung tissue | 0,01 | 0,009 | 0,036 | 0,016 | 0,26 | 0,008 | 0,02 | 0,035 |
| 859 | | 0,01 | 0,018 | 0,036 | 0,008 | 0,26 | 0,004 | 0,02 | 0,035 |
| 889 | Against muscle tissue | 0,01 | 0,006 | 0,01 | 0,004 | 0,0007 | 0,006 | 0,003 | 0,005 |
| 990 | | 0,01 | 0,006 | 0,01 | 0,004 | 0,0007 | 0,006 | 0,003 | 0,005 |
| 860 | Against normal fowl serum | 0,02 | 0,009 | 0,036 | 0,016 | 0,064 | 0,004 | 0,01 | 0,035 |
| 861 | | 0,02 | 0,009 | 0,036 | 0,008 | 0,028 | 0,004 | 0,02 | 0,035 |
| 862 | Against tissue of filterable Rous sarcoma | 0,02 | 0,009 | 0,036 | 0,008 | 0,033 | 0,008 | 0,005 | 0,018 |
| 863 | | 0,02 | 0,009 | 0,036 | 0,016 | 0,033 | 0,004 | 0,005 | 0,018 |
| 887 | Against methylcholanthrene tumor | 0,018 | 0,025 | — | 0,054 | 0,014 | 0,006 | 0,006 | 0,015 |
| 888 | | 0,018 | 0,025 | — | 0,027 | 0,014 | 0,006 | 0,006 | 0,015 |

[1]The protein content of the antigens was determined by Kjeldahl's method.

Antisera against normal fowl's organs (liver, kidney and muscle) and against normal fowl serum revealed components in homologous antigens that were not present in Rous sarcoma.

The 18 sera taken from the rabbits before immunization in general gave no precipitation reactions with either extracts from healthy fowl's organs or extracts from fowl sarcomas.

Side by side with the precipitation test in agar, we also used the crossed ring-precipitation test for differentiation of the specific Rous sarcoma antigen.

In the ring-precipitation test we used the same antigens and the same antisera as in the precipitation test in agar. The results of one crossed ring-precipitation experiment with antisera from the second cycle of immunization are given in the table.

All the antisera—antitumor and against healthy fowl's organs—reacted more strongly with normal fowl serum than with their own homologous antigen. Consequently, contamination with serum proteins, which are always present in the tissues to be tested, masks the antigenic differences between the individual organs and makes the results unreliable.

The antigens from muscle tissue are an exception.

The present work is the first stage in a study which we are undertaking of the antigenic structure of the Rous sarcoma. The specific antigen of this tumor was found earlier by means of the following reactions: virus neutralization [11, 13], anaphylaxis with desensitization [3, 8], complement fixation [6, 11], passive hemagglutination [5, 7] and precipitation in agar [2]. Most authors considered that this antigen is a virus antigen. In order to study the antigenic structure of the test tissue, the method of precipitation in agar shows great promise, for in practice it will reveal the complete antigen spectrum of the tissue and enable each antigen to be analyzed separately.

When Rous sarcoma is being compared with a homologous normal tissue, difficulty arises in the choice of an ade-

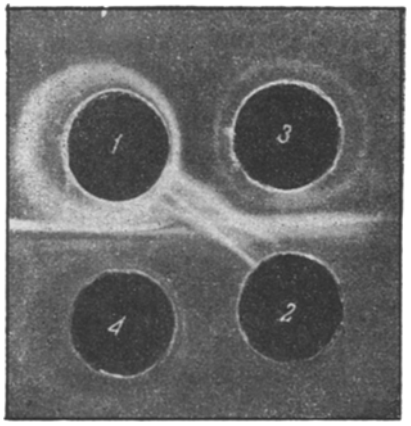


Fig. 3. Comparison of the systems: sarcoma-antisarcoma serum and lung–anti-lung serum. 1) original serum No. 858 against healthy fowl's lung tissue; 2) antigens from healthy fowl's lung tissue; 3) original serum No. 863 against Rous sarcoma tissue; 4) antigens from Rous sarcoma tissue.

quate control. Usually normal fowl serum and muscle are used as a control to this tumor, because this sarcoma grows in the muscle of the fowl [3, 8]. We consider that it is insufficient to restrict the comparison to muscle and normal fowl serum, for in addition to muscle tissue, the sarcoma is mainly composed of fibroblast-like cells, degenerative elements and an amorphous, mucoid substance. Our experiments actually showed that the sarcoma tissue differs from muscle not only in its specific antigen, but also in other antigens present in the organs of healthy fowls.

Besides muscle, we therefore used fibroblasts from chick embryos, grown in tissue culture, spleen, liver, kidney, lungs and normal fowl serum.

The use of the proteins of normal fowl serum as control preparations and, in particular, for the exhaustion of the immune sera in the Rous sarcoma, is of special importance. The serum proteins are extremely powerful antigens, which often mask the weaker precipitation lines of the tissue antigens, and the removal of antibodies against these proteins is therefore an essential condition for the detection of tissue antigens.

In this connection it must be pointed out that failure to differentiate the specific Rous sarcoma antigen by means of the ring-precipitation test is presumably due to the presence of serum proteins in the organs of healthy fowls.

A distinctive feature of the growth of the filterable sarcoma is its mucous character. In addition to the control preparations listed above, we therefore tested a tissue rich in tissue polysaccharides, namely the subcutaneous cellular tissue of a healthy chick. Finally, the last control preparation was antigen from sarcoma MKh-659. We consider that a combination of the antigens of all the selected tissues and organs creates a more adequate control to the Rous sarcoma than the antigens of each organ and tissue taken separately.

In work with non-inbred animals, there is always the possibility of detecting isoantigen instead of the specific tumor antigen.

This possibility was excluded in our experiments by comparing a tumor induced by a filtrate, and consequently arising from the tissues of the host, with the organs of the same fowl. In this case too, a specific antigen which was not an isoantigen was revealed.

By means of the precipitation test in agar we were thus able to detect an antigen which was not present in the organs and tissues of a healthy fowl, nor in sarcoma MKh-659.

Details concerning the nature of the specific Rous sarcoma antigen will be given in later communications.

## SUMMARY

With the aid of the precipitation reaction in agar and the ring-precipitation test the authors compared the antigenic composition of Rous sarcoma with that of the organs of healthy chickens (liver, kidney, spleen, subcutaneous cellular tissue, muscles, etc.), transplantable chicken sarcoma MKh-659 (primarily induced with methylcholanthrene), and normal chicken serum. Evidence was obtained that it is possible to detect the specific antigen in Rous sarcoma extracts only with the aid of precipitation in agar, while the ring precipitation reaction does not permit differentiation of this antigen.

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