DNA PRECIPITATION BY NORMAL RABBIT SERA

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The immunological characteristics of DNA have been studied by various methods: the complement fixation reaction (5, 6), anaphylactic reaction [1] and also precipitation reaction [9, 11].

In investigations in recent years the possibility of nonspecific DNA precipitation with nonimmune animal sera was noted [8, 10, 12]. In connection with this, it became necessary to investigate this fact in order to evaluate properly the data on the immunological properties of DNA.

The purpose of the present work was a study of the characteristics of the rabbit serum factor which causes precipitation of DNA preparations: the distribution of this factor in animals, individual variations in titer, its specificity and stability to storage, and also the effect of DNA polymerization and the presence of protein impurities in DNA preparations on their ability to precipitate.

TABLE 1. Physico-Chemical Characteristics of the DNA Preparations Obtained

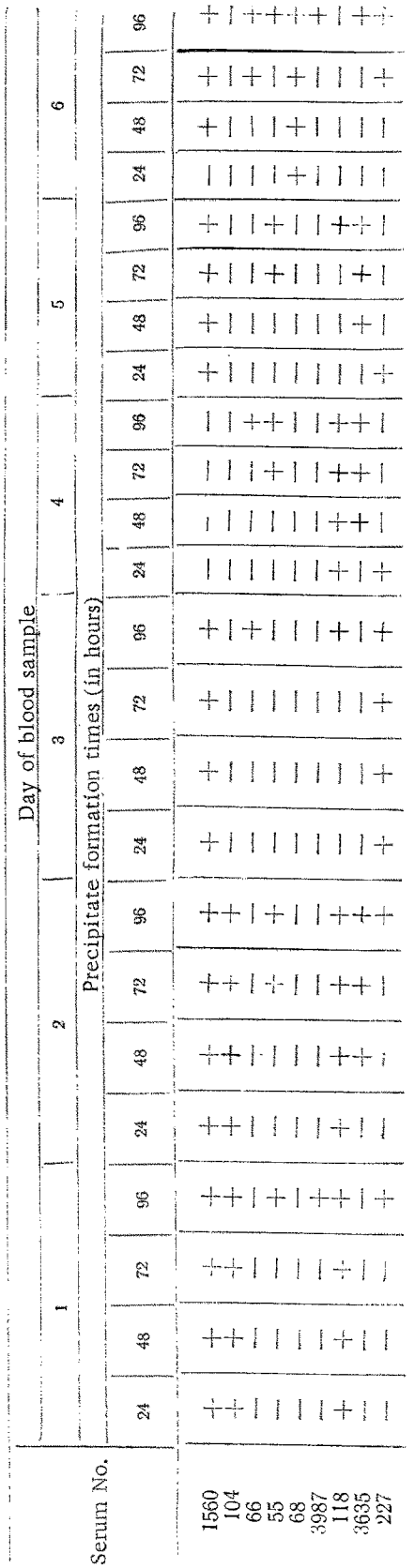
DNA source	N/P	R_{E270}	ε(P)	Molecular wt.
Chicken erythrocytes				
Preparation No. 1	1.68	0.55	6,100	$6.9 \cdot 10^6$
" No. 2	1.56	0.67	7,250	$8.4 \cdot 10^6$
No. 3	1.69	0.57	6.116	$9 \cdot 10^6$
Rabbit liver				
Preparation No. 1	1.66	0.55	6,800	$8.3 \cdot 10^6$
" No. 2	1.70	0.54	5,066	$9 \cdot 10^6$
Calf thymus				
Preparation No. 1	1.66	0.57	6,511	$7.8 \cdot 10^6$
" No. 2	1.68	0.55	6,110	$8.7 \cdot 10^6$
Rat spleen				
Preparation No. 1	1.60	0.57	6,762	$6.6 \cdot 10^6$

EXPERIMENTAL METHODS

DNA was isolated from chicken erythrocytes, rabbit liver, calf thymus and rat spleen. The material was frozen with dry ice immediately after slaughter of the animals and nucleoproteide obtained from it by the Mirsky-Pollister method. The nucleoproteide, reprecipitated 3-4 times, was deproteinized by Sevag's method. After deproteinization the DNA was precipitated with $1^1/_2$ volumes of ethyl alcohol. The precipitated threads were dissolved in 0.14 M NaCl and dialyzed against 0.14 M NaCl. The whole operation was carried out at 2°.

Phosphorus [2], nitrogen according to Konbe's method; $R_{E_{270}}$ [3] and the atomic extinction coefficient, to 1 g-atom phosphorus ε(P) [7] were determined in the DNA preparations. The relative amount of protein in the DNA preparations was judged by the ratio of the amount of nitrogen to the amount of phosphorus in these preparations. The data obtained is given in Table 1.

TABLE 2. DNA Precipitins in Normal Sera



The viscosity of the DNA solutions was determined in the Ostwald low-gradient viscosimeter. Molecular weight was calculated from the formula [4].

The specified preparations were used for observation of DNA precipitins in normal rabbit sera using the ring precipitation reaction, which was set up in the following way. 0.2 ml of DNA solution in concentrations of 1, 0.1, and 0.01 mg/ml was layered on 0.2 ml of whole serum. The test tubes were incubated for 2 h at 37°, and then placed in a refrigerator at 4°. Results were read after 24, 48, 72, and 96 h.

EXPERIMENTAL RESULTS

The presence of DNA precipitins in normal rabbit sera was determined, 1st, from samples of the animals' blood taken weekly for 2 months, and 2nd, from blood taken daily for 2 weeks. In both cases similar results were obtained, which indicates that the DNA precipitins in normal sera can appear and disappear. Typical results observed during daily blood sampling of the animals is given in Table 2. From this data, it is seen that in normal rabbit sera DNA precipitin titers fluctuate (precipitate formation time varied from 24 to 96 h).

These results were obtained from a study of the sera of 29 animals out of 38 in the experiment. We did not find DNA precipitins in the sera of 9 animals in this period.

DNA preparations isolated from various organs of different animals were used to study the specificity of the precipitation reaction: chicken erythrocytes, calf thymus, rabbit liver, rat spleen, etc.

It was shown that DNA preparations isolated from different sources react with normal sera at the same dilution (1:10), that is, in the given set-up of precipitation reaction, nucleic acid specificity does not appear. Investigation of the stability of "normal" precipitins during storage of the sera (at 4°) showed that the ability of the sera to give precipitates with DNA disappeared in 1-3 weeks depending on the original titers of the precipitins.

DNA fractions with different protein content which were obtained during deproteinization of one and the same preparation of desoxyribonucleoproteide were used to study the effect of protein contaminants in DNA preparations on the titer of their precipitates.

It was shown that the precipitate titer of nucleic acid preparations with rabbit sera was determined only by the DNA concentration and did not depend on the amount of protein in these fractions: preparations of DNA with different N/P values gave a precipitation reaction with normal sera at the same dilution (Table 3).

Similar results were obtained from 3 DNA preparations of various degrees of freedom from protein.

Along with this, the effect of the polymerization of DNA preparations on their ability to precipitate with sera was studied. For this purpose, polymerized DNA preparations were treated with crystalline DNA-ase in phosphate buffer at pH 6.45 in the presence of Mg at 37° for 17 h.

TABLE 3. Titer of Normal Rabbit Sera Precipitins to DNA Preparations with Different Amounts of Protein

Antigen	Serum No.			
	1560	104	66	55
DNA from chicken erythrocytes Molecular weight (8.5×10^6)	1:10	1:10	1:1	1:10
Fraction No. 1 N/p = 2.07				
Fraction No. 2 N/p = 1.56	1:10	1:10	1:1	1:10

Note: a small dilution of the DNA test antigen was made; original DNA concentration 1 mg/ml—1:1.

The activity of the enzyme was judged by the decrease in relative viscosity of 0.002% solutions of DNA; $\eta_{rel.}$ of 0.002% DNA solution before treatment with DNA-ase was 1.062; after treatment with DNA-ase it was 1.001.

From Table 4 it is seen that depolymerization of DNA by DNA-ase leads to a loss of the ability of DNA to precipitate with normal sera; in addition, this data indicates that protein contaminants do not take part in this reaction.

TABLE 4. Precipitation Reaction with Normal Rabbit Sera of DNA Preparations Before and After Treatment with DNA-ase

Serum No.	DNA from chicken erythrocytes (N/P = = 1.90)	
	Without DNA-ase treatment*	After DNA-ase treatment
104	+	—
1560	+	—
55	+	—

* DNA preparations without DNA-ase treatment were kept in pH 6.5 phosphate buffer in the presence of Mg ions for 17 h at 37°.

The investigation that was carried out shows that normal rabbit sera can precipitate DNA preparations from various sources at the same dilution, in addition, protein contaminants in the preparations have no effect on the DNA precipitation titer: the capacity of DNA to be precipitated is determined by their polymerization state.

Normal DNA precipitins are not stable to storage (at 4°).

SUMMARY

It was demonstrated that the DNA preparations can be precipitated by normal rabbit sera; no nucleic acid specificity was revealed in such cases. The capacity of DNA to precipitate with normal rabbit sera did not depend on the amount of protein admixtures in the DNA preparations and was determined by the polymeric state of the DNA molecule.

Normal precipitants to DNA are unstable when stored at +4°C.

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